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Action of Carbofuran Technical on Tissue Lipids of Freshwater Catfish, *Heteropneustes fossilis* (Bloch)

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Abstract

Female catfish (*Heteropneustes fossilis*, Bl.) were exposed to sublethal doses of carbofuran technical pesticide (0.5, 1.0 and 2.0 mg/l) for 30 days during the prespawning phase of the annual reproductive cycle (May-June). Cholesterol, total lipid and its fatty acid contents were determined in the liver and in the ovary. Cholesterol and total lipid increased significantly in the liver but remained unchanged in the ovary. This is an indication of the inhibitory effect of carbofuran on the mobilization of hepatic cholesterol and total lipid. In both organs, the predominant fatty acids in total lipid were palmitic ($C_{16:0}$), palmitolic ($C_{16:1}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), and arachidonic ($C_{20:4}$), both in the controlled and in the treated fish. However, eicosapentaenoic acid ($C_{20:5}$), and docosahexaenoic acid ($C_{22:6}$), were absent among fish receiving high doses of carbofuran. The polyunsaturated fatty acid ratio (w_3/w_6) was reduced among treated fish in comparison to the controlled ones. It is concluded that the lipid metabolism in catfish is affected by the technical grade of carbofuran.

Introduction

Hazards of environmental contamination through indiscriminate use of pesticides have created a problem throughout the world. In the mammalian system, pesticides even at very low concentration not only interfere with basal metabolism; these also affect liver weight and lipid concentration in tissues

and serum (Sanders et al. 1974; Gupta et al. 1986). In many teleosts, the relationship between the tissue lipid levels and the reproductive cycle has been reported (Singh and Singh, 1979; Singh and Singh, 1984). It is known that lipid is the major constituent of vitellogenin and a precursor of sex hormones which control the reproduction of fish. The lipid classes predominating in the gonads differ in the final stages of maturation, the testes having more sterol esters, phospholipids, and free fatty acids; whereas ovaries have more cholesterol and triglycerides (Shatunovskii and Novikov, 1971). Many pesticides are also lypophilic in nature. Lipids undergo rapid breakdown, resynthesis, and interconversion in response to external agents even like pesticide. Carbofuran (Fig.1) is a widely used carbamate pesticide in modern agriculture for controlling insect pests. Though carbofuran is not directly used in aquaculture it causes problems with agricultural run-off water particularly in tropical countries. This is more relevant in case of composite paddy cum pisiculture as practiced in many Asian countries.

Carbofuran, being lipid soluble is concentrated in the fatty tissues of fishes including liver and ovary. Whilst investigating the effects of carbofuran (CF) on the reproduction of an important food fish *H. fossilis* both *in vivo* and *in vitro* (Ghosh and Chatterjee, 1994; Chatterjee and Ghosh, 1995; Chatterjee et al. 1997), it became imperative to have a clear picture of lipid levels in relation to reproduction in response to this carbofuran (Fig. 1). Moreover, reports were available regarding organochlorine and organophosphate pesticides which affect different classes of lipid levels during the annual reproductive phases of fish, *H. fossilis* (Lal and Singh, 1986; 1987).

Studies have been done regarding the role of fatty acids of lipids in mammals. Increased amounts of palmitic, oleic and docosapentaenoic acids have been recorded among rats during normal maturation of the male gonads and after treatment with a low dose of cadmium (Davis et al. 1996; Davis and Coniglio, 1967). The spawning, oocyte fertilization, and hatching rate of embryos in *Penaeus monodon* are related with total w_3/w_6 type polyenoic fatty acids (Millamena, 1989). But no such data are available for the annual reproductive cycle of fish.

In the present communication we report the action of carbofuran on total lipid and its fatty acid composition and on the total cholesterol in the liver and

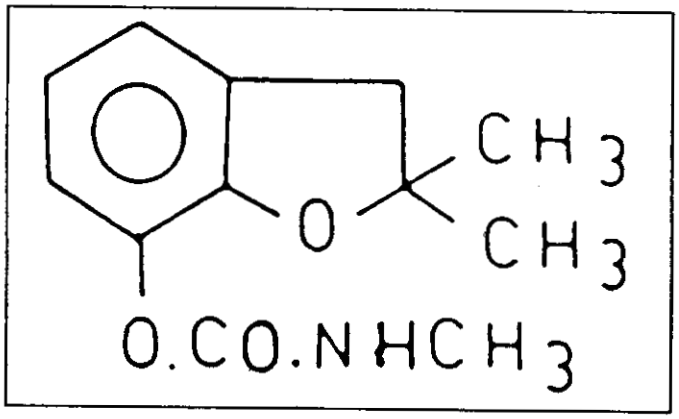

Fig. 1. Structure of carbofuran.

ovary of a female catfish during the prespawning phase of the reproductive cycle.

