REPRODUCTIVE TOXICITY OF PCB (AROCLOR 1242) TO FEMALE FRESHWATER FISH *CYPRINION WATSONI*

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ABSTRATCT:- *Reproductive dysfunction in fish as a result of contaminant exposure is of considerable current interest. Polychlorinated biphenyls (PCBs) are a class of synthetic, lipophilic, halogenated aromatics that are among the most ubiquitous and persistent environmental contaminants. Present study is aimed at assessing whether PCB (Aroclor 1242) had any potential to cause negative effects on certain reproductive parameters of female freshwater cyprinid fish, Cyprinion watsoni. In early quiescent period (August/September), the fish were exposed to 10, 30 and 50mg Aroclor 1242 per kg body weight for 30 days. Ovarian length, ovarian weight and GSI (Gonadosomatic index) decreased significantly (P<0.05) in fish exposed to Aroclor 1242. Serum cholesterol level in control fish was 9.14±0.21m mol/lit, this increased significantly in fish exposed to Aroclor 1242 at the dose of 30mg (10.68±0.44 m mol/lit., P<0.05) and 50mg (11.86±0.51m mol/lit., P<0.01). Estradiol 17 β, measured in serum pools, decreased significantly in fish exposed to 30 mg and 50 mg Aroclor 1242 (600.30 ± 104.14, 823.75 ± 211.95 pg/ml) respectively. Estradiol 17 β in 100mg ovarian tissue of control fish was 940.30 ± 113.11pg which show substantial and significant decrease (P < 0.01) in ovarian tissue of fish exposed to Aroclor 1242 at the dose of 10mg (256.62±128.31pg), 30mg (286.33±102.76pg) and 50mg (385.69±39.88pg) respectively. The results of this study indicate that Cyprinion watsoni exposed to PCB show reproductive impairments.*

Keywords: PCBs, Aroclor1242, fish, ovarian histology, Estradiol 17 β

INTRODUCTION

Polychlorinated biphenyls (PCBs) are a class of synthetic organochlorines lipophilic, halogenated aromatics [1] that are among the most ubiquitous and persistent environmental contaminants with a well known potential toxicity [2]. If released into the environment, PCBs persist for years because they are so resistant to breakdown by chemical or biological agents. Banned from production in the U.S.A. in 1976, but many still common in the environment (some have a half-life of over 1000 years) [3, 4, 5]. PCBs bioaccumulate in marine species and their levels increase with the age of the fish [2]. Reproductive dysfunction in aquatic species as a result of contaminant exposure is of considerable current interest. Numerous laboratory studies have shown that the potential exists for chemical contaminants such as PCBs to adversely affect the reproductive process of several fish species [6]. PCBs are known to accumulate in the fish ovary and therefore might be present in descendents of contaminated fish [7]. Disturbed reproductive and early life-stage-mortality have been observed in salmonids from the Baltic sea and the great lakes of North America and in perch *(Perca fluviatilis*) downstream of pulpmil effluents [8]. 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 [9]. 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 [10]. PCBs and many other pollutants adversely affect development and physiology by interfering with normal endocrine functions [11, 12, 13]. The importance of **gonadal** steroids (e.g, androgens, estrogens and progestins) in regulating the reproductive cycle in vertebrates, including fish, is well established [14]. Antunes et al. 2007 [15] studied the effects of Aroclor 1254 in adult tilapia and observed several reproductive alterations. In female teleost fish, for example, 17β estradiol initiates the production of yolk protein, or vitellogenin, by the liver. Vitellogenin is then transported in to the ovary, when it is taken up by developing oocytes. Biosynthesis of 17 β estradiol and other gonadal steroids take place in the follicle cells surrounding the oocytes. In female fish this involves conversion of cholesterol to testosterone through several intermediates. The testosterone produced is then converted to 17 β estradiol in the granulosa cells of the follicle [16]. Because of the critical role that gonadal steroids play in regulating reproductive development, any change in their production or metabolism could potentially disrupt the normal reproductive cycle. Waller and co-workers (1995) have showed that structural similarities exist between PCBs and17 β estradiol [17]. Depressed plasma estradiol levels and reduced ovarian estradiol production in vitro has been reported in English sole (*Pleuronectes Vetulus*) from water ways polluted with PCBs and other aromatic compounds [18, 19].

