

**THE EFFECTS OF A HIGHLY CHLORINATED BIPHENYL—DELOR 106—ON THE IN VIVO AND IN VITRO LUTEINIZING HORMONE SECRETION IN FEMALE PRUSSIAN CARP, *CARASSIUS GIBELIO* (ACTINOPTERYGII: CYPRINIFORMES: CYPRINIDAE)**

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**Background.** Endocrine disrupting chemicals (EDCs), among them polychlorinated biphenyls (PCBs), are able to change the hormonal regulation of reproduction at the hypothalamo-pituitary-gonadal (HPG) axis in many vertebrates. The aim of the presently reported study was to determine the effects of highly chlorinated PCBs mixture—Delor 106—on the in vivo and in vitro luteinizing hormone (LH) secretion from the pituitary gland of female Prussian carp, *Carassius gibelio* (Bloch, 1782), at the time of natural spawning.

**Materials and methods.** In the in vivo experiment we tested exposure to Delor 106: acute (one week at the concentration of  $700 \text{ ng} \cdot \text{L}^{-1}$ ) and chronic (5 weeks at increasing weekly concentrations, from 140 through  $700 \text{ ng} \cdot \text{L}^{-1}$ ). Blood samples from all females were taken before intoxication (0 week) and subsequently every week (week 1 through 5) till the end of the experiment. In the in vitro experiment enzymatically dispersed pituitary cells obtained from sexually mature Prussian carp females were incubated for 5 or 24 h in the presence of Delor 106 at the concentrations of: 1, 5, 10, 50, or  $100 \text{ ng} \cdot \text{mL}^{-1}$  of medium. LH levels in the incubation medium as well as in blood plasma were measured using ELISA method.

**Results.** Long-term (3 and 4 weeks) exposure to the Delor 106, gradually added to the water, caused a significant elevation of spontaneous LH secretion. Pituitary cells incubation with Delor 106 at the concentration range from 10 through  $100 \text{ ng} \cdot \text{mL}^{-1}$  of medium resulted in the significant increase of spontaneous LH secretion after 5 and 24 h.

**Conclusion.** The results obtained in this study show that highly chlorinated biphenyl—Delor 106—changes the secretion of luteinizing hormone in Prussian carp in the spawning season, acting, at least in part, directly at the pituitary level.

**Keywords:** PCBs mixture, reproduction, gonadotropins, cyprinids, endocrine disruptors

## INTRODUCTION

Polychlorinated biphenyls (PCBs) are considered endocrine disrupting chemicals (EDCs) because they have been blamed to change hormonal regulation of reproduction in many fish species (Khan et al. 2001, Coimbra et al. 2005, Khan and Thomas 2006, Coimbra and Reis-Henriques 2007, Daouk et al. 2011) and mammals (Gore et al. 2002, Gregoraszczyk et al. 2005, 2008, Kotwica et al. 2006, Dickerson and Gore 2007). Despite the ban on its production over 30 years ago, the PCBs are detected in the aquatic environment. PCBs are resistant to biodegradation and are lipophilic and that is why they easy accumulate in exposed organisms (Van Geest et al. 2011).

The negative effects of PCBs were observed at different life stages of the aquatic organisms: during their embryonic development (Socha et al. 2012), at larvae stages (Coimbra and Reis-Henriques 2007), as well as in the adults—inflicting usually disorders related to the endocrine system (Khan et al. 2001, Coimbra et al. 2005, Khan and Thomas 2006). In sexually mature fish the exposure to PCBs mixture leads to the neuroendocrine disorders at the hypothalamo-pituitary-gonadal (HPG) axis. For example, Aroclor 1254 (a common PCBs mixture) modulates gonadotropin secretion in Atlantic croaker, *Micropogonias undulatus* (Linnaeus, 1766), and common carp, *Cyprinus carpio* L. (see Khan and Thomas 2001, Socha et al. 2008b). Additionally it was demonstrated

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that, in Atlantic croaker, Aroclor 1254 influences the synthesis of gonadotropin-releasing hormone (GnRH) at the level of hypothalamus and significantly lowers the number of pituitary GnRH receptors, what in turn affects luteinizing hormone (LH) secretion. Therefore it is not surprising that in fish exposed to PCBs the weaker secretion of LH in response to GnRH analogue stimulation was observed (Khan and Thomas 2001, Khan et al. 2001).