Materials and Methods

Chemicals

Methanol, chloroform, acetone, ether, hexane, glacial acetic acid and concentrated sulfuric acid used were all of analytical grade (BDH, India). Silica Gel G (E. Merck, Darmstadt, West Germany) was also used.

Technical grade carbofuran (2,3-dihydro-2, 2-dimethyl-7-benzofuranyl methyl carbamate) was a gift from Rallis India Ltd., Calcutta. The composition of technical carbofuran is 75% (w/w) active ingredients and the remaining 25% is of inactive non-toxic carrier compounds.

Reference lipids–esters of palmitic, palmitolic, stearic, oleic, linoleic, linolenic, arachidonic, eicosapentaenoic, docosahexaenoic acids and cholesterol were purchased from Sigma Chemical Company (St. Louis, USA).

Maintenance of fish and treatment with carbofuran (CF)

The prespawning fish (May-June) were selected for the present experiment. Their maintenance and treatment with CF were the same as described earlier (Chatterjee et al. 1997). All the experiments were conducted at 25 ± 1 °C with 12h-L : 12h-D photoperiod for 30 days. During treatment with carbofuran (CF) at sublethal doses (0.5, 2.0 and 2.0 mg/l), each aquarium (40 x 25 x 25 cm) contained 10 fish with 15 l of water. Selection of sublethal doses (0.5, 1.0 mg/l) used in the experiment were recommended by the Indian Council for Agricultural Research (ICAR) for paddy and other crops. A little higher sublethal dose (2.0 mg/l) was also used in comparing the effects with those of lower recommended dose in Indian Agriculture.

Preparation of tissue samples

At the end of the experiment, exposed and controlled fish were sacrificed. The liver and ovary of the individual fish were dissected out, washed in 0.6% saline and weighed.

Lipid extraction and estimation

Liver and ovary were collected separately from the 10 individual fishes of each set for lipid extraction. The weight of each liver and ovary were about 0.5 g and 3.0 g respectively. Lipid was extracted following the method of Bligh and Dyer (1959) by using a mixture of chloroform and methanol (1 : 2) followed by a mixture of chloroform and water (1 : 1). The total lipid was weighed, dissolved in chloroform and preserved at -20 °C under nitrogen. Aliquots were taken for the estimation of total cholesterol using the method of Abell et al. (1952).

Estimation of the fatty acid composition of total lipid

Three out of the 10 samples in each set were used for determination of fatty acid composition. Mixed methyl esters were prepared from total lipid by methanolysis in the presence of methanol and sulfuric acid (Chalvardjian, 1966). The esters were separated by TLC using methyl ester standard (Oleic acid, $C_{18:1}$ w_9) The fatty acid composition of total lipid was evaluated from the esters comparing them with the reference lipids using gas chromatography.

Analysis of data

The data were subjected to statistical evaluation using analysis of variance (ANOVA) coupled with Duncan's multiple range test (Montogomery, 1976) and $P<0.05$ was considered significant.

Results

Effect of carbofuran technical (CF) on total lipid contents in the liver and ovary of catfish, Heteropneustes fossilis (Bloch)

Total lipid contents in the liver of Catfish, *H. fossilis* (Bl.) at the prespawning stage of the reproductive cycle maintained in tap water for 30 days were lower than those of the fish maintained in pesticide containing media (Fig. 2). The values were significantly different among fish treated with 1.0 mg/l (25% increase, $P<0.05$) and 2.0 mg/l CF (40% increase, $P<0.01$) but not in fish treated with 0.5 mg/l CF (7.65% increase). Further analysis of the data showed that total lipid in the liver of 2.0 mg/l CF treated fish was significantly higher ($P<0.01$) than that of the fish maintained in 0.5 mg/l CF but not of the fish in 1.0 mg/l CF. Total lipid in the ovaries remained unaltered even after 30 days of CF treatment (Fig. 3).

