

A STUDY ON CHRONIC EFFECT OF PROFENOFOS ON ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE ACTIVITIES AND PROTEIN CONTENTS IN VARIOUS TISSUES IN MAJOR CARPS

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Abstract

Background: Pesticides widely used for agricultural purposes are carbamates, organophosphates as well as organochlorines. Among these pesticides, organochlorine compounds have been extensively used to control disease vectors as well as agricultural pests. The objective of this experiment was to study the chronic effect of a commercial formulation of profenofos on acetylcholinesterase (AChE) and butylcholinesterase (BuChE) activity in various tissues in *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* fingerlings was investigated.

Materials and Methods: Healthy fingerlings of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* with an average body length 90 ± 6 mm and 30.00 ± 2.00 g body weight were collected from the fish seed hatchery and brought to the laboratory. Fishes were maintained in 70 liter glass aquaria (27 ± 1 °C, 2.70–2.80 ms and 8.85–9.40 pH). The fingerlings of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* were exposed to the 3 sub lethal concentrations of profenofos (0.038, 0.019, 0.012; 0.06, 0.03, 0.02 and 0.041, 0.020 and 0.013 mg/L) for 8 weeks. The control experiments were also performed with the addition of carrier solvent alone. Acetylcholinesterase and butyrylcholinesterase level were estimated according to the methodology of Ellman et al. (1961) and Kuster (2005). Total soluble proteins were determined by the Bradford (1976) standard method to assess enzymatic activity of the protein.

Results: The least activity of AChE was recorded in muscle samples of *Catla catla* (1.07 ± 0.040 μ moles/min/g of protein), exposed to the highest concentration of profenofos. BuChE activity was also reduced against various concentrations of profenofos. The AChE and BuChE activity was significantly inhibited even when exposed to a minimum concentration of this insecticide. Profenofos exposure affected the functioning of brain, blood, gills, muscle, kidneys and liver. We concluded that profenofos caused more inhibition in the liver for AChE and BuChE compared to other tissues.

Conclusion: It has been concluded that profenofos is very highly toxic to the *C. catla*, *L. rohita* and *C. mrigala* fingerlings, but further studies are required to assign a certain level of toxicity to the said pesticide. Considering the high toxicity of profenofos, it is suggested to handle the profenofos carefully using all the precautionary measures in order to minimize the harmful effects on non-target organisms.

Key words: Toxicity; Profenofos; esterase activity; issues; fish

Introduction

Nowadays, the concerns about pollutant residues and food safety are increasing. Among these residues, pesticides are widely detected toxic xenobiotics in many environments. They can mainly enter animals via feed consumption along the food chain (Prasad and Chhabra 2001; Nag et al., 2007; Aulakh et al., 2006; Mekonen et al., 2014). Pesticides widely used for agricultural purposes are carbamates, organophosphates as well as organochlorines. Among these pesticides, organochlorine compounds have been extensively used to control disease vectors as well as agricultural pests. They are highly persistent in environments and have been found in multiple environmental matrices; e.g., water, air, soil and organisms (Fang et al., 2014; Salem et al., 2009; Carrera et al., 2002; IPEN, 2008; Naqvi and Vaishnavi 1993). Many researches showed that the agricultural runoff is the major route of pesticide getting into the water. In addition, these organochlorine pesticides (OCPs) can be dispersed into various environments and bio-accumulated in food crops (Mumtaz et al., 2015; Park et al., 2011) and animal tissues (Nakata et al., 2002).

Varieties of substances are classified as OCPs, including DDT, endrin, as well as endosulfan. Profenofos is a broad spectrum insecticide and acaricide. It is non-systemic but has an excellent translaminar action. "Profenofos [CAS Number: 41198-08-7; Chemical name [O-(4-Bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate]; Empirical formula: C₁₁H₁₅BrClO₃PS; Molecular weight: 373.63] is a potentially ground water contaminating organophosphorous insecticide, slightly soluble in water (20.0 mg/L) and readily miscible in organic solvents. The substance is hydrolyzed with increasing pH, i.e. 50% loss in 93 days at pH 5, 14.6 days at pH 7 and 5.7 h at pH 9 along with chemically more unstable under alkaline conditions (Isamil et al., 2009)". Moreover, its half-life in the soil is about one week (Tomlin, 1994). The World Health Organization (1986) has considered Profenofos as a moderately hazardous (Toxicity class

