ACTA ICHTHYOLOGICA ET PISCATORIA (2012) 42 (1): 31–35 DOI: 10.3750/AIP2011.42.1.04

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)

Magdalena SOCHA*, Mirosława SOKOŁOWSKA-MIKOŁAJCZYK, Paweł SZCZERBIK, Jarosław CHYB, Tomasz MIKOŁAJCZYK, and Piotr EPLER

Department of Ichthyobiology and Fisheries, University of Agriculture in Kraków, Poland

Socha M., Sokołowska-Mikołajczyk M., Szczerbik P., Chyb J., Mikołajczyk 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 Ichthyol. Piscat. 42 (1): 31–35.

Background. Polychlorinated biphenyls (PCBs) are persistent and bioacumulative chemical pollutants which exert negative physiological effects on the reproductive system of mature male and female fish. PCBs present in the aquatic environment may also have an influence on developing embryos. The aim of this study was to investigate the effect of Aroclor 1254, a polychlorinated biphenyls mixture, on the embryonic development of Prussian carp, *Carassius gibelio* (Bloch, 1782), and common carp, *Cyprinus carpio* L.

Materials and methods. The samples of eggs obtained from 4 females of each species were divided into two dishes and incubated separately after fertilization (common carp) or activation (Prussian carp) with common carp sperm. The incubation, with Aroclor 1254 (1 or 10 ng · mL^{-1}) or in water with no PCB added, lasted for 4 days. The mortality, hatching rate, number of hatched larvae, and number of deformed larvae were observed.

Results. After 24 h of incubation of Prussian carp and common carp eggs there were no significant differences in the percentage of living eggs between the Aroclor 1254 treated groups and the control one. The lowest tested concentration of PCB (1 ng · mL^{-1}) accelerated the hatching of Prussian carp larvae at 75 h of incubation. The significant increase in the percentage of deformed larvae was observed only in the experiment with common carp eggs incubated with Aroclor 1254 at the concentration of 10 ng · mL^{-1}.

Conclusion. Results of the presented data showed that Aroclor 1254 (at tested concentrations) is not harmful for the development of activated Prussian carp eggs but teratogenic effect was observed in the case of common carp embryos.

Keywords: PCBs mixture, common carp, Prussian carp, embryonic development, reproduction

INTRODUCTION

Aroclor 1254, a polychlorinated biphenyls (PCBs) mixture, belongs to endocrine-disrupting chemicals (EDCs) which are able to affect the neuroendocrine axis (hypothalamo-pituitary-gonadal) involved in the regulation of reproductive function in fish (Khan et al. 2001, Khan and Thomas 2006) and in other vertebrates (Colborn et al. 1993, Gore 2001). The action of polychlorinated biphenyls as endocrine disruptors (usually through the oestrogen or androgen receptors) depends on the degree of their chlorination. Low chlorinated PCBs act in an estrogenic manner, and these with higher (>48%) chlorination act as weak estrogens or as oestrogen receptor antagonist (Kester et al. 2000, Gregoraszczuk et al. 2008). There are data showing that the exposure of adult fish to PCBs, leads to the changes in GnRH neurons, the key cells involved in the control of the secretion of luteinizing hormone (Khan and Thomas 2001). The impairment of LH secretion was found in Atlantic croaker, *Micropogonias undulatus* (Linnaeus, 1766), and common carp, *Cyprinus carpio*, after exposure to Aroclor 1254 (Khan and Thomas 2001, Socha et al. 2008a). Khan and Thomas (2001) explain that this neuroendocrine disruption of gonadotropin release is caused, at least in part, by inhibition of hypothalamic monoamine neurotransmitters (5-HT and DA). In vitro culture of pituitary carp cells in the presence of PCBs shows the possibility of direct action of PCB on LH release at the level of pituitary (Socha et al. 2008a). Beside the action on the hypothalamic-pituitary-gonadal axis PCBs are able to change other endocrine glands function linked to the reproduction. In experiments on Atlantic croaker and Nile tilapia,

* Correspondence: Dr Magdalena Socha, Katedra Ichtiobiologii i Rybactwa, Uniwersytet Rolniczy w Krakowie, ul. Spiczakowa 6, 30-199 Kraków-Mydlniki, Poland, phone: +48 12 6375176, fax: + 48 12 6385979, email: *m.socha@ur.krakow.pl*.