Taken together, these findings bolstered with the reports that xenobiotics like PCBs are suspected to have played a major role in the rarefaction of wild fish species this work is an ecotoxicological study of PCB contamination in a wild fish species, *Cyprinion watsoni*. This species belongs to cyprinid group, found in the streams of running water in hilly areas. The objective of this study was to investigate the potential effects of exposure to Aroclor1242 on several reproductive parameters.

MATERIALS & METHODS

Procurement and maintenance of fish

Samples of *cyprinion watsoni* were collected with cast nets from the Ramli stream near Quaid-i-Azam University (QAU), transported live to the experimental fish laboratory. The fish were weighed and the length was measured (from the tip of the snout to the implantation of the tail and from the implantation to the end of the fork). The fish were grouped in different experimental aquaria (total capacity: 60 lit) containing 40 lit of water and kept for acclimation for at least two weeks prior to the start of experiments. The fish were fed daily on tropical fish food and were maintained in a photoperiod of 12L: 12D using fluorescent tube lights and automatic timer clocks placed 24 inches above the water surface. The water was renewed after every alternate day. The water temperature was not controlled and it varied with the ambient laboratory conditions; temperature of water was recorded twice a day during the experimental period.

Polychlorinated biphenyl (PCB) used

Technical mixture Aroclor1242 (electrical grade, Lot KB05-612; Monsanto Company, St. Louis, MO, USA) a commercial PCB blend chlorinated to 42% was used.

Route of administration of doses

Aroclor1242 was administered via food. The dose groups were:

Control: Dose vehicle only.
Group I: 10mg Aroclor1242/kg body weight/day.
Group II: 30mg Aroclor1242/kg body weight/day.
Group III: 50mg Aroclor1242/kg body weight/day.

Final volume of the daily dose was held constant at 200µl test solution (Corn oil + Aroclor1242) containing 10, 30, and 50mg Aroclor1242. 200µl of test solution was mixed with 2 gm of tropical fish food (Small pellets, floating type) and sprinkled over the surface of water which was consumed by fish within 5 min.

Collection of blood and extraction of serum

Blood was obtained from caudal vein and serum was separated by using standard procedure.

Gonadosomatic index and condition factor

The ovaries of fish were dissected out following sacrifice, weighed to the nearest mg and measured (cm) (=Length and Breadth). Right ovary was freezed immediately at -20^{0}C for hormonal assay. Record of body weight and ovarian weight were used to determine Gonadosomatic index (GSI) which was calculated according to the following formula:

GSI = [Ovary weight (gm) / gutted body weight (gm)] 100

Condition factor was calculated according to following formula:

Condition factor = gutted body weight (g) / (Length) ^{3} cm.

Hormonal estimation

17β estradiol, was measured in serum pools and ovarian tissues from each experimental group by radioimmunoassay. Serum cholesterol levels were determined by enzymatic colorimetric method.

RESULTS

The fish were randomly dispersed in water column. No changes in feeding patterns or behavior were observed in fish fed on feed contaminated with Aroclor 1242 as compared to control fish during the course of experiments. During experimental period no dose dependent mortality was recorded. As is shown in Table (1) GSI, ovarian length and ovarian weight decreased significantly in fish exposed to Aroclor 1242.

Table 1. Effect of Araclor1242 on Gonadosomatic index, condition factor and other morphometric parameters.