Effect of carbofuran (CF) on fatty acid composition of total lipid in the liver and ovary of catfish, Heteropneustes fossilis (Bloch)

The fatty acid composition of total lipid in the liver and ovary of catfish was also affected by carbofuran treatments (Tables 1 and 2). The predominant fatty acids in the liver and ovary of control *H. fossilis* were palmitic ($C_{16:0}$), palmitolic ($C_{16:1}$ w_9), stearic ($C_{18:0}$), Oleic ($C_{18:1}$ w_9), linoleic ($C_{18:2}$ w_6), linolenic ($C_{18:3}$ w_3), and arachidonic ($C_{20:4}$ w_6) acids (Tables 1 and 2). Eicosapentaenoic ($C_{20:5}$ w_3) and docosahexaenoic ($C_{22:6}$ w_3) acids were also detected in the lipid component of both liver and ovary of this fish. Other fatty acids, $C_{18:4}$ w_3, $C_{20:2}$ w_6, $C_{20:3}$ w_6, $C_{22:4}$ w_6 and $C_{22:5}$ w_3 were below the detection limit in the control liver whereas traces of $C_{20:2}$ w_6 and $C_{20:3}$ w_6 fatty acids were present in the control ovary.

Pesticide treatment caused changes in the fatty acid components of total lipid in the liver and ovary of *H. fossilis* (Tables 1 and 2), compared to those

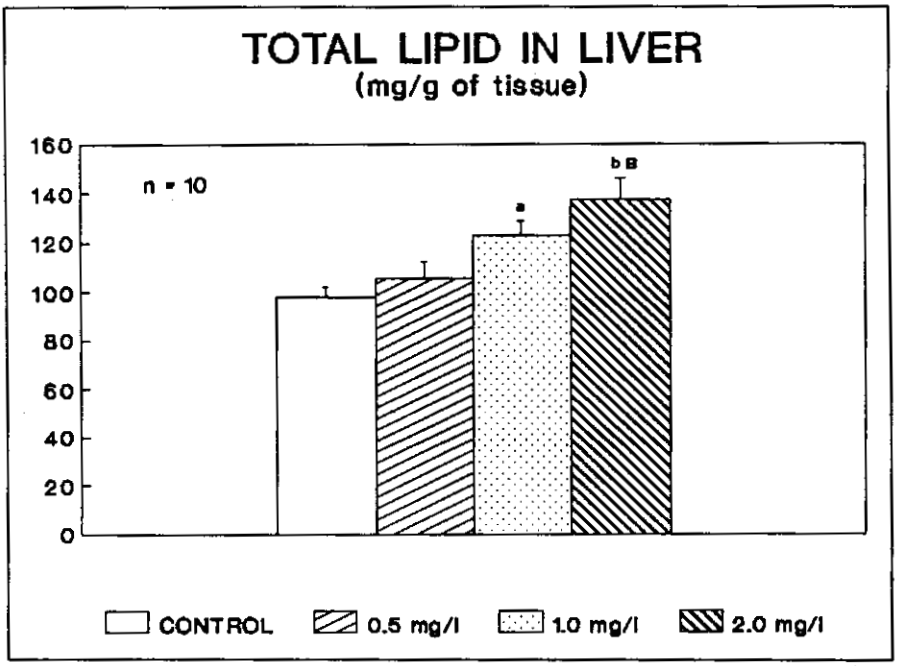

Fig. 2. Effect of carbofuran at different concentrations (0.5, 1.0 and 2.0 mg/l) on the total lipid content in the liver of female catfish, *Heteropneustes fossilis* (Bl.). The values are Mean ± SE. Each group summarizes data from ten fish. Data were statistically analyzed by analysis of variance followed by Duncan's Multiple Range Test. $P<0.05$ considered as significant.
[a]$p<0.05$, [b]$p<0.01$. Lower case letters indicate difference between the control and the carbofuran treated groups; capital letters between 0.5 and 1.0 or 2.0 mg/l group.