Oreochromis niloticus (L.), polychlorinated biphenyls were also found to alter thyroid status by changing circulating levels of T_4 and/or T_3 (LeRoy et al. 2006, Coimbra and Reis-Henriques 2007). Thyroid hormones are important in fish early development, growth, and reproduction (Power et al. 2001). PCBs are known to accumulate in the lipid-rich tissues—they were found in liver and gonads of many aquatic organisms. High PCB levels in ovary or eggs have been associated with reproductive failures such as decreased hatching success and high embryonic mortality in Baltic herring, *Clupea harengus* L., or lake trout, *Salvelinus namaycush* (Walbaum, 1792) (see: Mac et al. 1993, Hansen et al. 1985). These types of xenobiotics can be transmitted parentally or environmentally, and finally they affect the offspring. Despite a ban on manufacture of PCBs in the late 1970s, they are still present in the aquatic environment and may have an effect on developing embryos.

The aim of this study was to investigate the effect of Aroclor 1254, a polychlorinated biphenyls mixture with a 54% (weight) of chlorine, on the embryonic development of two cyprinid species: Prussian carp, *Carassius gibelio* (Bloch, 1782), and common carp, *Cyprinus carpio* L.

MATERIAL AND METHODS

The experiments were conducted at the Department of Ichthyobiology and Fisheries, University of Agriculture in Krakow, Poland in the end of June 2009.

For the first experiment the eggs were obtained from 4 Prussian carp (*Carassiuas gibelio*) females, stimulated previously with sGnRH analogue (Bachem Feinchemicalien AG, Switzerland) at the dose of 10 mg · kg^{-1} body weight. The samples of eggs obtained from each female were divided into two Petri dishes (about 150 eggs in each dish) and activated with common carp sperm. After 1 min the water in experimental groups was replaced with 1 or 10 ng · mL^{-1} of Aroclor1254 (Promochem, Poland), while in control group—with clean water (seasoned tap water supplemented with Fungi Stop Konzentrat (TETRA, Germany).

In the second experiment the eggs were obtained from 4 common carp (*Cyprinus carpio*) females, which were stimulated with Gonazon™ 32 mg · kg^{-1} body weight (Intervet International BV, Boxmeer, The Netherlands) to induce ovulation. The eggs were fertilized with 10 µL of common carp sperm. After 1 min the water in experimental groups was replaced with appropriate mixture of Aroclor 1254 (1, 10, 50, or 100 ng · mL^{-1} water), while in control group—with clean water. Fungi Stop at the concentration of 75 μL^{-1} of water was used to prevent fungal diseases of incubated eggs.

Water was changed twice a day in each dish. The incubation lasted for about 4 days. The mortality, hatching rate, number of hatched larvae, and number of deformed larvae (with vertebral curvatures, yolk sac malformation) were observed and counted. The results were analysed by means of nonparametric two-tailed Mann–Whitney test. The differences between the means were determined as significant for $P < 0.05$.

RESULTS

The effect of Aroclor 1254 on the development of activated Prussian carp eggs. After 24 h of incubation there was no significant difference in the percentage of live eggs between the Aroclor 1254 treated groups and the control one (Fig. 1). Statistically significant higher rate of hatching at 75 h of exposure to Aroclor at the concentration of 1 ng · mL^{-1} was noted (Fig. 2). There were no significant differences in the hatching rate after: 78, 81, 85, 89, and 99 h of exposure (Fig. 2). There was no significant difference in the percentage of deformed larvae between all groups of exposure (Fig. 3).

The efect of Aroclor 1254 on the development of fertilised common carp eggs. The proportion of live eggs ranged from 76.4% in the group incubated with Aroclor 1254 at the concentration of 10 ng · mL^{-1} to 80.28% in the control group. There was no significant difference in the percentage of living carp eggs between the experimental groups and the control one after the first day of incubation (Fig. 4).

Tested concentration of Aroclor 1254 (1, 10, 50 and 100 ng · mL^{-1} water) did not change the hatching rate of common carp larvae (Fig. 5). There was no significant time dependent effect on number of hatched larvae.

The highest percentage of deformed larvae was found in the group incubated with Aroclor 1254 at the concentration of 10 ng · mL^{-1} water (Fig. 6). The percentage of deformed larvae was significantly different in comparison with control group ($P < 0.05$).