There is a substantial evidence that in mammals, as well as in fish, polychlorinated biphenyls influence gonadal steroidogenesis (Yano and Matsuyama 1986, Wójtowicz et al. 2000, 2005, Benninghoff and Thomas 2005, Gregoraszczyk et al. 2005, Andric et al. 2006, Kotwica et al. 2006, Młynarczyk and Kotwica 2006). In fish, in addition to the observed disruptions in hormonal secretion at the HPG axis, exposure to PCBs mixtures suppresses ovarian follicle development, decreases ovarian growth, as well as decreases the quality of semen and eggs (Khan and Thomas 2001, Thomas and Doughty 2004, Coimbra and Reis-Henriques 2007, Socha et al. 2008a, Daouk et al. 2011, Kraugerud et al. 2012b).

The action of polychlorinated biphenyls might be estrogenic and/or anti-estrogenic (through estrogen receptor—ER and/or aryl hydrocarbon receptor—AhR) and is highly dependent on the number and position of chlorine atoms on the biphenyl rings (Jansen et al. 1993, Kester et al. 2000, Plíšková et al. 2005, Gregoraszczyk et al. 2005, 2008). In our study we used highly chlorinated biphenyl mixture—Delor 106—consisting of approximately 52.9% of hexa-, 19.4% hepta-, and 15% penta chlorobiphenyls (Taniyasu et al. 2003, Grabic et al. 2006)—to examine its *in vivo* effects, during the short- and the long-term exposure, on luteinizing hormone secretion in female Prussian carp, *Carassius gibelio* (Bloch, 1782), at the time of natural spawning. In the *in vitro* study the possible direct action of Delor 106 at the pituitary level on LH secretion (in a static culture of dispersed Prussian carp pituitary cells) was evaluated.

## MATERIAL AND METHODS

In the *in vivo* experiment 24 mature (2–3 year old) female Prussian carp, *Carassius gibelio*, were used. The fish were raised in the Fishery Station belonging to the Department of Ichthyobiology and Fisheries, University of Agriculture, Kraków, Poland. The average body weight of females was  $29.45 \pm 2.4$  g, and a mean gonadosomatic index (GSI) was  $8.6\% \pm 0.9\%$ . Fish were randomly divided into 3 groups (8 fish per group), individually weighed and marked. They were kept during 5 weeks in 3 aquaria (volume, 60 L); the temperature of water was approximately  $21 \pm 0.5^\circ\text{C}$ . Fish were exposed to a simulated, natural photoperiod (L : D = 16 : 8). Control fish were kept in fresh water (without addition of PCB). Second group was exposed to the maximal dose ( $700 \text{ ng} \cdot \text{L}^{-1}$  of water) of Delor 106 (a gift from the Institute of Public Health Ostrava, National Reference Laboratory for Analysis of POPs MH, Czech Republic) during the first week of the experiment. After this time the fish were transferred to the

aquaria with PCBs-free water for the next 4 weeks. Females in the third group were chronically (5 weeks) exposed to the weekly-increasing concentrations of Delor 106 ( $140, 280, 420, 560, \text{ and } 700 \text{ ng} \cdot \text{L}^{-1}$  of water, respectively). Every week the fish were anaesthetized with Propiscin (etomidate-based product, IRS, Zabieniec, Poland;  $3 \text{ mL} \cdot \text{L}^{-1}$  of water) (Lambooij et al. 2009, Kazuń i Siwicki 2012) and blood samples (approx. 100 mL) were taken from all fish by puncturing the caudal vein using heparinised 1-mL syringes. After blood centrifugation, the plasma samples were frozen at  $-20^\circ\text{C}$  until assayed. The levels of LH were analysed by the homologous ELISA assay according to the protocol proposed by Kah et al. (1989). The standard LH hormone and the specific antibody were kindly donated by Dr Bernard Breton (INRA, France).

For the *in vitro* experiment pituitaries were obtained from 16 sexually mature (GSI =  $9.5\% \pm 1.1\%$ ) female Prussian carp. The enzymatic dispersion of the pituitary glands was performed according to the method described by Mikołajczyk et al. (1990). In brief, collected pituitary glands were chopped into small pieces and subjected to dispersion for 6–8 h at  $20^\circ\text{C}$  in medium containing 0.1% (w/v) collagenase H (Boehringer Mannheim, Germany) and 1% BSA (Sigma-Aldrich, USA). After enzymatic dispersion cells were harvested by 10 min centrifugation ( $200 \text{ m} \cdot \text{s}^{-2}$  at  $20^\circ\text{C}$ ) and washed twice with preincubation medium containing 2% Ultrosor SF (Sepracor S.A., France) and 1% antibiotic-antimycotic (Sigma-Aldrich, USA). Cell viability test and cell counting were performed with a Thoma haemocytometer. Cells were resuspended in the pre-incubation medium and transferred into four 96-well microplates (Nunc A/S, Denmark) coated with Poly-L-lysine (Sigma-Aldrich, USA). Each well contained approximately  $5 \times 10^4$  cells in 250 mL of medium. Then the plates were sealed and incubated for 55 h at  $22^\circ\text{C}$ .