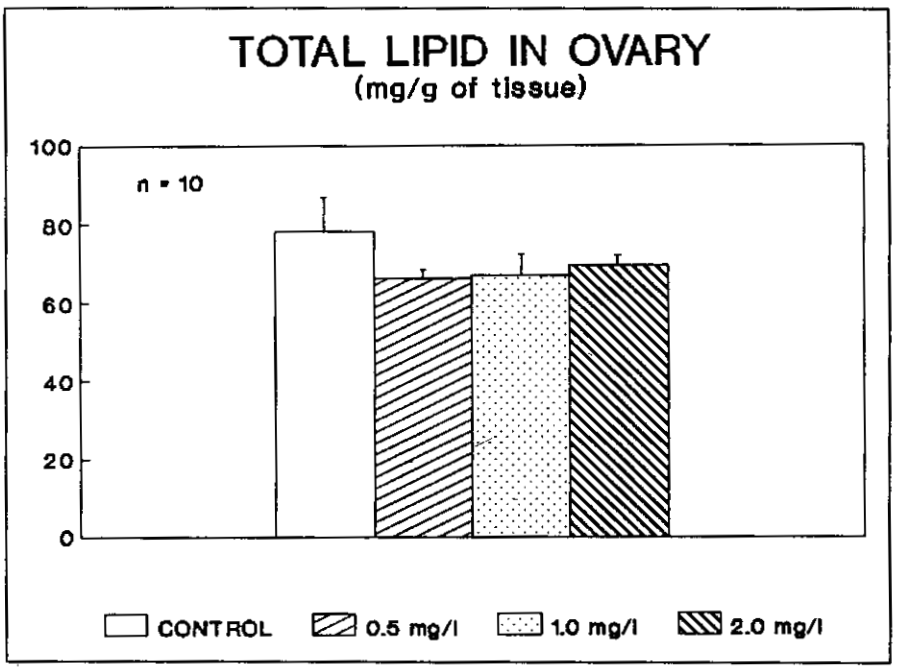

Fig. 3 . Effect of carbofuran at different concentrations (0.5, 1.0 and 2.0 mg/l) on the total lipid content in the ovary of female catfish, *Heteropneustes fossilis* (Bl.). The values are Mean ± SE. Each group summarizes data from ten fish. Data were statistically analyzed by analysis of variance.

Table 1. Fatty acid composition (in %) of total lipids of liver in the female catfish, *Heteropneustes fossilis* (Bl.).

*Fatty acids	Control	Carbofuran treated (mg/l)		
		0.5	1.0	2.0
14:0	8.54 ± 0.54	11.66 ± 1.04[a]	26.74 ± 0.23[c]	20.96 ± 0.43[c]
16:0	28.33 ± 0.45	39.65 ± 0.49[c]	24.61 ± 0.35[c]	19.52 ± 0.51[c]
$16:1w_9$	13.07 ± 0.31	9.19 ± 0.45[c]	9.77 ± 0.36[c]	7.51 ± 0.47[c]
18:0	3.67 ± 0.25	14.50 ± 0.65[c]	12.11 ± 0.21[c]	17.89 ± 0.40[c]
$18:1w_9$	12.76 ± 0.24	18.57 ± 0.51[c]	15.16 ± 0.34[c]	18.62 ± 0.39[c]
$18:2w_6$	7.74 ± 0.49	3.67 ± 0.27[c]	3.52 ± 0.06[c]	8.22 ± 0.12
$18:3w_3$	10.33 ± 0.23	0.75 ± 0.14[c]	2.26 ± 0.06[c]	3.68 ± 0.05[c]
$18:4w_3$	N.D.	0.37 ± 0.07	1.98 ± 0.05	N.D.
$20:2w_6$	N.D.	N.D.	N.D.	N.D.
$20:3w_6$	N.D.	N.D.	N.D.	N.D.
$20:4w_6$	3.62 ± 0.33	1.64 ± 0.42[b]	3.84 ± 0.22	2.63 ± 0.19[a]
$20:5w_3$	3.41 ± 0.23	N.D.	N.D.	N.D.
$22:4w_6$	N.D.	N.D.	N.D.	N.D.
$22:5w_3$	N.D.	N.D.	N.D.	N.D.
$22:6_{w3}$	8.53 ± 0.28	N.D.	N.D.	0.96 ± 0.03[c]

The values are Mean±SE of three samples. SE = Standard Error. [a]$p<0.05$, [b]$p<0.01$ and [c]$p<0.001$ as compared to control (Analysis of Variance, Duncan's Multiple Range Test). The values were considered statistically significant at $p<0.05$.
*The figure represents the carbon chain length : number of double bond. The w values denote the number of carbons between the terminal methyl group and double bond furthest removed from the carboxyl end.
N.D. = Not Detected.