DISCUSSION

Chemical pollutants (heavy metals, PCBs) present in aquatic environment can disrupt vital physiological processes (growth, reproduction, osmoregulation) in fish during their different life stages (Coimbra and Reis-Henriques 2007, Khan and Thomas 2001, Szczerbik et al. 2008). Exposure to PCBs may decline the population in many fish species due to decreased ovarian growth (Khan and Thomas 2001), induction of testicular lesions (Sangalang et al. 1981), decreased motility of sperm (Socha et al. 2008b), and finally due to the reduction in the number of spawned eggs (Örn et al. 1998, Heiden et al. 2006).

In this study we have focused on the influence of PCB mixture Aroclor 1254 on embryonic development and hatching of two freshwater species: Prussian and common carp. Aroclor 1254 was added after activation/fertilization of eggs with carp semen, to eliminate the effect of PCBs on sperm motility, because there are data showing that PCBs are able to decrease spermatozoa important parameters (Socha et al. 2008b). In both experiments there was no difference in the percentage of living eggs in the first 24 h of incubation; that period appears to be the most sensitive time for egg viability (Kime 1999). Concerning the hatching time, acceleration of hatching at 75 h at the lowest concentration of Aroclor 1254 (1 ng · mL^{-1}), was found only in *Carassius gibelio* eggs. In the second experiment with fertilised common carp eggs there were no differences of hatching frequency among all groups. Similar results

were obtained in zebrafish, *Danio rerio* (Hamilton, 1822), exposed to the mixture of PCB—no difference of hatching frequency or median hatching time were observed (Örn et al. 1998). In our study, the significantly higher number of deformed common carp larvae was observed after exposure to 10 ng · mL^{-1} of Aroclor 1254. It seems that common carp eggs are more sensitive to this kind of xenobiotics than eggs of Prussian carp. The lack of larvae deformation in *Carassius gibelio*, after exposure to the same concentration of Aroclor 1254 (1 and 10 ng · mL^{-1}) was noted. It is worth noticing that activated Prussian carp eggs were also resistant to cadmium, another waterborne pollutant (Szczerbik et al. 2008). It is known that the outermost layer of the chorion binds the greatest amount of PCBs, antibiotics, or other toxic chemicals and thereby impede entry of these xenobiotics (Kudo and Yazawa 1997, Mäenpää et al. 2004). These properties may explain why greater bioaccumulation occurs later in development of fish (Mäenpää et al. 2004) perhaps due to saturation of the chorion binding sites, and why hatched larvae are more sensitive to xenobiotics (Viant et al. 2006).

Summing up, our results showed that Aroclor 1254 (at tested concentrations) is not harmful to the development of activated Prussian carp and common carps eggs, but in

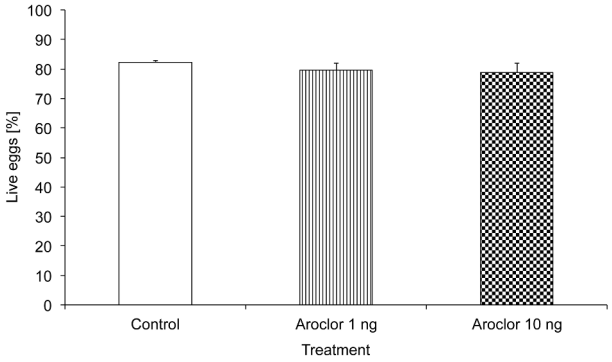

Fig. 1. The influence of Aroclor 1254 on the percentage of live eggs of Prussian carp, *Carassius gibelio*, after 24 h of incubation; Data are expressed as means ± SEM (n = 4)

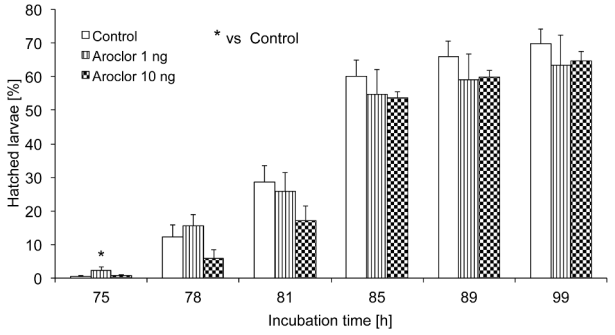

Fig. 2. The influence of Aroclor 1254 on hatching rate of activated eggs of Prussian carp, *Carassius gibelio*; Data are expressed as means ± SEM (n = 4)