II) pesticide and it has a moderate order of acute toxicity following an oral and dermal administration. Profenofos is extremely toxic to fish. The acute toxicity of profenofos is the inhibition of the acetylcholine and butylcholine esterase activity resulting in neuro toxicity to aquatic vertebrates and humans (Rusha et al., 2013). It can accumulate in foods, thus, raises more concerns for public health. It has been restricted and was banned in many countries such as European Union countries, Asia, etc. The objective of this study was to evaluate the effect of chronic toxicity of profenofos on acetylcholine esterase and butylcholine esterase activity in brain, liver, kidney, muscle and blood in *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*.

Materials and Methods

Fish and Its Maintenance

Healthy fingerlings of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* with an average body length 90 ± 6 mm and 30.00 ± 2.00 g body weight were collected from the Fish Seed Hatchery and brought to the laboratory. Fishes were maintained in 70 liter glass aquaria ($27 \pm 1^\circ\text{C}$, 2.70–2.80 ms and 8.85–9.40 pH). Fish specimens were acclimatized for to the laboratory conditions two weeks prior to pesticide exposure.

Procedure Adopted

“Profenofos 98% [O-(4-Bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate] was purchased from Ali Akbar Enterprises, Lahore, Pakistan. Profenofos was dissolved in methanol (Analytical grade, Merck) as 1/5th, 1/10th and 1/15th part of LC₅₀ (predetermined). To confirm the solubility of profenofos in water, a 1-ppm concentration of profenofos was dissolved in the solvent and the test water sample was prepared and was confirmed with HPLC (Model L7400). Fish were exposed to these lower concentrations of pesticides in triplicates with 20 fish at each concentration for a period of 60 days. The fingerlings of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* were exposed to the 3 sub-lethal concentrations of profenofos (0.038, 0.019, 0.012; 0.06, 0.03, 0.02 and 0.041, 0.020 and 0.013 mg/L). The control experiments were also performed with the addition of carrier solvent alone. Fish were fed daily with commercial diet at the rate of 3 % of their body weight in two factions at an interval of 8 hours. The water was changed after every 4 days to maintain a continuous supply of pesticides to the fish. The fish were exposed to pesticides in a static bioassay system and were continuously observed. Fish were removed from each aquarium at the end of the experiment and anesthetized with MS-222 (Finquel®). They were dissected to remove the brain, gills, liver, kidney and muscle samples which were quickly removed, frozen in liquid nitrogen and were stored at -20°C . Acetylcholinesterase and butrylcholinesterase level were estimated according to the methodology of Ellman et al. (1961) and Kuster (2005). Total soluble proteins were determined by the Bradford (1976) standard method to assess enzymatic activity of the protein. The AChE and BuChE activity were expressed as a specific activity (normal substrate hydrolyzed /min mg protein)” (Ghazala, 2014).

The data collected in this study were statistical analyses with the help of Minitab software. The differences among treatments were tested using ANOVA followed by Turkey’s HSD test.

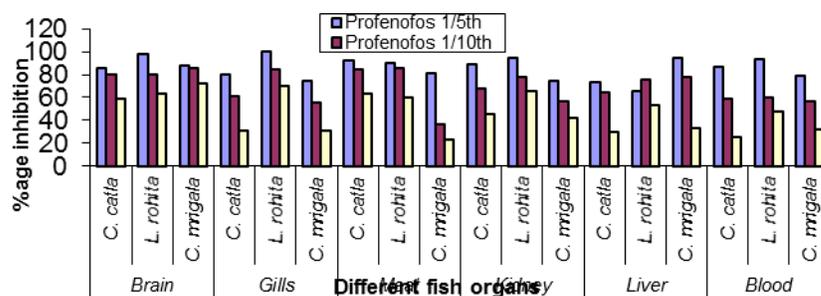


Figure 1: Effect of various concentrations of profenofos on Acetylcholinesterase activity inhibition %age, in different organs of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*.