Table 2. Fatty acid composition (in %) of total lipids of ovary in the female catfish, *Heteropneustes fossilis* (Bl.).

*Fatty acids	Control	Carbofuran treated (mg/l)		
		0.5	1.0	2.0
14:0	15.43 ± 0.78	3.32 ± 0.16[c]	10.84 ± 0.18[c]	9.79 ± 0.35[c]
16:0	27.49 ± 0.52	31.57 ± 0.58[c]	24.48 ± 0.37[b]	24.54 ± 0.42[b]
$16:1w_9$	6.64 ± 0.39	1.91 ± 0.34[c]	12.09 ± 0.16[c]	6.99 ± 0.12
18:0	18.14 ± 0.30	15.21 ± 0.23[c]	16.90 ± 0.38[a]	21.72 ± 0.64[c]
$18:1w_9$	18.05 ± 0.40	25.20 ± 0.54[c]	23.92 ± 0.24[c]	23.31 ± 0.64[c]
$18:2w_6$	4.99 ± 0.24	6.57 ± 0.56[a]	5.95 ± 0.12[b]	6.23 ± 0.37[a]
$18:3w_3$	1.77 ± 0.14	0.96 ± 0.04[c]	0.12 ± 0.02[c]	0.92 ± 0.02[c]
$18:4w_3$	N.D.	1.05 ± 0.07	N.D.	0.68 ± 0.05
$20:2w_6$	1.36 ± 0.22	1.34 ± 0.09	0.07 ± 0.03[c]	0.75 ± 0.04[a]
$20:3w_6$	0.50 ± 0.01	1.43 ± 0.04[c]	5.63 ± 0.37[c]	0.53 ± 0.05
$20:4w_6$	3.64 ± 0.27	7.40 ± 0.55[c]	N.D.	4.47 ± 0.03[a]
$20:5w_3$	0.43 ± 0.04	0.81 ± 0.18	N.D.	0.07 ± 0.02[c]
$20:4w_6$	N.D.	0.28 ± 0.04	N.D.	N.D.
$22:5w_3$	N.D.	0.31 ± 0.06	N.D.	N.D.
$22:6_{w3}$	1.56 ± 0.19	2.64 ± 0.16[a]	N.D.	N.D.

The values are Mean±SE of three samples. SE = Standard Error. [a]$p<0.05$, [b]$p<0.01$ and [c]$p<0.001$ as compared to control (Analysis of Variance, Duncan's Multiple Range Test). The values were considered statistically significant at $p<0.05$.
* The figure represents the carbon chain length : number of double bond. The w values denote the number of carbons between the terminal methyl group and double bond furthest removed from the carboxyl end.
N.D. = Not Detected.

of the control (fish maintained in tap water). Fatty acids $C_{14:0}$, $C_{18:0}$, $C_{18:1}$ w_9, in liver lipid (Table 1) and $C_{18:1}$ w_9 and $C_{20:4}$ w_6 in ovarian lipid (Table 2) were increased after CF treatment. Fatty acids $C_{16:1}$ w_9, $C_{18:2}$ w_6, $C_{18:3}$ w_3, $C_{20:5}$ w_3 and $C_{22:6}$ w_3 in liver lipid as well as $C_{14:0}$, $C_{18:3}$ w_3 and $C_{20:2}$ w_6 in ovarian lipid decreased in comparison to their respective control values (Tables 1 and 2).

Different fatty acid classes after treatment with carbofuran

Levels of saturated fatty acid classes increased in the liver and decreased in the ovary of pesticide treated fish (Table 3). Among the unsaturated fatty acids, the level of total polyunsaturated fatty acids decreased remarkably in the liver although total monoenes of this tissue remained unchanged (Table 3). In case of the ovary, monoenes increased in 1.0 mg/l and 2.0 mg/l concentrations of CF whereas polyunsaturated fatty acids remained unchanged except in 0.5 mg/l concentration in this tissue.

Among the polyunsaturated fatty acid classes, total w_3 acids were reduced in both ovary and liver of pesticide treated fish and w_6 acids remained unaltered (Table 3). Thus the ratio of total w_3/w_6 fatty acid was reduced in both tissues.

