# Impact of Cypermethrin on Selected Enzymes in Tissues of *Heterobranchus bidorsalis*

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**Abstract**

*Heterobranchus bidorsalis* (mean total length 31.50 ± 2.32 cm SD; mean weight 241.25 ± 30.39 g SD) was exposed to cypermethrin (0.005, 0.0075, 0.010, 0.125 and 0.0150 p.p.m.) for 23 days to determine the activity of transaminases (alanine transaminase, ALT; aspartate transaminase, AST) the phosphatase, alkaline phosphatase, ALP in the gill, kidney, liver and muscle tissue. The activity of ALT in the gill at 0.005 and 0.010 p.p.m. were lower ($P > 0.05$) than the control, whereas elevated activity above the control were recorded at the other concentrations. AST activity was excited 11.36–220.45% above the control value at all the concentrations. ALP activity was inhibited below the control value with the highest inhibition, 40.46% at 0.010 p.p.m. ALT activities in the kidney at all the exposure concentrations were elevated 33.33% and 66.67% above the control at 0.005–0.010 p.p.m. and 0.0125–0.0150 p.p.m., respectively. Inhibition below the control was recorded in all the exposure concentrations for AST and ALP. ALT and AST activities in the liver were inhibited below their respective control values. ALP activity was inhibited at 0.0075 and 0.0150 p.p.m. (44.12 and 23.53%, respectively, below control value), but excited at the other concentrations with a peak, 33.83% at 0.010 p.p.m. In the muscle, 12.68% and 23.94% elevation above the control were recorded at 0.0125 and 0.0150 p.p.m., respectively, for ALT with a decrease in the lower concentrations. There was excitation of AST activity at 0.0050, 0.010 and 0.015 p.p.m., and inhibition at 0.0075 and 0.0125 p.p.m. relative to the control value. There was general inhibition of ALP activity in the muscle of treated fish below the control except at 0.010 p.p.m. The usefulness of the enzymes as biomarkers of cypermethrin toxicity appeared to be concentration and tissue dependent, and can be effectively used to assess the impact of the agrochemical on the fish.

## Introduction

Pesticides are known to affect all members of an ecosystem from the smallest invertebrates to birds and humans, and their toxicities in both urban and agricultural settings are responsible for the death of many birds and fishes, and smaller aquatic animals that fishes depend on for food (Khan *et al*., 2003). On application, pesticides are carried away by rain flood, wind drift, precipitation and runoffs to ponds, lakes and rivers (Richardson, 1998). In these environments, they cause disruptive changes that are harmful to non-target aquatic species.

Cypermethrin is a synthetic pyrethroid used to kill insects on cotton and vegetable crops. It has eight isomers and all of its containers is a combination of the various isomers (Cox, 1996). It kills target organisms by the disruption of the normal functioning of the nervous system, thereby, causing excitability and convulsion through the inhibition of gamma-aminobutyric acid receptors in the nervous system (Ramadan, 1988). The pesticide has been reported to cause mortality and behavioural changes in *Tilapia guineensis* juveniles (Chindah *et al*., 2000), and post fingerlings of *Clarias*

*gariepinus* (Gabriel & Kparobo, 2002) at concentrations as low as 0.0125 and 0.034 p.p.m., respectively. It inhibited acetylcholinesterase activities and reduced protein contents in the kidney and liver of the amphibian, *Rana tigrina* (Khan *et al*., 2003).

Cypermethrin altered biochemical variables in the plasma/serum of rainbow trout (Velisek *et al*., 2006), disrupted electrolytes (calcium, phosphorus and sodium) balance and total protein in the same fish (Atamanalp *et al*., 2002). It has also been observed to inhibit succinate dehydrogenase and ATPase activities in the brain, kidney and liver of *Labeo rohita* fingerlings (Das & Mukhejee, 2003). Besides, in the same fish species, Philip & Rajasree (1996) observed that the agrochemical caused an increase in transamination and oxidative deamination, manifested by elevation in the activities of AST and ALT, and glutamate dehydrogenase. Interestingly, these changes occurred at very low concentrations of the chemical, indicating its high toxicity to fish.

