Bull. Environ. Contam. Toxicol. (1992) 48:23–30

Environmental Contamination and Toxicology

Impact of γ-BHC on Lipid Class Levels and Their Modulation by Reproductive Hormones in the Freshwater Catfish, *Heteropneustes fossilis*

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Toxic substances are known to suppress gonadal growth (Singh 1988; Kocan and Landolt 1989), steroidogenesis (Freeman and Idler 1975; Singh and Singh 1987) and lipid metabolism (Lal and Singh 1987a; Singh and Singh 1990b; Singh 1991). Singh and Singh (1982) have observed that aldrin and hexadrin significantly reduced gonadotropin releasing hormone-like factor in the hypothalamus. Meagre attempts have been made to observe the effects of pesticides on phospholipids (vitellogenin precursor) and cholesterol (steroids precursor) metabolism as well as modulation of the impact of pesticides by ovine Luteinizing Hormone-Releasing Hormone (oLH-RH) and Mystus gonadotropin (mGTH) on above lipids.

Therefore, in the present study an attempt has been made to explore the effect of sublethal concentration of an organochlorine agricultural pesticide γ-BHC (1,2,3,4,5,6-hexachlorocyclohexane) on phospholipids, free cholesterol and esterified cholesterol levels in liver, plasma, and ovary during reproductively active phases,i.e.,preparatory and prespawning phases in the female catfish, Heteropneustes fossilis. Modulatory role of oLH-RH and mGTH on pesticides induced changes on above lipids were also evaluated.

MATERIALS AND METHODS

Adult female specimens of H. fossilis of weight range 65-75g and total length 21-23 cm were collected from the ponds around Varanasi, India, during preparatory (March 3rd wk, photoperiod 11.5L:12.5D, temperature 28±2°C) and prespawning phase (May 3rd wk, photoperiod 13.0L:11.0D, temperature 30±2 °C). They were brought to the laboratory and acclimated in outdoor cisterns of size 2x1x1m, circulated with dechlorinated tap water having pH 7.6, temperature 28±2°C, hardness 154 mg/L (as calcium carbonate), alkalinity 68 mg/L (as

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calcium carbonate), dissolved oxygen 7.2 mg/L and conductivity 0.56 mmho for 10 d prior to experimentation. They were fed ad libitum minced goat liver comprising 20% protein, 5% lipid, 15% carbohydrate and the rest 60% being water, minerals and vitamins etc.

Analytical grade chemicals were obtained from Glaxo Laboratory (India). Solvents were redistilled before use. Silica gel G 60 containing 13% gypsum was obtained from E.Merck, Germany. Lipid standards - lecithin, cholesterol and cholesterol oleate were obtained from Sigma Chemical Co. (USA). Partially purified mGTH-fraction II G100 used here was purified in author's laboratory.

After 10 d of acclimation fish were divided into 11 batches each comprising 5-8 fish, in glass aquarium of size 60x40x50 cm having 90 L water. The first batch served as a control and was maintained in tap water with 1.0 mL of acetone. The second batch was exposed to 16 mg L^{-1} γ-BHC (Singh and Singh 1986). The pesticide was dissolved in 1.0 mL acetone and then it was added to the aquarium water. The details of the treatments of the remaining nine batches are given in Table 1. During the experiment, fish were fed every 4th d when aquarium water was changed with freshwater having similar concentration of the above pesticide. All experiments lasted for 28 d. Five fish from each batch of all experiments (11 batches) were bled on 29th d by caudal incision and blood was collected separately in heparinized glass culture tubes and centrifuged at 2000 g for 15 min at 4°C, and plasma separated and kept frozen at -80°C until assayed for various lipids. After decapitation, liver and ovary were extirpated, washed in saline (0.6%), blotted and stored frozen at -80°C.

Plasma 100 µL and 50 mg (10% homogenate) of tissue were thawed and extracted two times with 4 mL distilled chloroform:methanol (2:1). The details of thin layer chromatography have been described elsewhere (Singh and Singh 1990a).

Lipids are expressed as mean±SEM having units mg/mL plasma and mg/g of tissue. Data were analysed by two-way ANOVA followed by Newman-Keuls' Multiple range 't' test (Bruning and Kintz 1977).