Results and Discussion

Profenofos inhibited the esterase activity in all exposure concentrations in *C. catla*, *L. rohita* and *C. mrigala*. The least activity of acetylcholinesterase (AChE) was calculated in the muscle of *C. catla* (1.07 ± 0.040 $\mu\text{moles/min/g}$ of protein), exposed to the highest concentration of this pesticide (Table 1). The highest activity was observed in the liver tissues, i.e. 85.65 ± 0.029 $\mu\text{moles/min/g}$ of protein.

The comparison of the means showed a highly significant difference among all concentrations and between the exposure concentrations and control group. The highest activity of AChE in the brain of *L. rohita* and *C. mrigala* was recorded against the exposure with least concentrations of profenofos (0.02 and 0.013 mg/L, respectively). Statistically significant ($P < 0.05$) differences among all exposure concentrations and control groups were observed (Table 1). The maximum inhibition percentage of AChE activity was observed in the gills of *L. rohita* exposed to the highest (0.06 mg/L) exposure concentration of profenofos (Fig. 1). There was a relationship between exposure concentrations and inhibition of AChE activity in the studied fish species.

Table 1: Comparison of means (\pm S.E.) for acetylcholinesterase activity ($\mu\text{mol}/\text{min}/\text{g}$ protein), of control group and three exposure concentrations (mg/L=ppm) of profenofos in different tissues of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*.

Fish Spp.	Treatments (mg/L)	Brain	Gills	muscle	Kidney	Liver	Blood
<i>Catla catla</i>	Control	34.55 \pm 0.03A	14.24 \pm 0.14A	13.80 \pm 0.17A	36.39 \pm 0.05A	121.99 \pm 0.01A	22.40 \pm 0.06A
	0.038	4.87 \pm 0.04D	2.90 \pm 0.52D	1.07 \pm 0.04D	4.19 \pm 0.11D	31.98 \pm 0.06D	2.98 \pm 0.01D
	0.019	6.88 \pm 0.05C	5.56 \pm 0.32C	2.09 \pm 0.05C	11.70 \pm 0.40C	43.87 \pm 0.04C	9.34 \pm 0.02C
	0.012	14.09 \pm 0.05B	9.80 \pm 0.46B	5.13 \pm 0.07B	19.74 \pm 0.02B	85.65 \pm 0.03B	16.71 \pm 0.01B
<i>Labeo rohita</i>	Control	40.44 \pm 0.02A	20.36 \pm 0.03A	18.09 \pm 0.05A	24.39 \pm 0.05A	98.06 \pm 0.03A	26.52 \pm 0.01A
	0.06	1.04 \pm 0.02D	0.6 \pm 0.03D	1.89 \pm 0.05D	1.35 \pm 0.20D	4.24 \pm 0.02D	1.65 \pm 0.03D
	0.03	7.99 \pm 0.05C	3.24 \pm 0.14C	2.64 \pm 0.02C	6.87 \pm 0.04C	23.59 \pm 0.10C	10.50 \pm 0.17C
	0.02	14.87 \pm 0.04B	6.02 \pm 0.13B	7.34 \pm 0.20B	8.34 \pm 0.20B	45.78 \pm 0.01B	13.78 \pm 0.02B
<i>Cirrhinus mrigala</i>	Control	24.55 \pm 0.03A	10.36 \pm 0.21A	6.13 \pm 0.09A	8.61 \pm 0.11A	132.66 \pm 0.01A	30.98 \pm 0.01A
	0.041	3.00 \pm 0.11D	2.70 \pm 0.40D	1.14 \pm 0.08D	2.17 \pm 0.09D	7.41 \pm 0.01D	6.70 \pm 0.06D
	0.020	3.60 \pm 0.06C	4.56 \pm 0.03C	3.87 \pm 0.04C	3.70 \pm 0.40C	29.83 \pm 0.07C	13.45 \pm 0.09C
	0.013	6.78 \pm 0.04B	7.19 \pm 0.11B	4.67 \pm 0.04B	5.00 \pm 0.29B	89.00 \pm 0.29B	20.98 \pm 0.01B

Means with different letters for each fish in a column are highly significantly different ($P < 0.01$). S.E. =standard error