Since the presence of the toxicant in water has been found to alter the physiology and biochemistry of fish there is, therefore, the need to examine the enzymatic changes associated with cypermethrin-polluted environment in selected organs (gill, liver, kidney) and muscle tissue of *Heterobranchus bidorsalis* under laboratory conditions.

## Materials and methods

*Heterobranchus bidorsalis* (mean total length 31.50 ± 2.32 cm SD; mean weight 241.25 ± 30.39 g SD) were obtained and transported to the Laboratory, Department of Chemistry, Rivers State University of Science and Technology, Port Harcourt. They were acclimated individually to laboratory conditions for 7 days in a 30-litre aquarium with 10 litres effective volume. The top of the aquaria were covered with perforate lid to avoid escape of fish. The fish was fed daily with a 35% crude protein diet at 1% biomass. The length and weight of the fish was recorded.

The fish was exposed to graded concentrations of cypermethrin (0.005, 0.0075, 0.010, 0.125 and 0.0150 p.p.m.) in borehole water (characteristics: temperature, 26.51 ± 2.10 °C; dissolved oxygen, 4.51 ± 0.51 mg/l; *p*H, 6.49 ± 0.21; alkalinity, 17.30 ± 3.16 mg/l and hardness, 16.00 ± 2.06 mg/l**),** and a control for 23 days. Each treatment level was replicated four times. Fresh solutions were prepared daily and introduced after the aquaria were washed with a piece of foam, and the wastes and old solution siphoned away with a hose.

At the end of the experiment, the fish were killed with a blow on the head, and organs of interest (kidney, liver and gill), and tissues of muscle were removed after dissection of the fish. Samples (0.5 g) of organs and muscle tissue were homogenised in mortar and then thoroughly mixed with 5 ml physiology saline. This was centrifuged for 10 min at the rate of 3000 r.p.m. The supernatant was decanted into plain bottles and then stored at -2 °C in a refrigerator for analysis.

The samples were analysed for the transaminases (aspartate transaminase, AST; alanine transaminase, ALT) and alkaline phospatase, ALP. AST and ALT were analyzed by the method of Reitman & Frankel (1957), while ALP was analysed according to the method described by King & Armstrong (1934). The data obtained were subjected to a one-way analysis of variance, ANOVA, and Duncan's multiple range test

(DMRT) was used to separate the means where differences existed (Zar, 1984).

## Results

The activity of ALT in the gill at 0.005 and 0.010 p.p.m. were lower ($P > 0.05$) than the control, 123.75 ± 14.36IU/l. Elevated activities above the control were recorded at the other concentrations with a peak ($P < 0.05$), 176.25 ± 25.00IU/l at 0.125 p.p.m., 42.42% rise above the control value. AST at all the concentrations had elevated activities 11.36–220.45% above that of the control value, 55.00 ± 14.00 IU/l. ALP activity was inhibited below the control value, 821.25 ± 84.401IU/l in the exposed fish with the highest inhibition, 40.46% (487.50 ± 264.751IU/l) occurring at 0.010 p.p.m. (Table 1). ALT activities in the kidney at all the exposure concentrations were elevated 33.33% and 66.67% above the control, 30.00 ± 11.55 IU/l at 0.005–0.010 p.p.m. and 0.0125–0.0150 p.p.m., respec-tively. Inhibition below the control was recorded in all the exposure concentrations for AST and ALP (Table 2). ALP activity was much higher than that of the ALT and ALT in the kidney, although general inhibition was recorded in all the exposure concen-trations.