RESULTS AND DISCUSSION

Intraperitoneal administration of lower doses of oLH-RH and mGTH (0.1 and 1 µg/fish) with simultaneous exposure of γ-BHC were ineffective to abolish the impact of γ-BHC on lipids, but their higher doses (10 and 20 µg/fish) effectively reversed the impact of

Table 1. Treatments of H. fossilis with γ-BHC and hormones. Number of fish per treatment = 8.

Batches	γ-BHC 16mg L^{-1}	Saline injection*	
		Hormone	Amount (μg)
1	0	0	0
2	x	0	0
3	x	saline only	0
4	x	oLH-RH	0.1
5	x	oLH-RH	1.0
6	x	oLH-RH	10.0
7	x	oLH-RH	20.0
8	x	mGTH	0.1
9	x	mGTH	1.0
10	x	mGTH	10.0
11	x	mGTH	20.0

*Control batch 3 was injected with saline only. For batch 4-11 hormones were dissolved in saline (0.6%, NaCl) and injected intraperitoneally on alternate days of different concentrations as above in 0.1 mL volume simultaneously with γ-BHC exposure (16 mg L^{-1}) at sub-lethal concentration for 4 wk.
0 = not treated, x = treated.

γ-BHC in general. Henceforth only higher doses of oLH-RH and mGTH have been taken into account.

In general γ-BHC exposure decreased the levels of phospholipid (PL) levels whereas simultaneous administration of oLH-RH and mGTH caused the recovery of its level partially or fully in the studied tissues during both the phases (Table 2).

Hepatic free cholesterol (CF) decreased and increased upon γ-BHC exposure during preparatory and prespawning phases, respectively (Table 3). With simultaneous administration of oLH-RH and mGTH CF recovered its level fully during preparatory phases, but during prespawning phases these drugs increased its concentration further. Plasma CF remained unchanged in response to γ-BHC during preparatory phase but was increased during prespawning phase which was elevated further more by oLH-RH and mGTH treatments during both the phases. CF concentration in ovary were decreased by γ-BHC during both the phases which were decreased further by simultaneous administration of oLH-RH and mGTH (Table 3).

γ-BHC increased hepatic esterified cholesterol (CE) but had no effect on its plasma and ovarian levels

Table 2. Effect of γ-BHC, γ-BHC + oLH-RH, and γ-BHC + mGTH on concentrations of phospholipids in liver, plasma and ovary during preparatory and prespawning phases of the annual reproductive cycle in the freshwater female catfish, H. fossilis (values are, in mg/g tissue of /ml plasma, mean± SEM, n = 5).

Batches	Concentration of phospholipids		
	Liver	Plasma	Ovary
	Preparatory phase		
1	8.21±0.149	2.31±0.098	5.25±0.124
2	3.14±0.114[a]	1.31±0.017[a]	2.20±0.051[a]
3	3.20±0.093	1.37±0.015	2.27±0.057
4	3.22±0.093	1.37±0.031	2.25±0.066
5	3.20±0.076	1.37±0.016	2.25±0.056
6	4.88±0.180[b]	3.55±0.088[b]	4.59±0.107[b]
7	5.71±0.214[b]	5.43±0.119[b]	7.14±0.174[b]
8	3.27±0.085	1.35±0.013	2.25±0.056
9	3.21±0.074	1.36±0.012	2.27±0.036
10	5.96±0.201[b]	3.67±0.031[b]	6.09±0.170[b]
11	6.75±0.205[b]	3.76±0.088[b]	4.23±0.105[b]
	Prespawning phase		
1	3.84±0.230	4.43±0.051	9.58±0.351
2	1.92±0.081[a]	2.26±0.033[a]	4.07±0.173[a]
3	1.88±0.078	2.28±0.039	4.05±0.174
4	1.84±0.079	2.27±0.025	4.10±0.180
5	1.95±0.103	2.28±0.029	4.18±0.192
6	6.74±0.261[b]	3.42±0.066[b]	8.24±0.214[b]
7	8.65±0.257[b]	4.63±0.093[b]	7.13±0.165[b]
8	1.89±0.075	2.26±0.024	4.19±0.174
9	1.82±0.078	2.25±0.031	4.21±0.190
10	9.39±0.397[b]	4.52±0.096[b]	8.45±0.230[b]
11	7.79±0.203[b]	3.60±0.080[b]	9.04±0.257[b]