Butyrylcholinesterase (BuChE) activity in *C. catla*, *L. rohita* and *C. mrigala* decreased when exposed to various concentrations of profenofos. In *C. catla*, the activity of BuChE was recorded as 1.77 \pm 0.043 $\mu\text{mol}/\text{min}/\text{g}$ protein in the brain, against the 0.019 and 0.038 mg/L concentration of profenofos. Significantly different BuChE activity (12.78 \pm 0.043 $\mu\text{mol}/\text{min}/\text{g}$ Protein) was observed against the least concentration of profenofos (1/15th of LC₅₀) (Tables 2). An overall comparison showed that BuChE activity was found to be higher in control groups. The sequence of BuChE activity was as follows: liver > blood > kidneys > brain > flesh > gills in *C. catla*. In *L. rohita* the sequence of BuChE activity was as follows: liver > blood > brain > kidney > flesh > gills. In *C. mrigala* the sequence of BuChE activity was as follows: liver > blood > kidney > flesh > brain > gills (Table 2). A non-significant ($P > 0.05$) difference was observed for BuChE activity in the muscle and gills of *C. catla* and *L. rohita*. BuChE activity was highly significantly different ($P < 0.01$) in brain, muscles, liver, kidneys, gills and blood of *C. catla*, *L. rohita* and *C. mrigala* compared to control groups. The highest inhibition in BuChE activity was observed in the liver of *C. mrigala* (95%) against the exposure of the high concentration of profenofos (Table 2 and Fig. 2). Protein contents were also reduced in different organs of three fish species against the exposure of profenofos. An increase in soluble protein contents was recorded in the brain and gills of *L. rohita*, in blood of *C. catla* and liver of *C. mrigala* (Fig. 3).

The response of biomarkers in fish may vary depending on species, pollution and sex differences. In this study, however, AChE and BuChE activities in blood, muscle, liver, kidney and gills were decreased. Our results were in line with the previously reported findings of Stegeman et al. (1992), Chamber, and Boone (2002). Cholinesterases level is changed because of stress response when exposed to various insecticides, pesticides, halogenated aromatics and certain types of dioxins (Stegeman, 1992; Boer et al., 1993). Sensitivity of fish to pesticide exposure, especially organophosphate is dependent on the level of brain AChE activity. In this study, cholinesterase activity was inhibited up to less than 50% of the normal. In *C. catla* the inhibition of AChE was less than 50%, except in muscle samples exposed to the lowest concentration of profenofos. "Reduction of brain AChE activity by 20% or more in birds, fish or invertebrates indicates exposure to OPs or carbamate pesticides, and a 50% or greater reduction is indicative of a life-threatening situation (Wright and Welbourn 2002)". In the current study, similar response was recorded after exposure to profenofos. A strong inhibition in AChE activity was observed in the muscle of *C. catla* after exposure with profenofos (Toni et al., 2010). During this investigation, among all the studied tissue maximum inhibition of AChE activity was recorded in the brain of *C. catla*, *L. rohita* and *C. mrigala* and dose-effect relationships were observed. It has been assumed the inhibition of cholinesterase activity in either nervous system or muscle as an adverse effect of profenofos in these fish species. These findings were in line with the already-reported results of Padilla (1995). Inhibition in the cholinesterase activities reported in various organisms and organs with a focus on brain tissues (Penallopis et al., 2003). The observed difference in potencies was ascribed to variations in the rate at which dephosphorylation and decarbamylation take place in different OPs and carbamates (Ecobichon, 2001).

In *L. rohita* AChE was inhibited after exposure to different concentrations of profenofos. In *C. mrigala*, the sequence of AChE activity was as follows: Liver > blood > brain > gills > kidney > flesh (Table 1). During this study, the *L. rohita* exposed to different concentration demonstrated the greatest inhibition in AChE in the kidneys. The AChE and BuChE activities in blood were significantly

($P \leq 0.05$) reduced compared to control group in these fish species after 60 days exposure with profenofos. The maximum inhibition in AChE activity in fish has been reported in the brain (Jafery and Keizer, 1995). In this study, more than 90% of AChE and BuChE inhibition observed in the brain and blood compared to other tissues. In this study, the muscle were least affected. These findings were in line with Straus and Chamber (1995). A reduction in AChE and BuChE activity in gills caused suffocation and reduced respiratory activity (Chamber and Carr, 1995).