ALT and AST activities in the liver of treated fish were inhibited below their respective control values (ALT, 182.50 ± 48.05IU/l; AST, 380.00 ± 150.00IU/l) with the highest inhibition, 68.42%, occurring at 0.012 p.p.m. for AST. ALP activity was inhibited at 0.0075 and 0.0150 p.p.m. (44.12 and 23.53%, respectively, below control value), but excited at the other concentrations with a peak, 33.83% at 0.010 p p.m. (Table 3). In the muscle, 12.68% and 23.94% elevation above the control (88.75 ± 21.36IU/l) were recorded at 0.0125 and 0.0150 p.p.m., respectively for ALT. However, decrease in activity was observed in the lower concentrations. The toxicant caused excitation of AST activity at 0.0050, 0.010 and 0.015 p.p.m., but inhibition at 0.0075 and 0.0125 p.p.m. relative to the control value, 362.50 ± 106.89IU/l (Table 4). There was general inhibition of ALP activity in the muscle of treated fish below the control, 56.25 ± 13.15 IU/l except at 0.010 p.p.m. (Table 4).

## Discussion

Normally, elevation of ALT and AST activities reflects hepatic disease because of its biological location. Elevation of both AST and ALT may also reflect some inflammatory disease or liver injury (Ayalogu *et al*., 2001). These enzymes which are generally found in the functional organs (liver, heart, gill and kidney) and muscle tissue, and always leak into the blood when there is cellular damage (Heath, 1991; Pari & Amali, 2005). The increase might be as a result of hepatocellular damage, a consequence of cypermethrin toxicity. During stress, fish need more energy to detoxify, biotransform and excrete the toxicants with the view of minimizing the toxic effects. This is achieved by the use of carbohydrate, the principal and immediate energy source during chronic stress (Umminger, 1977). The depletion of protein fraction in fish may be due to the degradation of carbohydrate. Thus, the interplay between the carbohydrate and protein synthesis (transamination) in this study might be due to degradation and possible utilization of the product for metabolic processes.

Biochemical changes fall within the secondary alterations that occur in fishes in

TABLE 1
*ALT, AST and ALP in the gill of* Heterobranchus bidorsalis *exposed to various concentrations of cypermethrin for 23 days*

| *Cypermethrin concentration (p.p.m.)* | *ALT (IU/l)* | *Percent control* | *AST (IU/l)* | *Percent control* | *ALP (IU/l)* | *Percent control* |
|---|---|---|---|---|---|---|
| 0.00 | 123.75 ± 14.36$^{bc}$ | 100.00 | 55.00 ± 40.00$^{c}$ | 100.00 | 821.25 ± 8.44$^{a}$ | 100.00 |
| 0.005 | 115.00 ± 11.55$^{c}$ | 92.93 | 67.50 ± 47.17$^{c}$ | 122.73 | 725.00 ± 24.09$^{a}$ | 88.28 |
| 0.0075 | 127.50 ± 6.58b$^{c}$ | 103.03 | 110.00 ± 0.00$^{bc}$ | 200.00 | 683.75 ± 38.08$^{a}$ | 83.33 |
| 0.010 | 120.00 ± 10.00$^{bc}$ | 96.98 | 61.25 ± 30.92$^{c}$ | 111.36 | 487.50 ± 26.47$^{a}$ | 59.36 |
| 0.0125 | 176.25 ± 25.00$^{a}$ | 142.42 | 232.50 ± 89.12$^{a}$ | 422.73 | 796.25 ± 26.57$^{a}$ | 96.96 |
| 0.0150 | 146.25 ± 33.26$^{b}$ | 118.18 | 176.25 ± 47.15$^{ab}$ | 320.45 | 666.25 ± 15.08$^{a}$ | 81.13 |

Means with the same superscript in the same column are not significantly different ($P < 0.05$).

TABLE 2
*ALT, AST and ALP in the kidney of* Heterobranchus bidorsalis *exposed to various concentrations of cypermethrin for 23 days*