On the third day of incubation the pre-incubation medium was replaced with medium containing Delor 106 (at concentrations: 1, 5, 10, 50, and  $100 \text{ ng} \cdot \text{mL}^{-1}$  medium). Control wells were filled up with medium without any supplementation of PCBs. Two identical microplates were prepared. One of them was incubated for 5 h and the other for 24 h at  $22^\circ\text{C}$ . At the end of the incubation periods the plates were centrifuged ( $20 \text{ m} \cdot \text{s}^{-2}$  for 10 min at  $20^\circ\text{C}$ ), and the media were collected and frozen at  $-20^\circ\text{C}$  until LH determination by ELISA.

The experiments were conducted according to the research protocols approved by the Local Animal Ethics Committee in Krakow, Poland.

The mean LH levels from both experiments were analysed using a nonparametric two-tailed Mann–Whitney U-test. The differences between the means were determined as significant for  $P \leq 0.05$ .

## RESULTS

**The effect of short- and long-term exposure to Delor 106 on LH secretion in female Prussian carp *in vivo*.** Before the exposure to Delor 106 (week 0) there were no

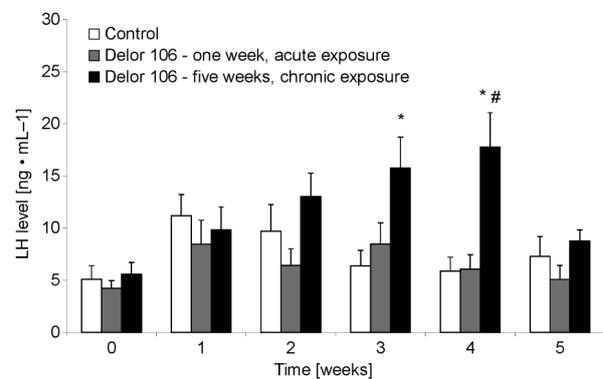
significant differences in LH levels among the groups. After first week of exposure both tested concentrations of Delor 106 (700 and 140 ng · L<sup>-1</sup>) did not change the LH secretion. No significant changes in LH release were also observed after second week of both acute and chronic exposure. The significant ( $P \leq 0.05$ ) stimulation of LH release was observed after 3 weeks of exposure in the third group with gradually increased concentration of Delor 106 in comparison to the control group (Fig. 1). The significant ( $P \leq 0.05$ ) elevation of LH secretion in this group was also observed a week later (after 4 weeks of exposure) in comparison to the control group and to the acute exposure. At the end of experiment (after 5 weeks) there were no significant differences in gonadotropin level among all groups.

**The effect of Delor106 on LH secretion from dispersed Prussian carp pituitary cells.** The incubation of female Prussian carp pituitary cells in medium containing Delor 106 caused the dose-dependent increase of LH release. After 5 and 24 h of incubation, Delor 106 at the concentration of: 10, 50, or 100 ng · mL<sup>-1</sup> significantly ( $P \leq 0.05$ ) stimulated luteinizing hormone secretion in comparison to the control group (Figs. 2 and 3). After 24 h of incubation also the lower concentration (5 ng · mL<sup>-1</sup>) of Delor 106 caused a significant ( $P \leq 0.05$ ) increase of LH release compared with the control group (Fig. 3). The smallest concentration (1 ng · mL<sup>-1</sup>) of Delor 106, at both times of incubations, had no effect on LH secretion (Figs. 2 and 3).