Effect of carbofuran (CF) on total cholesterol contents in the liver and ovary of catfish, Heteropneustes fossilis (Bloch)

Total cholesterol content in the liver of the catfish was less than that in the ovary when the results were expressed per 100 mg tissue (Figs. 4 and 5). Treatment of fish with carbofuran (0.5-2.0 mg/l) enhanced liver cholesterol content to about 2-2.5 fold in comparison to that of controlled fish (Fig. 4). Increases were more or less similar in all three concentrations of carbofuran used in this study. Ovarian cholesterol remained unaffected after pesticide treatment to controlled fish (Fig. 5).

Discussion

Carbofuran, like many pesticides, is lipid soluble and accumulates easily in tissues. The total lipid content in the liver increased significantly in fish treated with 1.0 and 2.0 mg/l (Fig. 2), but total lipid content in the ovary remained unaltered (Fig. 3). The increase in liver lipid content in the experimental fish did not change the total body weight of the fish suggesting that the general health of the experimental fish remained unaffected at sublethal concentration of CF treatment. The increase in lipid content in fish liver might possibly be due to increased lipogenesis or less lipid mobilization. In mice, intraperitoneal administration of furadan (commercial form of carbofuran) for 42 days (0.125-0.500 mg/kg) resulted in an increase of total lipid, cholesterol (free and esterified), triglyceride and phospholipid and its fractions (lecithin, lysolecithin, phosphatidyl ethanolamine and lysophosphatidyl ethanolamine) in liver, kidney and serum with corresponding decrease of lipase activity in the liver

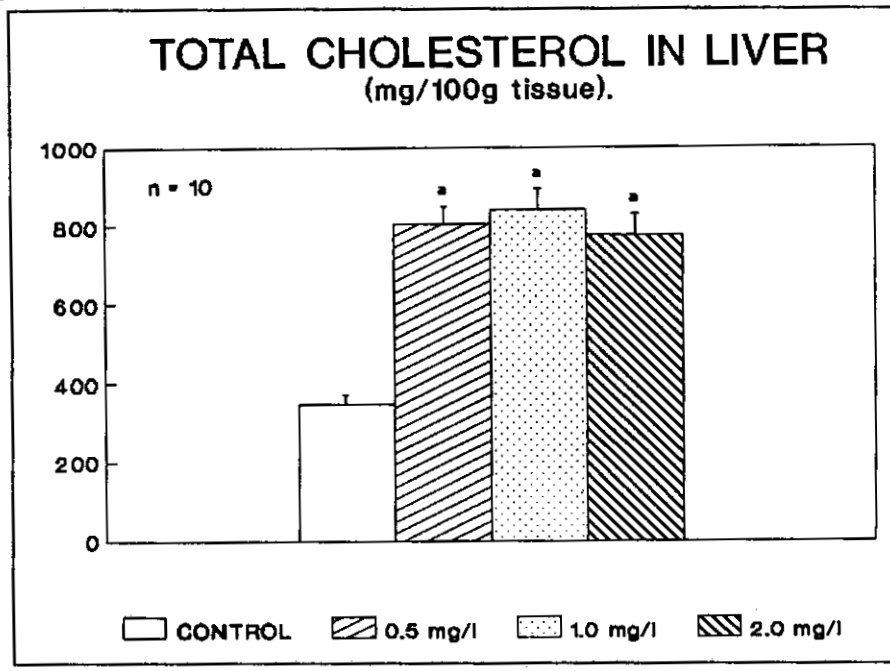

Fig. 4. Effect of carbofuran at different concentrations (0.5, 1.0 and 2.0 mg/l). on the total cholesterol content in the liver of female catfish, *Heteropneustes fossilis* (Bl.). The values are Mean ± SE. Each group summarizes data from ten fish. Data were statistically analyzed by analysis of variance followed by Duncan's Multiple Range Test. $P<0.05$ considered as significant.
"$p<0.01$, "a" is used for comparison between the controlled and the carbofuran treated groups.