| *Cypermethrin concentration (p.p.m.)* | *ALT (IU/l)* | *Percent control* | *AST (IU/l)* | *Percent control* | *ALP (IU/l)* | *Percent control* |
|---|---|---|---|---|---|---|
| 0.00 | 30.00 ± 11.55$^{a}$ | 100.00 | 367.50 ± 12.99$^{a}$ | 100.00 | 1197.50 ± 9.57$^{a}$ | 100.00 |
| 0.005 | 40.00 ± 16.33$^{a}$ | 133.33 | 342.50 ± 10.51$^{ab}$ | 93.20 | 1203.75 ± 6.29$^{a}$ | 100.52 |
| 0.0075 | 40.00 ± 16.33$^{a}$ | 133.33 | 183.75 ± 6.13$^{ab}$ | 50.00 | 1142.50 ± 9.56$^{a}$ | 95.41 |
| 0.010 | 40.00 ± 23.09$^{a}$ | 133.33 | 323.75 ± 16.42$^{ab}$ | 88.10 | 765.00 ± 53.49$^{b}$ | 63.13 |
| 0.0125 | 50.00 ± 11.55$^{a}$ | 166.67 | 273.75 ± 12.31$^{ab}$ | 74.49 | 1042.50 ± 15.84$^{b}$ | 87.06 |
| 0.0150 | 50.00 ± 20.00$^{a}$ | 166.67 | 167.50 ± 9.75$^{b}$ | 45.58 | 1196.25 ± 14.36$^{a}$ | 99.90 |

Means with the same superscript in the same column are not significantly different ($P < 0.05$)

TABLE 3
*ALT, AST and ALP in the liver of* Heterobranchus bidorsalis *exposed to various concentrations of cypermethrin for 23 days*

| *Cypermethrin concentration (p.p.m.)* | *ALT (IU/l)* | *Percent control* | *AST (IU/l)* | *Percent control* | *ALP (IU/l)* | *Percent control* |
|---|---|---|---|---|---|---|
| 0.00 | 182.50 ± 48.05$^{a}$ | 100.00 | 380.00 ± 15.00$^{a}$ | 100.00 | 85.00 ± 17.80$^{ab}$ | 100.00 |
| 0.005 | 153.75 ± 37.73$^{ab}$ | 84.25 | 152.50 ± 32.79$^{b}$ | 40.13 | 95.00 ± 4.71$^{ab}$ | 111.76 |
| 0.0075 | 130.00 ± 19.15$^{b}$ | 71.23 | 282.50 ± 14.18$^{ab}$ | 74.34 | 47.50 ± 2.89$^{b}$ | 55.88 |
| 0.010 | 165.00 ± 29.15$^{ab}$ | 90.41 | 277.50 ± 13.90$^{ab}$ | 73.03 | 113.75 ± 6.78$^{a}$ | 133.82 |
| 0.0125 | 136.25 ± 7.50$^{ab}$ | 74.66 | 120.00 ± 6.03$^{b}$ | 31.58 | 97.50 ± 2.90$^{ab}$ | 114.71 |
| 0.0150 | 135.00 ± 27.39$^{ab}$ | 73.97 | 267.50 ± 14.12$^{ab}$ | 70.39 | 65.00 ± 2.80$^{ab}$ | 76.47 |

Means with the same superscript in the same column are not significantly different ($P < 0.05$)

TABLE 4
*ALT, AST and ALP in the muscle of* Heterobranchus bidorsalis *exposed to various concentrations of cypermethrin for 23 days*

| *Cypermethrin concentration (p.p.m.)* | *ALT (IU/l)* | *Percent control* | *AST (IU/l)* | *Percent control* | *ALP (IU/l)* | *Percent control* |
|---|---|---|---|---|---|---|
| 0.00 | 88.75 ± 21.36[a] | 100.00 | 362.50 ± 10.68[c] | 100.00 | 56.25 ± 13.15[c] | 100.00 |
| 0.005 | 78.75 ± 12.50[b] | 88.73 | 377.50 ± 9.28[c] | 104.14 | 31.25 ± 6.29[d] | 55.56 |
| 0.0075 | 82.50 ± 30.69[a] | 92.96 | 275.00 ± 17.16 | 75.86 | 36.25 ± 9.46[d] | 64.44 |
| 0.010 | 88.75 ± 7.50[a] | 100.00 | 390.00 ± 8.75[c] | 107.59 | 67.50 ± 6.61[c] | 120.00 |
| 0.0125 | 100.00 ± 10.00[a] | 112.68 | 277.50 ± 12.43[d] | 76.55 | 36.25 ± 8.54[d] | 64.44 |
| 0.0150 | 110.00 ± 19.15[c] | 123.94 | 398.75 ± 11.25[c] | 110.00 | 32.50 ± 2.22[d] | 57.78 |