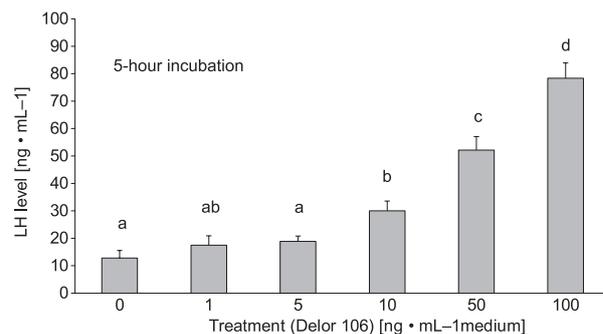
## DISCUSSION

Our results demonstrate the effect of a highly chlorinated biphenyl—Delor 106—on LH secretion at the time of Prussian carp natural spawning. In vivo exposure to Delor 106, added to the water, resulted in changes of LH secretion only in the case of chronic treatment of PCBs mixture—the elevation of LH secretion was observed after 3-week exposure to Delor 106 in comparison to the control group (Fig. 1). After 4 weeks LH level reached the maximum (17.76 ng · mL<sup>-1</sup>) and was still significantly different from the control group and also from the group exposed acutely, for one week, to the maximal (700 ng · L<sup>-1</sup> of water) dose of Delor 106. This maximal dose of Delor 106 did not change the release of LH after one week of acute exposure as well as till the end of experiment (Fig. 1). These results may suggest that the chronic exposure to the small doses of PCBs is more disrupting for Prussian carp HPG axis, because this kind of intoxication alters gonadotropin secretion in females. The stimulatory effect of Delor 106 on the spontaneous LH secretion in Prussian carp is in contrast to the data presented for male Atlantic croaker exposed to another PCBs mixture—Aroclor 1254—given for 30 days in a diet, in which the observed LH levels were significantly lower than in the control group (Khan and Thomas 2001). It is worth noticing that the difference in these results may be due to the sex of experimental fish, as well as, the composition of this two PCB mixtures (Aroclor 1254 and Delor 106) and their tested doses. The reduced base level of LH, related with

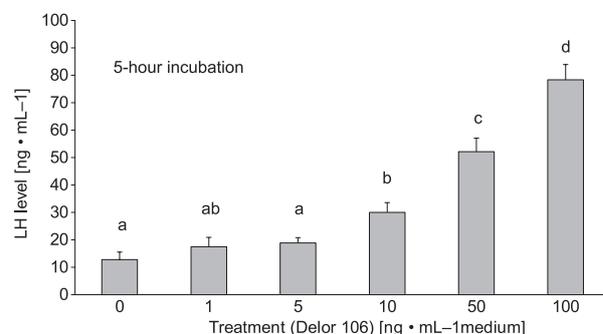
the sex of animals, was observed also in higher vertebrates (rat, goat) following exposure to PCB mixtures or single congeners (Desaulniers et al. 1999, Oskam et al. 2005, Yamamoto et al. 2005). Desaulniers et al. (1999) showed



**Fig. 1.** The effect of short- and long-term exposure to Delor 106 on LH secretion in female Prussian carp, *Carassius gibelio*; Each value represents the mean  $\pm$  SEM ( $n = 8$ ); \* vs control, # vs Delor 106, one week, acute exposure ( $P \leq 0.05$ )



**Fig. 2.** The effect of Delor 106 on LH secretion from dispersed pituitary cells of female Prussian carp, *Carassius gibelio*, after 5 h of incubation; Bars represent means and SEM are given as vertical error bars ( $n = 5$ ); Groups bearing the same letter are not significantly different ( $P \leq 0.05$ )



**Fig. 3.** The effect of Delor 106 on LH secretion from dispersed pituitary cells of female Prussian carp, *Carassius gibelio*, after 24 h of incubation; Bars represent means and SEM are given as vertical error bars ( $n = 5$ ); Groups bearing the same letter are not significantly different ( $P \leq 0.05$ )

that lower circulating LH level in male rats treated with PCB 126 was associated with increased content of LH in the pituitary gland. These results support PCBs-induced alternation of the secretory mechanisms of the gonadotroph cells. It is known that the secretion of luteinizing hormone from the pituitary gland is estrogen-sensitive process, depended on the level of blood steroid hormones. As mentioned in the introduction, the action of PCBs might be both estrogenic and antiestrogenic, what is highly dependent on the molecular weight and the structure of biphenyls. In the case of Delor 106 the composition of chlorobiphenyls homologues consists mostly of hexa-, hepta-, and penta-chlorobiphenyls, whereas in Aroclor 1254 the most dominant (over 50%) are pentachlorobiphenyl congeners, such as dioxin-like congeners PCB126 (Taniyasu et al. 2003). Antiestrogenicity was found in the case of hexa-, hepta-, and octachlorinated biphenyls resulting, for example, in the inhibition of estrogen receptor (ER) activation in human males (Plíšková et al. 2005). Probably the stimulation of LH secretion in Prussian carp after chronic exposure to Delor 106 was caused by this PCBs mixture antiestrogenic properties, which were previously demonstrated on mammals, carried on the *theca interna* and *granulosa* cells from porcine ovary after single exposure to Delor 106 (Gregoraszczyk et al. 2005). It seems possible that the changed activation of estrogen receptor (ER) in Delor 106 treated Prussian carp females in the spawning season, might be responsible for the observed LH stimulation. But to support possible changes in ER activity after Delor 106 exposure it would be important to provide further study with the evaluation of the level of other steroid hormones (estradiol, progesterone) and estradiol-stimulated vitellogenin secretion.