Batch 1 (Normal control) vs batch 2 (γ-BHC exposed) were compared by Student's 't' test; a=P<0.001. Batch 3 (γ-BHC exposure + saline injection control) vs batch 4-11 (γ-BHC exposure + hormone treated) were compared by Newman Keuls' Multiple-Range test; b=P<0.05.

during preparatory phase (Table 4). After treatment with oLH-RH and mGTH, CE returned to its hepatic normal level, but in plasma and ovary these hormones decreased the concentration of CE further. During

Table 3. Effect of γ-BHC, γ-BHC + oLH-RH, and γ-BHC + mGTH on concentrations of free cholesterol in liver, plasma and ovary during preparatory and prespawning phases of the annual reproductive cycle in the freshwater female catfish, H. fossilis (values are, in mg/g tissue or/mL plasma, mean±SEM, n = 5)

Batches	Concentration of Free cholesterol		
	Liver	Plasma	Ovary
	Preparatory phase		
1	3.62±0.044	0.49±0.024	2.62±0.078
2	1.83±0.029[a]	0.49±0.019	0.98±0.047[a]
3	1.79±0.034	0.50±0.022	1.06±0.051
4	1.75±0.047	0.53±0.029	1.04±0.058
5	1.81±0.032	0.51±0.026	1.10±0.058
6	4.04±0.078[b]	0.57±0.021	0.76±0.049[b]
7	4.89±0.115[b]	0.63±0.031[b]	0.53±0.030[b]
8	1.78±0.042	0.49±0.019	1.14±0.056
9	1.82±0.043	0.48±0.024	1.15±0.045
10	4.10±0.071[b]	0.63±0.034[b]	0.87±0.026[b]
11	5.30±0.125[b]	0.74±0.045[b]	0.66±0.023[b]
	Prespawning phase		
1	2.41±0.033	0.88±0.038	1.66±0.052
2	3.50±0.055[a]	1.06±0.066[b]	0.51±0.027[a]
3	3.42±0.066	1.03±0.048	0.53±0.023
4	3.56±0.058	1.03±0.053	0.55±0.023
5	3.58±0.060	1.06±0.065	0.54±0.024
6	5.33±0.191[b]	2.59±0.124[b]	0.32±0.017[b]
7	4.53±0.069[b]	1.63±0.064[b]	0.34±0.018[b]
8	3.35±0.055	1.02±0.049	0.56±0.025
9	3.40±0.055	1.06±0.062	0.55±0.025
10	5.31±0.130[b]	2.70±0.120[b]	0.33±0.022[b]
11	4.13±0.069[b]	1.44±0.052[b]	0.34±0.016

Batch 1 (Normal control) vs batch 2 (γ-BHC exposed) were compared by Student's 't' test; a = $P<0.001$, b = $P<0.05$. Batch 3 (γ-BHC exposure + saline injection control) vs batch 4-11 (γ-BHC exposure + hormone treated) were compared by Newman Keuls' Multiple-Range test; b = $P<0.05$.

prespawning phase, γ-BHC increased hepatic and ovarian CE but decreased its concentration in plasma. The hormones oLH-RH and mGTH caused the return of CE to its normal level in liver fully and partially in ovary. Plasma CE was decreased further by these

Table 4. Effect of γ-BHC, γ-BHC + oLH-RH, and γ-BHC + mGTH on concentrations of esterified cholesterol in liver, plasma and ovary during preparatory and prespawning phases of the annual reproductive cycle in the freshwater female catfish, H. fossilis (values are, in mg/g tissue or/mL plasma, mean±SEM, n = 5)