Means with the same superscript in the same column are not significantly different ($P < 0.05$)

an attempt to maintain equilibrium in the presence of environmental stressors (Wedemeyer & McLeay, 1981). It is also common knowledge that contaminants/pollutants perturb the internal integrity of biochemical/physiological process in fish (Gabriel & George, 2005). Both elevation and depression of enzyme activities were recorded in the experimental fish due to cypermethrin in toxicity. However, increase in the transaminases in the various tissues depicts effective or efficient utilization of amino acids for metabolic processes and also an indication of the augmentation for stress (Tiwari & Singh, 2004). The increase in AST and ALT activities is to gain more energy so as to tolerate the stress condition due to higher demand for carbohydrate and its precursors to keep both the glycolytic pathway and TCA cycles at sustained levels to cope with the energy demands during the toxicant-induced stress (Tiwari & Singh, 2004).

An increase in AST and ALT indicates tissue damage (Ayalogu *et al*., 2001) and effective transamination (the principal pathway for the synthesis and deamination of amino acids), thereby, allowing or catalyzing the inter-conversion of strategic compound like a-ketoglutarate and alanine to pyruvic and glutamic acid, which are the link between carbohydrate and protein metabolism (Salah El-Deen & Rogers, 1993; Knox & Greengard, 1965). AST and ALT, in conjunction with LDH, have been found to be involved in gluconeogenesis from amino acids, and the effects of changes in the activities of the transaminases (Suseela *et al*., 2007; Rashatwar & Ilyas, 1983). Increase in the transaminases is an immune mechanism, which occurs at the initial stages of diseased condition (Chang *et al*., 2005).

On the other hand, the decrease of AST and ALT in some of the concentrations may be a repressive mode to counter the effect of cypermethrin on the fish. Decrease of the activities of AST, ALT and ALP is a form of protection offered to protect the structural integrity of hepatocellular membrane (Pari & Amali, 2005). It also suggests that there were no damage to the parenchymatous tissues or skeletal muscles (Luskova *et al*., 2002) and

that the permeability and integrity of cell membranes were intact. The excitation and inhibition recorded in the organs and muscle tissue indicate the concentration-dependent enzymatic res-ponses of the enzymes in the target tissues of the experimental fish under sub-lethal cypermethrin toxicity.

The phosphatases (ALP and ACP) are important biomarkers because they are involved in adaptive cellular response to the potential cytotoxicity and genotoxicity of pollutants (Lohner *et al*., 2001). The increase in some of the concentrations may be as a result of liver damage or arrested bone growth (Ayalogu *et al*., 2001; Mayne, 2002). According to Vorbrodt (1959), phosphatase plays an important role in the transport of metabolites across membranes. The rate of transport appeared to be more pronounced in the gills and kidney in comparison to the liver, considering the level of ALP activity in these organs. This may be due to the strategic roles these organs play in the management of toxicants and their metabolic wastes.

ALP is a microsomal enzyme, which is involved in membrane transport because of its high concentration in vertebrate kidney and its action on a number of phospho-monoesters of organic materials such as glucose (Edquist *et al*., 1992). Decline in ALP activity may result from fall in the rate of synthesis of glycogen caused by lowered metabolic demands and electrolytic imbalance due to tissue overhydration (Shaffi, 1979). Decrease in ALP may reflect a change in endoplasmic mass known to occur in the cell membrane (Edquist *et al*., 1992), since it also function in the conversion of energy compounds NADP to NAD (Morton, 1955). Therefore, declined ALP activity could result in biosynthetic shifts and energy metabolism pathway of the exposed organism (Ovuru & Mgbere, 2000). Results from the present work indicate this may happen in wild fish exposed to cypermethrin.

The enzymatic responses to sub-lethal cypermethrin toxicity in *Heterobranchus bidorsalis* seem to be concentration-dependent and their usefulness as biomarkers appeared to be related to the organ studied.

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