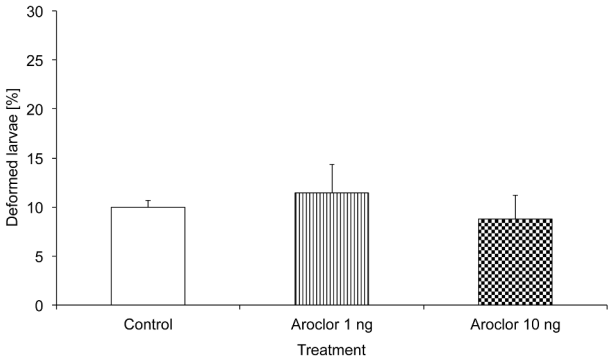

Fig. 3. The effects of Aroclor 1254 on the number of deformed larvae of Prussian carp, *Carassius gibelio*; Data are expressed as means ± SEM (n = 4)

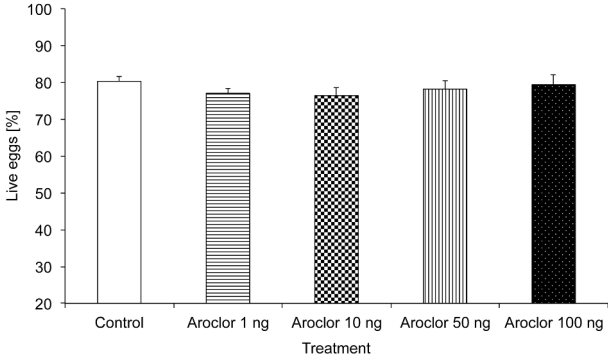

Fig. 4. The influence of Aroclor 1254 on the percentage of live eggs of common carp, *Cyprinus carpio*, after the first day of incubation; Data are expressed as means ± SEM (n = 4)

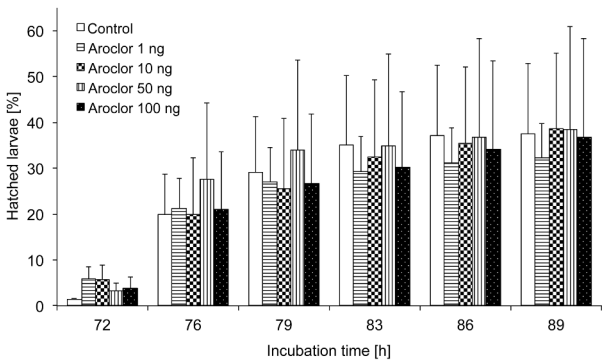

Fig. 5. The influence of Aroclor 1254 on hatching rate of fertilised eggs of common carp, *Cyprinus carpio*; Data are expressed as means ± SEM (n = 4)

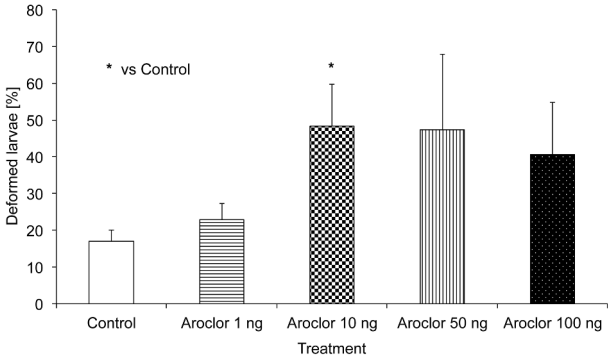

Fig. 6. The effects of Aroclor 1254 on the number of deformed larvae of common carp, *Cyprinus carpio*; Data are expressed as means ± SEM (n = 4)

the case of common carp embryos, PCB mixture may increase the teratogenic effect. Since fish larvae can be more sensitive to contaminants than developing embryos it is important to evaluate the impact of Aroclor 1254 during early life stages of Prussian carp and common carp.

ACKNOWLEDGEMENTS

This work has been financially supported by the Ministry of Science and Higher Education of Poland; grant N311 032 32/2322.