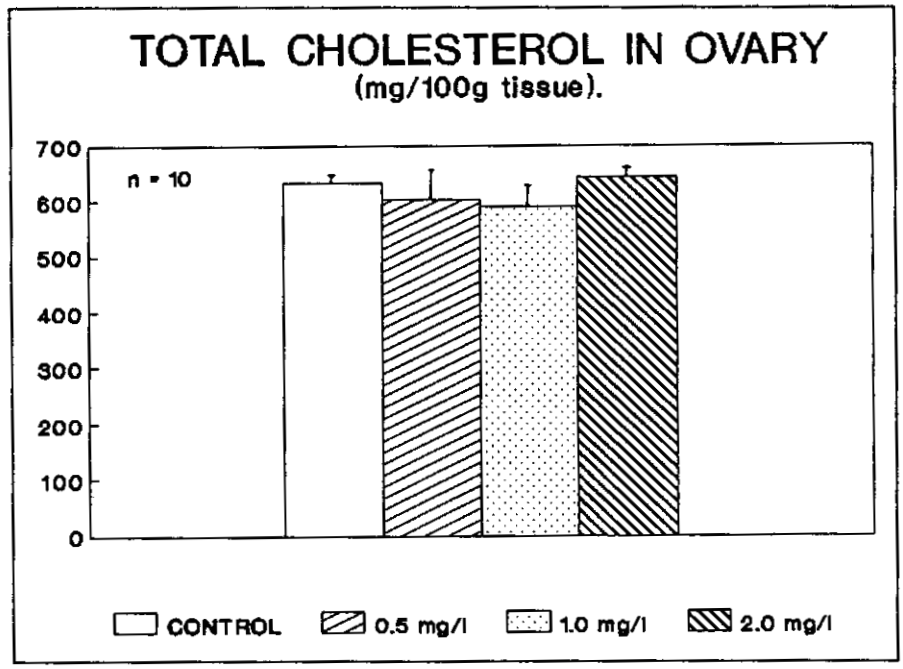

Fig. 5. Effect of carbofuran at different concentrations (0.5, 1.0 and 2.0 mg/l). on the total cholesterol content in the ovary of female catfish, *Heteropneustes fossilis* (Bl.). The values are Mean ± SE. Each group summarizes data from ten fish. Data were statistically analyzed by analysis of variance.

Table 3 . Fatty acid classes of liver and ovary of female catfish, *Heteropneustes fossilis* (Bl.).

Total fatty acid classes	Liver				Ovary			
	Control	Carbofuran treated (mg/l)			Control	Carbofuran treated (mg/l)		
		0.5	1.0	2.0		0.5	1.0	2.0
Saturates	40.54 ± 0.16	66.12 ± 1.21^{c}	63.46 ± 0.40^{c}	58.37 ± 0.29^{c}	61.06 ± 0.55	50.10 ± 0.44^{c}	52.31 ± 0.58^{c}	56.07 ± 0.69^{c}
Unsatu.	59.46 ± 0.16	33.88 ± 1.21^{c}	36.53 ± 0.40^{c}	41.62 ± 0.29^{c}	38.93 ± 0.55	49.90 ± 0.40^{c}	47.69 ± 0.58^{c}	43.94 ± 0.71^{c}
Monoenes	25.83 ± 0.29	27.77 ± 0.96	24.93 ± 0.07^{a}	26.13 ± 0.07	24.69 ± 0.48	27.11 ± 0.26^{b}	36.02 ± 0.32^{c}	30.30 ± 0.56^{c}
Polyenes	33.62 ± 0.20	6.44 ± 0.10^{c}	11.47 ± 0.18^{c}	15.49 ± 0.22^{c}	14.24 ± 0.09	22.79 ± 0.43^{b}	11.67 ± 0.30^{c}	13.64 ± 0.45
w_3	22.06 ± 0.25	1.12 ± 0.13^{c}	4.24 ± 0.02^{c}	4.64 ± 0.08^{c}	3.77 ± 0.26	4.59 ± 0.91	0.08 ± 0.04^{c}	1.66 ± 0.03^{c}
w_6	11.35 ± 0.18	5.32 ± 0.22	7.22 ± 0.19^{c}	10.84 ± 0.21^{c}	10.49 ± 0.28	16.92 ± 0.65^{c}	11.66 ± 0.37^{a}	11.79 ± 0.26^{b}
w_3/w_6	1.96 ± 0.06	0.21 ± 0.03^{c}	0.59 ± 0.01^{c}	0.43 ± 0.01^{c}	0.36 ± 0.04	0.29 ± 0.06	0.01 ± 0.003^{c}	0.14 ± 0.006^{c}

$^{a}p<0.05$, $^{b}p<0.01$ and $^{c}p<0.001$ as compared to control (Analysis of Variance, Duncan's Multiple Range Test). The values were considered statistically significant at $p<0.05$.

(Gupta et al. 1986). It can therefore be assumed that the increased amount of total lipid in the liver of the experimental catfish after CF treatment obtained in this study was the result of less degradation of the lipids due to a decrease in lipase activity rather than new synthesis.