Groups	Body Weight (gm)	Standard Body Length (cm)	Ovarian Weight (mg)	Ovarian Length (cm)		GSI	CF
				Right Ovary	Left Ovary		
Control (n=13)	12.70±1.62	10.92±0.36	259.5±93.9	2.29±0.16	2.31±0.14	1.72± 0.26	0.0105± 0.0013
10mg/kg body weight/day (n=8)	12.85±1.39	10.59±0.31	154.0±244.6 *	1.89±0.14 **	1.90±0.12 **	1.18±0.11 ***	0.0105±0.00 037
30mg/kg body weight/day (n=11)	12.86±0.97	11.04±0.30	131.6±14.9 **	2.18±0.10	2.21±0.13	1.004±0.063 ****	0.0094±0.00 023
50mg/kg body weight/day (n=12)	11.72±0.37	10.65±0.18	131.9±0.005 ***	2.00±0.09 *	2.03±0.09 *	1.13±0.05 ***	0.0097±0.00 031

Mean (±SE); Student's 't' test, * P < 0.05, ** P < 0.02, ***P < 0.01; **** P < 0.001

Estradiol and Cholesterol

Serum cholesterol in control fish was 9.14±0.21 m mol/lit, this increased after PCB treatment. Estradiol17β was measured in serum pools from each experimental group, decreased significantly in fish exposed to 30mg and 50mg Aroclor 1242 (600.30±104.14, 823.75±211.95 pg/ml) respectively as compared to control fish. Estradiol 17β in 100mg ovarian tissue of control fish was 940.30±113.11pg which show substantial and significant decrease (P < 0.01) in ovarian tissue of fish exposed to Aroclor1242 at the dose of 10mg (256.62 128.31pg), 30mg (286.33 102.76) and 50mg (385.69 39.88pg).

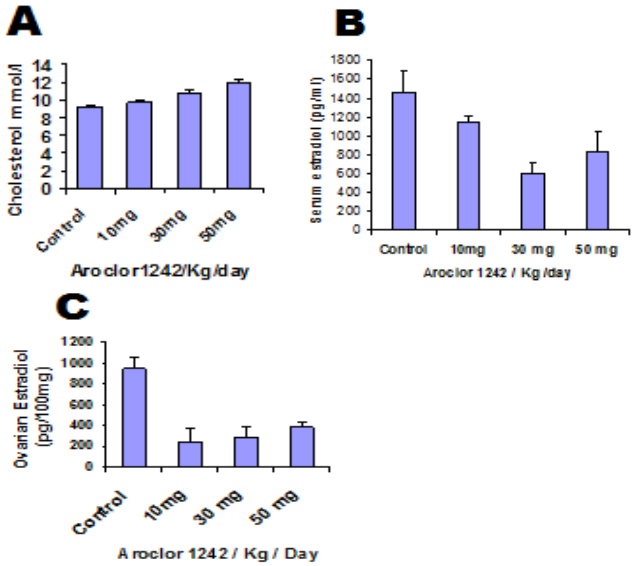

Fig.1: Effect of Aroclor 1242 on serum cholesterol (A), serum estradiol (B) and ovarian estradiol (C).