Taking into consideration the results obtained in the in vivo exposure to Delor106 only, it is not possible to determine the exact site of PCBs mixture action. Changes in LH secretion might be caused by the action of Delor 106 at the level of hypothalamus and/or pituitary, where the estrogen receptors are also present (Kah et al. 1997, Muriach et al. 2008). Khan et al. 2001 showed also the adverse effect of PCBs on the most important hypothalamic factor controlling the synthesis and secretion of LH—the gonadotropin-releasing hormone (GnRH). Pituitary sensitivity to GnRH stimulation is known to be modulated by estrogens, so exposure to different PCBs, which exert estrogenic and/or anti-estrogenic activities, may change pituitary sensitivity to GnRH, resulting in disruption of GnRH-stimulated LH secretion (Khan and Thomas 2001, Desotelle et al. 2005, Kraugerud et al. 2012a).

The results of in vitro experiments with pituitary cells of Prussian carp, incubated with Delor 106, showing dose-dependent elevation of spontaneous LH release after 5 and 24 h of incubation with Delor 106 at the concentration of: 10, 50, and 100 ng · mL<sup>-1</sup> (Fig. 2) let to conclude that Delor 106 acts also at the pituitary level. Our previous in vitro study on dispersed common carp pituitary cells, exposed to Aroclor 1254, also showed the direct

action of PCBs on this gland (Socha et al. 2008b). It was found that Aroclor 1254 (5 ng · mL<sup>-1</sup>) stimulated spontaneous LH secretion in females, but not in males, what could be linked to the different sensitivity of the pituitary cells to endocrine disrupting factors. Several other exogenous environmental factors similar to PCBs, including the most toxic halogenated aromatic hydrocarbons—TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin)—are known to affect secretion of pituitary gonadotropins in pigs and rats (Li et al. 1997, Petroff et al. 2003, Jablonska et al. 2011). The dose-dependent LH elevation caused by TCDD was observed in immature female rats (Li et al. 1997). The stimulatory effect of TCDD on in vitro LH release was also found during the follicular phase of porcine estrous cycle (Jablonska et al. 2011). Taking into consideration the possible antiestrogenic activity of TCDD (Buchanan et al. 2000) as well as of Delor 106, which consists also of dioxin-like congeners PCB126 (Taniyasu et al. 2003), the stimulation of LH secretion in Prussian carp females observed in our study might be due to the activation of the similar ways at the pituitary level. Further studies are needed to explain the effects of polychlorinated biphenyls on both female and male Prussian carp reproduction cycle.

In summary, our results showed that:

The chronic exposure to a highly chlorinated biphenyl—Delor 106—increases the secretion of luteinizing hormone in *Carassius gibelio* females at the time of natural spawning;

Delor 106 stimulates LH secretion acting, at least in part, directly at the pituitary level.

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#### REFERENCES

- Andric N.L., Kostic T.S., Zoric S.N., Stanic B.D., Andric S.A., Kovacevic R.Z.** 2006. Effect of a PCB-based transformer oil on testicular steroidogenesis and xenobiotic-metabolizing enzymes. *Reproductive Toxicology* **22** (1): 102–110. DOI: 10.1016/j.reprotox.2005.12.002
- Benninghoff A.D., Thomas P.** 2005. Involvement of calcium and calmodulin in the regulation of ovarian steroidogenesis in Atlantic croaker (*Micropogonias undulatus*) and modulation by Aroclor 1254. *General and Comparative Endocrinology* **144** (3): 211–223. DOI: 10.1016/j.ygcen.2005.06.005
- Buchanan D.L., Sato T., Peterson R.E., Cooke P.S.** 2000. Antiestrogenic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in mouse uterus: Critical role of the aryl hydrocarbon receptor in stromal tissue. *Toxicological Sciences* **57** (2): 302–311. DOI: 10.1093/toxsci/57.2.302
- Coimbra A.M., Reis-Henriques M.A.** 2007. Tilapia larvae Aroclor 1254 exposure: Effects on gonads and circulating thyroid hormones during adulthood. *Bulletin of Environmental Contamination and Toxicology* **79** (5): 488–493. DOI: 10.1007/s00128-007-9288-2