The blood AChE and BuChE activities reduced in *C. catla*, *L. rohita* and *C. mrigala* after exposure to different concentrations of profenofos (Table 1-2). Straus and Chamber (1995) previously reported similar fluctuation in AChE activity in blood in catfish. Recovery of esterase activity after pesticide intoxication requires different intervals. *In vivo* studies a period of one week is required to recover brain esterase activity in fish after thiobencarb exposure (Babu et al., 1989). During this investigation, no recovery pattern was found in any of the sampled tissues. However, the least variation in AChE and BuChE activities recorded in the control group. The difference in the rate of inhibition in AChE and BuChE activity among different tissues in these fish species could be due to variable molecular forms of these enzymes, such as substrate specific AChE and BuChE and other enzymes called pseudo-cholinesterases that can hydrolyze acetylcholine (Massoulie et al., 1993).

In this study, a different pattern of BuChE activity was observed in the studied tissues of *C. catla*, *L. rohita* and *C. mrigala* after the chronic exposure of different concentrations of profenofos. BuChE was found to be highest in the liver of testing fish species in contrast to AChE activity. The minimum BuChE was observed in gills of all the fish species. BuChE inhibition to the greatest extent observed in blood of *C. catla* and *L. rohita* followed by the brain of *C. mrigala* after the exposure of the highest concentrations of profenofos. Statistically non-significant ($P \leq 0.05$) differences were observed for BuChE activity in the brain between *L. rohita* and *C. mrigala*. The extent of AChE and BuChE inhibition observed explicit that *C. catla*, *L. rohita* and *C. mrigala* are a useful fish species in assessing polluted aquatic environment by profenofos and other cholinesterase pollutants. The findings of this study, for AChE and BChE in major carps against this pesticide may be helpful to use it as a useful biomarker to identify such pollutants.

Table 2: Comparison of means (\pm S.E.) for butyrylcholinesterase activity ($\mu\text{mol}/\text{min}/\text{g}$ protein), of control group and three exposure concentrations ($\text{mg}/\text{L}=\text{ppm}$) of profenofos in different tissues of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*.

Fish Spp.	Treatments (mg/L)	Brain	Gills	muscle	Kidney	Liver	Blood
<i>Catla catla</i>	Control	25.67 \pm 0.04A	7.71 \pm 0.08A	19.03 \pm 0.02A	29.05 \pm 0.03A	158.55 \pm 0.03A	52.00 \pm 0.57A
	0.038	1.77 \pm 0.04D	1.00 \pm 0.06D	2.50 \pm 0.29D	5.79 \pm 0.06D	15.79 \pm 0.05D	5.89 \pm 0.05D
	0.019	1.77 \pm 0.05D	2.56 \pm 0.03C	8.71 \pm 0.06C	8.52 \pm 0.01C	39.87 \pm 0.04C	13.00 \pm 0.29C
	0.012	12.78 \pm 0.04B	5.89 \pm 0.05B	12.90 \pm 0.50B	19.34 \pm 0.19B	98.00 \pm 0.29B	29.80 \pm 0.11B
<i>Labeo rohita</i>	Control	40.43 \pm 0.05A	7.28 \pm 0.16A	19.03 \pm 0.02A	40.18 \pm 0.10A	167.00 \pm 0.29A	49.08 \pm 0.05A
	0.06	8.38 \pm 0.05D	2.50 \pm 0.29C	1.89 \pm 0.05D	4.37 \pm 0.04D	16.94 \pm 0.03D	8.65 \pm 0.02D
	0.03	25.87 \pm 0.04C	4.38 \pm 0.05B	4.73 \pm 0.02C	10.67 \pm 0.04C	32.62 \pm 0.01C	14.87 \pm 0.02C
	0.02	28.78 \pm 0.05B	4.78 \pm 0.04B	12.45 \pm 0.26B	12.92 \pm 0.07B	94.37 \pm 0.04B	23.67 \pm 0.01B
<i>Cirrhinus mrigala</i>	Control	7.17 \pm 0.04A	6.78 \pm 0.05A	17.93 \pm 0.02A	22.96 \pm 0.11A	156.66 \pm 0.03A	41.87 \pm 0.02A
	0.041	2.50 \pm 0.29C	2.01 \pm 0.12D	1.96 \pm 0.03D	4.51 \pm 0.06D	6.40 \pm 0.11D	5.60 \pm 0.06D
	0.020	2.72 \pm 0.01C	3.98 \pm 0.05C	6.40 \pm 0.23C	9.80 \pm 0.11C	47.00 \pm 0.58C	16.09 \pm 0.05C
	0.013	5.37 \pm 0.04B	4.75 \pm 0.03B	13.12 \pm 0.07B	15.05 \pm 0.03B	95.65 \pm 0.03B	20.50 \pm 0.059