DISCUSSION

The aim of the present study was to determine whether PCB (Aroclor 1242) had any potential to cause negative effects on certain reproductive and developmental parameters of *Cyprinion watsoni.* The use of *Cyprinion watsoni* as a test organism is ecologically relevant in hilly areas like Islamabad owing to their abundance in fresh water ecosystem. Furthermore, it is small, easy to handle in aquaria, and suitable for use in many disciplines e.g. Developmental Biology, Reproductive Physiology Endocrinology and Ecotoxicology. It has been shown that the exposure route is of major importance for the uptake of xenobiotics in fish [20]. In this study, ingestion of contaminated food was chosen since the contaminant investigated is highly lipid soluble. So by feeding fish with contaminated food we can effectively deliver lipophilic xenobistics like PCBs in a relevant way to evaluate their biological impact. Although fish size has been identified as an important determinant of reproductive success in fish [21], neither fish length nor fish body weight appeared to be effected by PCB. However, PCB treatment resulted in significantly reduced ovarian weight and length. Rodents treated with PCBs and commercial Aroclors exhibited a wide range of estrogenic effects including precocious puberty increases in the ovarian wet weight and water imbibition [22]. Studies with other fish species showed that PCBs exposure may lead to population decline due to decreased ovary growth [23]. A condition factor was calculated for all groups of experimental fish so the effect of emaciation on ovarian development could be distinguished from any potential effects of PCB exposure. So the condition factor is a generalized indicator of the overall health or "plumpness" of a fish and can reflect the integrated effect of nutritional status and metabolic stress [24]. In our study the condition factor of fish exposed to Aroclor 1242 was almost similar to that of control fish. Similar results were obtained in a study in which no significant intersite differences were found in either condition factor or length weight relationship in English sole (*Pleuronectes vetulus*) from reference and contaminated sites [25]. However, another study showed that the condition factor was significantly higher in Pigeon River (a high gradient fifth order stream receiving pulp mill effluents) red breast sunfish females than in females of the reference sunfish ($P < 0.05$) [26]. Gonadosomatic index is an indicator of the level of gonadal development. In present study significant decrease in GSI due to PCB treatment could be accounted for variation in ovarian maturity stage. Variations in GSI have been reported in English sole (*Parophrys vetulus*) where GSI was significantly lower in fish from Duwamish waterway (heavily contaminated, sediment aromatic hydrocarbons (AHs) and PCB concentrations were high) than in fish from Sinclair Inlet (with relatively low levels of AHs and PCBs in the sediment) and was significantly lower in fish from both Duwamish Waterway and Eagle Harbor (with extremely high AHs and low PCB concentrations) than in fish from Sinclair Inlet in 1987 [18]. Similarly significant intersite differences in GSI in female winter flounder (*Pseudopleuronectes americans)* from reference site and from sites heavily contaminated with PCBs [27]. In female fish biosynthesis of 17β estradiol involves conversion of cholesterol to testosterone to 17β estradiol through several intermediates. Because of the critical role that cholesterol play as precursor of gonadal steroids, any change in their production or metabolism could potentially disrupt the normal reproductive cycle [16]. PCBs can alter the patterns of synthesis of reproductive hormones [28]. Altered steroid biosynthesis was reported in Atlantic Cod (*Gadus morrhua*) fed on diet containing 1-50 mg Aroclor 1254 [29]. These findings tend to corroborate our observations in *Cyprinion watsoni.* Plasma estradiol concentrations declined in rock sole and flathead sole treated with Prudhoe Bay crude oil (PCBO) [25]. Estradiol alongwith other ovarian steroids, is an important regulator of vitellogenic growth of the devleoping oocytes [26]. It is well established that estradiol stimulates the liver to produce vitellogenin [30]. Our findings suggest that reduced ovarian steroidogenesis may account for decreased serum estradiol levels in fish exposed to Aroclor 1242. However, other mechanisms, such as increased excretion of 17β-estradiol metabolites [31] or altered pituitary or hypothalmic function [32].

REFERENCES

[1] Fielden, M.R., I. Chen, B. Chittim, S.H. Safe, T.T. Zacharewski, "Examination of the estrogenecity of 2,4,6,2`,6`-Pentachloro-biphenyl (PCB 104), its hydroxylated metabolite 2,4,6,2`,4`,6`-Pentachloro-4-Biphenylol (Ho-PCB 104), and a further chlorinated Derivative, 2,4,6,2`4`,6`-Hexachlorobiphenyl (PCB 155)", *Environ. Health. Perspect*. **105**: 1238-1248(1997)

[2] Domingo, J.L., A. Bocio, "Levels of PCDD/PCDFs and PCBs in edible marine species and human intake: A literature review", *Environ. Int.* doi:**10**.1016/j.envint.2006.12.004(2007).