- Coimbra A.M., Reis-Henriques M.A., Darras V.M.** 2005. Circulating thyroid hormone levels and iodothyronine deiodinase activities in Nile tilapia (*Oreochromis niloticus*) following dietary exposure to Endosulfan and Aroclor 1254. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology* **141** (1): 8–14. DOI: 10.1016/j.cca.2005.04.006
- Daouk T., Larcher T., Rouspard F., Lyphout L., Rigaud C., Ledevin M., Loizeau V., Cousin X.** 2011. Long-term food-exposure of zebrafish to PCB mixtures mimicking some environmental situations induces ovary pathology and impairs reproduction ability. *Aquatic Toxicology* **105** (3–4): 270–278. DOI: 10.1016/j.aquatox.2011.06.021
- Desaulniers D., Leingartner K., Wade M., Fintelman E., Yagminas A., Foster W.G.** 1999. Effects of acute exposure to PCBs 126 and 153 on anterior pituitary and thyroid hormones and FSH isoforms in adult Sprague Dawley male rats. *Toxicology Sciences* **47** (2): 158–169. DOI: 10.1093/toxsci/47.2.158
- Desotelle J., Splett C., Scheffen J.** 2005. PCB exposure alters anterior pituitary sensitivity to gonadotropin-releasing hormone (GnRH) in adult female rats. *Bios* **76** (2): 68–76. DOI: 10.1893/0005-3155(2005)076[0068:RAPEAA]2.0.CO;2
- Dickerson S.M., Gore A.C.** 2007. Estrogenic environmental endocrine-disrupting chemical effects on reproductive neuroendocrine function and dysfunction across the life cycle. *Reviews in Endocrine and Metabolic Disorders* **8** (2): 143–159. DOI: 10.1007/s11154-007-9048-y
- Gore A.C., Wu T.J., Oung T., Lee J.B., Woller M.J.** 2002. A novel mechanism for endocrine-disrupting effects of polychlorinated biphenyls: Direct effects on gonadotropin-releasing hormone neurones. *Journal of Neuroendocrinology* **14** (10): 814–823. DOI: 10.1046/j.1365-2826.2002.00845.x
- Grabic R., Hansen L.G., Ptak A., Crhova S., Gregoraszcuk E.L.** 2006. Differential accumulation of low-chlorinated (Delor 103) and high-chlorinated (Delor 106) biphenyls in human placental tissue and opposite effects on conversion of DHEA to E2. *Chemosphere* **62** (4): 573–580. DOI: 10.1016/j.chemosphere.2005.06.027
- Gregoraszcuk E.L., Ptak A., Karniewska M., Ropstad E.** 2008. Action of defined mixtures of PCBs, p,p'-DDT and its metabolite p,p'-DDE, on co-culture of porcine theca and granulosa cells: Steroid secretion, cell proliferation and apoptosis. *Reproductive Toxicology* **26** (2): 170–174. DOI: 10.1016/j.reprotox.2008.07.003
- Gregoraszcuk E., Zemla M., Ptak A., Grabic R.** 2005. The action of low- and high-chlorinated biphenyl mixture on the prepubertal porcine ovary: Steroid secretion and cell apoptosis. *Endocrine Regulation* **39** (2): 33–41.
- Jablonska O., Piasecka J., Petroff B.K., Nynca A., Siawrys G., Wąsowska B., Zmijewska A., Lewczuk B., Ciereszko R.E.** 2011. In vitro effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on ovarian, pituitary, and pineal function in pigs. *Theriogenology* **76** (5): 921–932. DOI: 10.1016/j.theriogenology.2011.04.023