Batches	Concentration of Esterified cholesterol		
	Liver	Plasma	Ovary
	Preparatory phase		
1	1.41±0.061	0.64±0.014	1.75±0.028
2	2.33±0.109[a]	0.61±0.015	1.73±0.020
3	2.51±0.110	0.61±0.016	1.79±0.030
4	2.55±0.124	0.61±0.013	1.82±0.032
5	2.45±0.090	0.60±0.015	1.77±0.020
6	0.82±0.015[b]	0.59±0.011	0.74±0.002[b]
7	1.16±0.063[b]	0.54±0.016[b]	0.63±0.015[b]
8	2.42±0.107	0.61±0.010	1.74±0.038
9	2.51±0.108	0.60±0.013	1.78±0.027
10	0.99±0.035[b]	0.57±0.015[b]	0.78±0.013[b]
11	1.08±0.040[b]	0.52±0.011[b]	0.63±0.016[b]
	Prespawning phase		
1	1.10±0.018	1.52±0.034	2.15±0.034
2	2.78±0.051[a]	1.22±0.031[a]	4.19±0.084[a]
3	2.73±0.035	1.23±0.029	4.21±0.065
4	2.69±0.037	1.24±0.025	4.15±0.079
5	2.77±0.036	1.27±0.023	4.32±0.107
6	1.23±0.010[b]	0.92±0.021[b]	3.71±0.060[b]
7	1.14±0.013[b]	0.92±0.026[b]	3.05±0.050[b]
8	2.71±0.039	1.24±0.016	4.18±0.067
9	2.72±0.034	1.22±0.026	4.27±0.082
10	1.23±0.012[b]	0.91±0.017[b]	3.14±0.135[b]
11	1.15±0.013[b]	0.93±0.029[b]	3.24±0.138[b]

Batch 1 (Normal control) vs batch 12 (γ-BHC exposed) were compared by Student's 't' test; a = $P<0.001$. Batch 3 (γ-BHC exposure + saline injection control) vs batch 4-11 (γ-BHC exposure + hormone treated) were compared by Newman Keuls' Multiple-Range test; b = $P<0.05$.

drugs(Table 4).

In the present study, decreased concentrations of phospholipids in liver, plasma, and ovary in response

to ,-BHC exposure during preparatory and prespawning phases revealed that this pesticide arrested lipid synthesis in liver, and translocation of hepatic lipid to ovary subsequently. Decrease in hepatic diglycerides level (precursor of triglycerides and PL) caused the reduction in PL levels in studied tissue in response to ,-BHC (Singh 1988). Here it can be contemplated that the inhibition of hepatic lipogenesis and translocation of hepatic lipid to ovary is brought about by impairing steroid metabolism. This contention is supported by a number of earlier reports where it has been well established that steroids play a vital role in regulation of lipid metabolism in teleosts in relation to reproduction (Wiegand and Peter 1980; Lal and Singh 1987b; Singh and Singh 1990a). Lal and Singh (1987a) have reported decreased free fatty acids, mono-, di-, triglycerides and PL during preparatory and prespawning phases after 28 days ,-BHC exposure. These findings supported the observations reported presently.

Increases in CE and decrease in CF in ovary ,-BHC exposure have demonstrated that hydrolysis of CE to CF is arrested. This is further evidenced by the decrease in CF and CE, where CE is hydrolysed into CF and utilised in sex steroid biosynthesis which elevated plasma levels of sex steroids hormones after oLH-RH and mGTH treatments (Singh and Singh 1990b).

Arrested lipogenesis in present species in response to ,-BHC exposure appears to be because of ,-BHC provoked disturbances in the activity of gonadotropin releasing hormone (GnRH) at hypothalamus and gonadotropin (GTH) at pituitary level. This suggestion gets support from the fact that administration of oLH-RH and mGTH simultaneously with ,-BHC exposure reversed the impact of ,-BHC on studied lipids though there was some difference in the magnitude of their recovery. Singh and Singh (1982) have also reported reduced GnRH-like factor in hypothalamus and GTH potency of pitiuitary and serum in response to cythion, parathion 50, hexadrin and aldrin in H. fossilis.

On the basis of results obtained here, it is suggested that ,-BHC arrested hepatic lipogenesis as well as translocation of liver lipid to ovary by impairing GnRH and GTH acting through hypothalamo-hypophyseal-ovarian axis in this species.

Acknowledgments. UGC Teacher Fellowship [F.34-10/81 (D-1)] to PBS is appreciated. We thank KS for her help in the preparation of this manuscript.

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Received October 10, 1990; accepted July 9, 1991.