REFERENCES

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

Colborn T., von Saal F.S., Soto A.M. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environmental Health Perspectives **101** (5): 378–384. DOI: 10.2307/3431890

Gore A.C. 2001. Environmental toxicant effects on neuroendocrine function. Endocrine **14** (2): 235–246. DOI: 10.1385/ENDO:14:2:235

Gregoraszczuk 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

Hansen P.D., von Westernhagen H., Rosenthal H. 1985. Chlorinated hydrocarbons and hatching success in Baltic herring spring spawners. Marine Environmental Research **15** (1): 59–76. DOI: 10.1016/0141-1136(85)90038-8

Heiden T.K., Carvan M.J., Hutz R.J. 2006. Inhibition of follicular development, vitellogenesis, and serum 17 beta-estradiol concentrations in zebrafish following chronic, sublethal dietary exposure to 2,3,7,8 – tetrachlorodibenzo-p-dioxin. Toxicological Sciences **90** (2): 490–499. DOI: 10.1093/toxsci/kfj085

Kester M.H.A., Bulduk S., Tibboel D., Meinl 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): 251–159. 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

Kime D.E. 1999. Endocrine disruption in fish. Kluwer Academic Publishers, Boston, USA.

Kudo S., Yazawa S. 1997. Binding of antibiotics to glycoproteins of the vitelline and fertilization envelopes of cherry salmon eggs. The Histochemical Journal **29** (8): 607–616. DOI: 10.1023/A:1026436311921

LeRoy K.D., Thomas P., Khan I.A. 2006. Thyroid hormone status of Atlantic croaker exposed to Aroclor 1254 and selected PCB congeners. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology **144** (3): 263–271. DOI: 10.1016/j.cbpc.2006.09.005

Mac M.J., Schwartz T.R., Edsal C.C., Frank A.M. 1993. Polychlorinated biphenyls in Great Lakes lake trout and their eggs: Relation to survival and congener composition 1979–1988. Journal of Great Lakes Research **19** (4): 752–765.

Mäenpää K.A., Penttinen O.-P., Kukkonen J.V.K. 2004. Pentachlorophenol (PCP) bioaccumulation and effect on heat production on salmon eggs at different stages of development. Aquatic Toxicology **68** (1): 75–85. DOI: 10.1016/j.aquatox.2004.02.004

Örn S., Andersson P.L., Förlin L., Tysklind M., Norrgren L. 1998. The impact on reproduction of an orally administrated mixture of selected PCBs in zebrafish (*Danio rerio*). Archives of Environmental Contamination and Toxicology **35** (1): 52–57. DOI: 10.1007/s002449900348

Power D.M., Llewellyn L., Faustino M., Nowell M.A., Björnsson B.Th. Einarsdottir I.E., Canario A.V.M., Sweeney G.E. 2001. Thyroid hormones in growth and development of fish. Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology **133** (4): 447–459. DOI: 10.1016/S1532-0456(01)00271-X

Sangalang G.B., Freeman H.C., Crowell R. 1981. Testicular abnormalities in cod (*Gadus morhua*) fed Aroclor 1254. Archives of Environmental Contamination and Toxicology **10** (5): 617–626. DOI: 10.1007/BF01054884

Socha M., Sokołowska-Mikołajczyk M., Szczerbik P., Mikołajczyk T., Chyb J., Duc M., Epler P. 2008a. 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, Calgary 22–27 June 2008, Canada.

Socha M., Sokolowska-Mikolajczyk M., Szczerbik P., Chyb J., Dietrich G., Kowalski R., Grabic R., Epler P. 2008b. The influence of polychlorinated biphenyls (PCBs) on computer analysed sperm motility of common carp (*Cyprinus carpio* L.) in vitro. Cybium **32** (Suppl. 2): 197.

Szczerbik P., Mikołajczyk T., Sokołowska-Mikołajczyk M., Socha M., Chyb J., Epler P. 2008. The influence of cadmium on Prussian carp oocyte maturation, development of eggs and hatching. Czech Journal of Animal Science **53** (1): 36–44.

Viant M.R., Pincetich C.A., Tjeerdema R.S. 2006. Metabolic effects of dinoseb, diazinon and esfenvalerate in eyed eggs and alevins of Chinook salmon (*Oncorhynchus* tshawytscha) determined by ^{1}H NMR metabolomics. Aquatic Toxicology **77** (4): 359–371. DOI: 10.1016/j.aquatox.2006.01.009

Received: 2 November 2011
Accepted: 28 March 2012
Published electronically: 31 March 2012