[3]. Gray, L. E., J. Ostby, R.L. Cooper, W.R. Kelce, "The estrogenic and antiandrogenic pesticide methoxychlor alters the reproductive tract and behaviour without affecting pituitary size or LH and prolactin secretion in male rats", *Toxicology and Industrial Health*, **15**, 37e47, (1999a)

[4]. Cheek, A. O., K. Kow, J. Chen, J.A. McLachlan, "Potential mechanisms of thyroid disruption in humans: interaction of organochlorine compounds with thyroid receptor, transthyretin, and thyroid-binding globulin", *Environmental Health Perspectives***,** **107**, 273e278(1999)

[5]. Korach, K. S., P. Sarver, K. Chae, J.A. McLachlan, J.D. McKinney,"Estrogen receptor-binding activity of polychlorinated hydroxybiphenyls: conformationally restricted structural probes", *Molecular Pharmacology*, **33**, 120e126(1988)

[6]. Guillette L.J. Jr, M.P. Gunderson, "Alterations in development of reproductive and endocrine systems of wildlife populations exposed to endocrine-disrupting contaminants", *Reproduction.* **122(6)**:857-64 (2001)

[7]. Monosson E., J.T. Ashley, A.E. McElroy, D. Woltering, A.A. Elskus, "PCB congener distributions in muscle, liver and gonad of Fundulus heteroclitus from the lower Hudson River Estuary and Newark Bay", *Chemosphere.* **52(4)**:777-87(2003)

[8]. Howell, M. W., A.D. Black, S.A. Bortone, "Abnormal expression of secondary sex characters in a population of mosquitofish, *Gambusia affinis holbrooki*: evidence for environmentally- induced masculinization",*Copeia*,**4**, 676e681. (1980)

[9]. Heiden T.K., R.J. Hutz, M.J. Carvan III, "Accumulation, Tissue Distribution, and Maternal Transfer of Dietary 2,3,7,8,-Tetrachlorodibenzo-p Dioxin: Impacts on Reproductive Success of Zebrafish", *Toxicological Sciences* **87(2)**, 497–507(2005)

[10]. Heiden T.K., M.J. Carvan III, R.J. Hutz, "Inhibition of Follicular Development, Vitellogenesis, and Serum 17b-Estradiol Concentrations in Zebrafish Following Chronic, Sublethal Dietary Exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin", *Toxicological Sciences***, 90(2)**, 490–499(2006)

[11]. Crews, D., E. Willingham, J.K. Skipper, "Endocrine disruptors: present issues, future directions", *Quarterly Reviews of Biology*, 75, 243e260(2000)

[12]. Guillette, L. J., Jr, D.A. Crain, M.P. Gunderson, S.A.E. Kools, M.R. Milnes, E.F. Orlando, A.A. Rooney, A.R. Woodward, "Alligators and endocrine disrupting contaminants: a current perspective", *American Zoologist*, **40**, 438e452(2000)

[13]. Gray, L. E., Jr, J. Ostby, J. Furr, C.J. Wolf, C. Lambright, L. Parks, D.N. Veeramachaneni, V. Wilson, M. Price, A. Hotchkiss, E. Orlando, L. Guillette, "Effects of environmental antiandrogens on reproductive development in experimental animals" *Human Reproduction*, **7**, 248e264 (2001)

[14]. Wallace, R.A., "Vitellogenesis and oocyte growth in non-mammalian vertebrates", *Dev. Biol*. **1**, 127-177.

[15]. Antunes, P., J. Amado, C. Vale, O. Gil, "Influence of the chemical structure on mobility of PCB congeners in female and male sardine (*Sardina pilchardus*) from Portuguese coast", *Chemosphere* 69:395-402(2007)

[16]. Nagahama, Y., "Cytodifferentiation of ovarian follicles during oocyte growth and maturation", In: G. Eguchi, et al, Eds: *Regulatory mechanisms in development processes*. Elsevier Scientific, Amsterdam, pp. 9-24(1988)

[17]. Waller, C.L., D.L .Minor, J.D. McKinney, "Using three dimensional quantitative structure activity relationships to examine estrogen receptor binding affinities of polychlorinated hydroxybiphenyls" *Environ. Health Perspect*, **103**, 702-707(1995)

[18]. Johnson, L.L., D. Misitano, S.Y. Sol, G.M. Nelson, B. French, G.M. Yalitalo, T. Hom, "Contaminant effects on ovarian development and spawning in Rock sole from Pudget Sound, Washington", *Trans. Am. Fish. Society* **127**: 375-392.