Oocytes of teleosts import lipids from the liver during the vitellogenic stage (Weigand and Peter, 1980), although there are reports about extrahepatic sources of ovarian lipids in teleost fish and the capacity of the ovary itself to synthesize lipid endogenously (Weigand and Idler, 1982; Mommsen and Walsh, 1988). In the present experimental fish, the total ovarian lipid remained unaltered (statistically no significant changes) after the treatment of CF (Fig.3), although the control values were 12-18% higher than in the pesticide treated fish (Fig.-3). These findings suggest that the ovary in this experimental fish is at least partly dependent on the liver with respect to its lipid component.

The lipids in fish, as in other aquatic animals, consisted of more unsaturated fatty acids (UFA) (20:4 w_6, 20:5 w_3, 22:6 w_3). In *H. fossilis*, UFA were measured both in the liver and the ovary (Tables 1 and 2). There were more polyunsaturated fatty acids (PUFA) in the liver than in the ovary (Table 3) but their significance was not explored during vitellogenesis. In rainbow trout, the major source of PUFA in the ovary are the circulating lipoproteins (Fremont et al. 1984). Among PUFA, arachidonic acid ($C_{20:4}$) which acts as a precursor of prostaglandin (Ichinose et al. 1987), was also identified in the liver and ovary of the catfish (Tables 1 and 2). Prostaglandin plays a significant role in fish reproduction. Therefore, any alteration in the level of UFA, might affect fish reproduction. The inhibitory effect of CF on gonadal maturation and fertilization of eggs of *H. fossilis* (Ghosh and Chatterjee, 1994; Chatterjee and Ghosh, 1995; Chatterjee et al. 1997) might be correlated with the changes in the fatty acid levels. The total w_3/w_6 type polyenoic acids have an important role on the spawning, oocyte fertilization, and hatching rate of embryos in *Penaeus monodon* (Millamena, 1989). In the present experiment, it was observed that w_3/w_6 fatty acids ratio in both liver and ovary of the CF treated fish was diminished (Table 3). These results suggested that CF induced impaired ovarian function may be the results of the diminished level of w_3 fatty acid particularly linolenic acid ($C_{18:3}$ w_3), which is the precursor of w_3 fatty acid series (Montgomery et al. 1980) and reflecting the diminished ratio of w_3/w_6 fatty acids.

Like total lipid, the cholesterol concentration also increased in the liver but remained unaltered in the ovary after pesticide treatment (Figs. 4 and 5). Inhibitory influence of pesticides in cholesterol metabolism reflected the steroid hormone levels in fish. There are reports that cythion, an organophosphorous pesticide and hexadrin, an organochlorine pesticide, affected lipid and cholesterol metabolism in the liver and ovary of *H. fossilis* (Singh and Singh, 1980a, 1980b). The ovarian cholesterol increased with the organochlorine or organophosphorus pesticides after short-term (96 h) or long-term exposure. Deleterious effects of metal pollutants (cadmium and mercury) on the transport of circulating cholesterol to ovary and on the gonadal steroidogenesis in sexually mature common carp *Cyprinus carpio* were reported by Mukherjee et al. (1994). In the case of mice, carbamate pesticide (furadon) increased cholesterol concentrations

in the liver and kidney (Gupta et al. 1986) which suggests that influence of carbamate pesticides in lipid metabolic pathways was different from organochlorine and organophosphorus pesticides.

The alteration in cholesterol concentrations in the liver and ovary by CF will affect the steroid hormone level in the present experimental fish. The production of estrogen, the female sex steroid hormone, in the ovary of fish was greatly affected by CF (Chatterjee and Ghosh, Communicated). In fish, ovary is the major site for the formation of estrogen (Fostier et al. 1983). It was also reported that both the granulosa and the thecal cells of the growing oocytes synthesized steroid hormones. Thecal cells also produced estradiol by synthesizing testosterone which was transferred and aromatized to estrogen in granulosa cells (Chieffi and Pierantoni, 1987; Kagawa et al. 1982). It has already been reported by us that CF affected ovarian structure with large number of Stage I oocytes (Chatterjee et al. 1997).

Conclusion

The results suggest that carbofuran interferes estrogen level and consequently affects vitellogenesis in fish due to impaired lipid metabolism. Ultimately, fish production will be hampered.

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