Means with different letters for each fish in a column are highly significantly different ($P < 0.01$). S.E=standard error

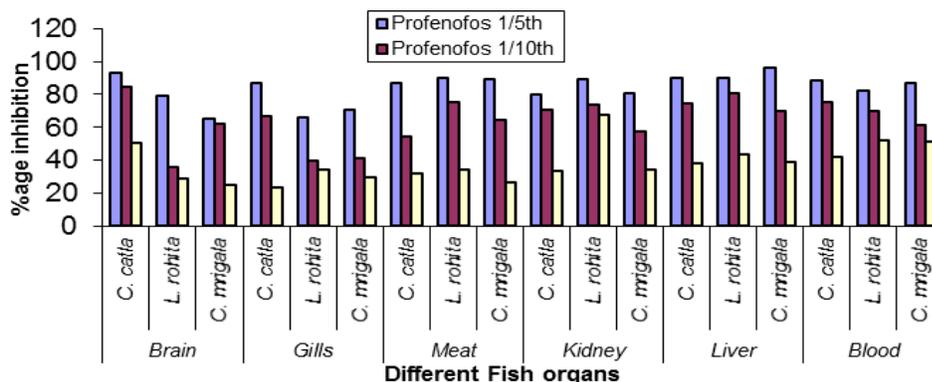


Figure 2: Effect of various concentrations of profenofos on Butyrylcholinesterase activity inhibition %age, in different organs of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*.

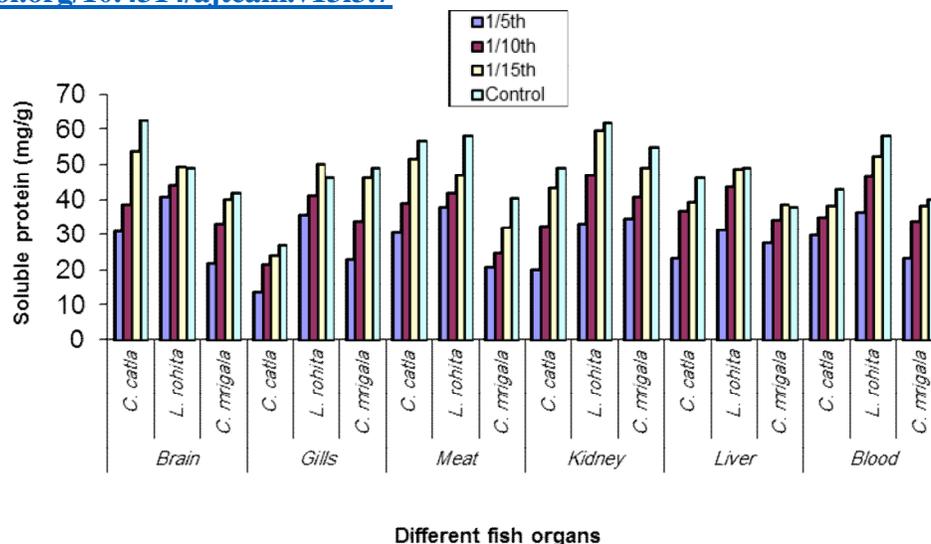


Figure 3: Total soluble protein in different organs and tissues of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* from various exposure concentrations of profenofos and control group

Conclusions

We concluded that profenofos is very highly toxic to the *C. catla*, *L. rohita* and *C. mrigala* fingerlings, but further studies are required to assign a certain level of toxicity to the said pesticide. Considering the high toxicity of profenofos, it is suggested to handle the profenofos carefully using all the precautionary measures in order to minimize the harmful effects on non-target organisms.

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