[19]. Johnson, L.L., S.Y. Sol, D.P. Lomax, C.A. Sloan, E. Casillas, "Fecundity and egg weight in English, *Pleuronectes vetulus*, from Pudget Sound, Washington: influence of nutritional status and chemical contaminants", *Fishery Bulletin*, **95**: 231-249(1997)

[20]. Ekelund, R., "Bioaccumulation and biomagnification of hydrophobic persistent compounds as exemplified by hexachlorobenzene. In: Chemicals in the aquatic environment. Advanced hazard assessment", edited by L. Landner, Springer-Verlag, Heidelberg, pp. 128-149(1989)

[21]. Collier, T. K., J. E. Stein, H. R. Sanborn, T. Hom, M. S. Myers, U. Varanasi, "Field studies of reproductive success in English sole (*Parophrys vetulus*): correlations with bioindicators of maternal contaminant exposure" *Science Tot. Environ*. **116**: 169-185 (1992)

[22]. Jansen, H.T., P.S. Cooke, J. Porcelli, T.C. Liu, L.G. Hansen, "Estrogenic and antiestrogenic actions of PCBs in the female rat: In vitro and in vivo studies", *Reprod. Toxicol*. **7**, 237-248(1993)

[23]. Khan I.A., S. Mathews, K. Okuzawa, H. Kagawa, P. Thomas, "Alterations in the GnRH-LH system in relation to gonadal stage and Aroclor 1254 exposure in Atlantic croaker", *Comp Biochem Physiol B Biochem Mol Biol*. **129**(2-3):251-9(2001)

[24]. Adams, S.M., R.B. McLean, "Estimation of largemouth bass. *Micropterus salmoides lacepede*, growth using the liver somatic index and physiological variables", *J. Fish Biol*. **26**, 111-126(1985)

[25]. Johnson, L.L., Stein, J.E., Hom, T., Collier, T.K., Sol, S. and Varanasi, U. 1995. Effects of exposure to Prudhoe Bay crude oil on reproductive function in gravid female flatfish. *Environ. Sciences*, 3,2: 067-081.

[26]. Adams, S.M., Crumby, W.D., Greeley, M.S., Shugart, L.R. and Saylor, C.F. 1992. Responses of fish populations and communities to pulp mill effluents: A holistic assessment. *Ecotoxicol. Environ. Safety* **24**: 347-360.

[27]. Johnson, L.L., J.E. Stein, T.K. Collier, E. Cassillas, U. Varanasi, "Indicators of reproductive development in prespawning female winter flounder *(Pleuronectes americanus)* from Urban and non-urban estuaries in the northeast United States", *Sci. Total Environ*. **141**: 241-260(1994)

[28]. Kime, D.E., "The effects of pollution on reproduction in fish", *R. Fish. Biol. Fish*. **5**, 52-96(1995)

[29]. Freeman, H.C., G. Sangalang, B. Flemming, "The sublethal effects of a polychlorinated biphenyl (Aroclor 1254) diet on the Atlantic Cod (*Gadus morhua*)", *Sci. Total Environ*., **24**:1-11(1982)

[30]. Ng, T.B., D.R. Idler, "Yolk formation and differentiation in teleost fishes", In Fish Physiology: Vol. IX. Reproduction Part A, ed. W.S. Hoar, D.H. Randall and E.M. Donaldson. Academic Press, New York, NY, pp. 373-403(1983)

[31]. Stein, J.E., T. Hom, H.R. Sanborn, U. Varanasi, "Effects of exposure to a contaminated sediment extract on the metabolism and disposition of estradiol-17β in English sole *(Parophrys vetulus)*" *Comp. Biochem. Physiol*. **99C**: 231-240(1991)

[32]. Thomas, P., "Effects of Aroclor 1254 and cadmium on reproductive endocrine function and ovarian growth in Atlantic croaker", *Mar. Environ. Res.* **24**: 285-289(1989)