- Jansen H.T., Cooke P.S., Porcelli J., Liu T.-C., Hansen L.G.** 1993. Estrogenic and antiestrogenic actions of PCBs in the female rat: In vitro and in vivo studies. *Reproductive Toxicology* **7** (3): 237–248. DOI: 10.1016/0890-6238(93)90230-5
- Kah O., Anglade I., Linard B., Pakdel F., Salbert G., Bailhache T., Ducouret B., Saligaut C., Le Goff P., Valotaire Y., Jégo P.** 1997. Estrogen receptors in the brain-pituitary complex and the neuroendocrine regulation of gonadotropin release in rainbow trout. *Fish Physiology and Biochemistry* **17** (1–6): 53–62. DOI: 10.1023/A:1007749715473
- Kah O., Ponent A., Rodriguez J.N., Calas A., Breton B.** 1989. Development of enzyme-linked immunosorbent assay for goldfish gonadotropin. *Biology of Reproduction* **40**: 68–73. DOI: 10.1095/biolreprod41.1.68
- Kazuń K., Siwicki A.K.** 2012. Propiscin – a safe new anaesthetic for fish. *Archives of Polish Fisheries* **20** (173–177). DOI: 10.2478/v10086-012-0021-3
- Kester M.H.A., Bulduk S., Tibboel D., Meinel W., Glatt H., Falany C.N., Coughtrie M.W.H., Bergman A., Safe S.H., Kuiper G.G.J.M., Schuur A.G., Brouwer A., Visser T.J.** 2000. Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: A novel pathway explaining the estrogenic activity of PCBs. *Endocrinology* **141** (5): 1897–1900. DOI: 10.1210/en.141.5.1897
- Khan I.A., Mathews S., Okuzawa K., Kagawa H., Thomas P.** 2001. Alterations in the GnRH-LH system in relation to gonadal stage and Aroclor 1254 exposure in Atlantic croaker. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **129** (2–3): 251–259. DOI: 10.1016/S1096-4959(01)00318-9
- Khan I.A., Thomas P.** 2001. Disruption of neuroendocrine control of luteinizing hormone secretion by Aroclor 1254 involves inhibition of hypothalamic tryptophan hydroxylase activity. *Biology of Reproduction* **64** (3): 955–964. DOI: 10.1095/biolreprod64.3.955
- Khan I.A., Thomas P.** 2006. PCB congener-specific distribution of reproductive neuroendocrine function in Atlantic croaker. *Marine Environmental Research* **62** (Suppl. 1): S25–S28. DOI: 10.1016/j.marenvres.2006.04.029
- Kotwica J., Wróbel M., Młynarczuk J.** 2006. The influence of polychlorinated biphenyls (PCBs) and phytoestrogens in vitro on functioning of reproductive tract in cow. *Reproductive Biology* **6** (Suppl. 1): 189–94.
- Kraugerud M., Aleksandersen M., Nyengaard J.R., Østby G.C., Gutleb A.C., Dahl E., Berg V., Farstad W., Schweder T., Skaare J.U., Ropstad E.** 2012a. In utero and lactational exposure to PCB 118 and PCB 153 alter ovarian follicular dynamics and GnRH-induced luteinizing hormone secretion in female lambs. *Environmental Toxicology* **27** (11): 623–634. DOI: 10.1002/tox.20679
- Kraugerud M., Doughty R.W., Lyche J.L., Berg V., Tremoen N.H., Aleström P., Aleksandersen M., Ropstad E.** 2012b. Natural mixtures of persistent organic pollutants (POPs) suppress ovarian follicle development, liver vitellogenin immunostaining and hepatocyte proliferation in female zebrafish (*Danio rerio*). *Aquatic Toxicology* **116–117**: 16–23. DOI: 10.1016/j.aquatox.2012.02.031
- Lambooij B., Pilarczyk M., Białowas H., Reimert H., André G., Van De Vis H.** 2009. Anaesthetic properties of Propiscin

- (Etomidaat) and 2-phenoxyethanol in the common carp (*Cyprinus carpio* L.), neural and behavioural measures. *Aquaculture Research* **40** (11): 1328–1333. DOI: 10.1111/j.1365-2109.2009.02233.x
- Li X., Johnson D.C., Rozman K.K.** 1997. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) increases release of luteinizing hormone and follicle-stimulating hormone from the pituitary of immature female rats in vivo and in vitro. *Toxicology and Applied Pharmacology* **142** (2): 264–269. DOI: 10.1006/taap.1996.8044
- Mikolajczyk T., Weil C., Epler P., Breton B.** 1990. Involvement of voltage-dependent calcium channels (VDCC) in the action of GnRH on GtH release in common carp (*Cyprinus carpio* L): comparison with K<sup>+</sup> action. *Reproduction Nutrition Development* **30** (5): 619–628. DOI: 10.1051/rnd:19900507
- Młynarczuk J., Kotwica J.** 2006. Influence of polychlorinated biphenyls on LH-stimulated secretion of progesterone and oxytocin from bovine luteal cells. *Polish Journal of Veterinary Sciences* **9** (2): 101–108.
- Muriach B., Carrillo M., Zanuy S., Cerdá-Reverter J.M.** 2008. Distribution of estrogen receptor 2 mRNAs (Esr2a and Esr2b) in the brain and pituitary of the sea bass (*Dicentrarchus labrax*). *Brain Research* **1210**: 126–141. DOI: 10.1016/j.brainres.2008.02.053
- Oskam I.C., Lyche J.L., Krogenæs A., Thomassen R., Skaare J.U., Wiger R., Dahl E., Sweeney T., Stien A., Ropstad E.** 2005. Effects of long-term maternal exposure to low doses of PCB126 and PCB153 on the reproductive system and related hormones of young male goats. *Reproduction* **130** (5): 731–742. DOI: 10.1530/rep.1.00690
- Petroff B.K., Crouch C.R., Hunter D.M., Wierman M.E., Gao X.** 2003. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) stimulates gonadotropin secretion in the immature female Sprague-Dawley rat through a pentobarbital- and estradiol-sensitive mechanism but does not alter gonadotropin-releasing hormone (GnRH) secretion by immortalized GnRH neurons in vitro. *Biology of Reproduction* **68** (6): 2100–2106. DOI: 10.1095/biolreprod.102.010439
- Plíšková M., Vondráček J., Canton R.F., Nera J., Kočan A., Petrik J., Trnovec T., Sanderson T., van den Berg M., Machala M.** 2005. Impact of polychlorinated biphenyls contamination on estrogenic activity in human male serum. *Environmental Health Perspectives* **113** (10): 1277–1284. DOI: 10.1289/ehp.7745
- Socha M., Sokolowska-Mikolajczyk M., Szczerbik P., Chyb J., Mikolajczyk T., Epler P.** 2012. The effect of polychlorinated biphenyls mixture (Aroclor 1254) on the embryonic development and hatching of Prussian carp, *Carassius gibelio*, and common carp, *Cyprinus carpio* (Actinopterygii: Cypriniformes: Cyprinidae). *Acta Ichthyologica et Piscatoria* **42** (1): 31–35. DOI: 10.3750/AIP2011.42.1.04
- Socha M., Sokolowska-Mikolajczyk M., Szczerbik P., Chyb J., Dietrich G., Kowalski R., Grabic R., Epler P.** 2008a. The influence of polychlorinated biphenyls (PCBs) on computer analysed sperm motility of common carp (*Cyprinus carpio* L.) in vitro. *Cybiurn* **32** (Suppl. 2): 197.
- Socha M., Sokolowska-Mikolajczyk M., Szczerbik P., Mikolajczyk T., Chyb J., Duc M., Epler P.** 2008b. Effect of Aroclor 1254 (PCB) on spontaneous and sGnRHa-stimulated LH secretion from male and female carp (*Cyprinus carpio* L.) pituitary gland. p. 142. *In: Proceeding 6th International Symposium of Fish Endocrinology*, 22–27 June 2008, Calgary, Canada.
- Taniyasu S., Kannan K., Holoubek I., Ansorgova A., Horii Y., Hanari N., Yamashita N., Aldous K.M.** 2003. Isomer-specific analysis of chlorinated biphenyls, naphthalenes and dibenzofurans in Delor: polychlorinated biphenyl preparations from the former Czechoslovakia. *Environmental Pollution* **126** (2): 169–178. DOI: 10.1016/S0269-7491(03)00207-0
- Thomas P., Doughty K.** 2004. Disruption of rapid, nongenomic steroid actions by environmental chemicals: Interference with progestin stimulation of sperm motility in Atlantic croaker. *Environmental Science and Technology* **38** (23): 6328–6332. DOI: 10.1021/es0403662
- Van Geest J.L., Mackay D., Poirier D.G., Sibley P.K., Solomon K.R.** 2011. Accumulation and depuration of polychlorinated biphenyls from field-collected sediment in three freshwater organisms. *Environmental Science and Technology* **45** (16): 7011–7018. DOI: 10.1021/es1043744
- Wójtowicz A.K., Goch M., Gregoraszczyk E.L.** 2005. Polychlorinated biphenyls (PCB 153 and PCB 126) action on conversion of 20-hydroxylated cholesterol to progesterone, androstenedione to testosterone, and testosterone to estradiol 17beta. *Experimental and Clinical Endocrinology and Diabetes* **113** (8): 464–470. DOI: 10.1055/s-2005-865776
- Wójtowicz A.K., Gregoraszczyk E.L., Lyche J.L., Ropstad E.** 2000. Time dependent and cell-specific action of polychlorinated biphenyls (PCB 153 and PCB 126) on steroid secretion by porcine *theca* and *granulosa* cells in mono- and coculture. *Journal of Physiology and Pharmacology* **51** (3): 555–568.
- Yamamoto M., Narita A., Kagohata M., Shirai M., Akahori F., Aroshima K.** 2005. Effects of maternal exposure to 3,3',4,4',5-pentachlorobiphenyl (PCB126) or 3,3',4,4',5,5'-hexachlorobiphenyl (PCB169) on testicular steroidogenesis and spermatogenesis in male offspring rats. *Journal of Andrology* **26** (2): 205–214. DOI: 10.1002/j.1939-4640.2005.tb01087.x
- Yano T., Matsuyama H.** 1986. Stimulatory effect of PCB on the metabolism of sex hormones in carp hepatopancreas. *Bulletin of the Japanese Society of Scientific Fisheries* **52** (10): 1847–1852.

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