Corpuscles of Stannius of a teleost \textit{Heteropneustes fossilis} following intoxication with a pyrethroid (cypermethrin)

Diwakar MISHRA$^1$, Sarojni TRIPATHI$^1$, Sunil K. SRIVASTAV$^1$, Nobuo SUZUKI$^2$ and Ajai K. SRIVASTAV$^1*$

$^1$. Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur 273009, India.
$^2$. Noto Marine Laboratory, Institute of Nature and Environmental Technology, Kanazawa University, Ogi, Noto-cho, Ishikawa 927-0553, Japan
$^*$Corresponding author: Ajai K. Srivastav, e-mail: ajaiksrivastav@hotmail.com

**Abstract.** Fresh water fish, \textit{Heteropneustes fossilis} were subjected to 5.76 $\mu$g/L (0.8 of 96 h 50% lethal concentration, LC50) and 1.44 $\mu$g/L (0.2 of 96 h LC50) solution of cypermethrin for short-term (96 h) and long-term (28 days), respectively. After short-term cypermethrin exposure, the plasma calcium levels of \textit{Heteropneustes fossilis} showed a decrease which persisted for 96 h. In long-term exposed fish, following 7 days exposure to cypermethrin there was a decrease in the plasma calcium level. This decrease persisted progressively up to 28 days. Up to 72 h following cypermethrin exposure there was no change in the AF-positive and AF-negative cells of CS. Increased granulation in the AF-positive cells of CS was recorded following 96 h exposure to cypermethrin. The nuclear volume of AF-positive cells remained unaffected. The AF-negative cells of CS had increased nuclear volume only at 96 h cypermethrin exposure. In cypermethrin-treated fish the CS remained unaffected up to 7 days. The nuclear volume of AF-positive cells decreased at 14 days. Increased granulation in the AF-positive cells was noticed following 21 days of exposure to cypermethrin. The nuclear volume of these cells further decreased. These changes were exaggerated and few degenerating cells were encountered after 28 days. No change was observed in the AF-negative cells of CS until 14 days of cypermethrin exposure. These cells exhibited an increase in their nuclear volume after 21 and 28 days treatment.

**Keywords:** cypermethrin; pyrethroid; corpuscles of Stannius; fish; \textit{Heteropneustes fossilis}; plasma calcium

**Introduction**

Pyrethroid insecticides are used preferably over organochlorine and organophosphates due to their potent insecticidal properties and are practically non-toxic to most non-target animals, especially mammals (Haya 1989). Pyrethroids have a short-life in most animals as they are readily metabolized. Fish are an exception, since they seem to be deficient in the enzyme system that hydrolyses pyrethroid (Haya 1989). Due to their lipophilicity, pyrethroids have a high rate of gill absorption, which in turn would be a contributing factor in the sensitivity of the fish to aqueous pyrethroid exposures. The toxicity of pyrethroids for the fish has been assessed by several investigators (Mishra et al. 2002, Begum 2007, Thomas et al. 2008, El-Sayed & Saad 2008).

Cypermethrin (CY), a synthetic pyrethroid insecticide has contact and stomach action properties which are extremely toxic to fish. CY is metabolized and eliminated more slowly by rainbow trout than by birds and mammals (Bradbury & Coats 1989) which may explain this compound’s higher toxicity in fish than in other vertebrates. It is a unique insecticide used mainly as residual treatment for the control of flies, mosquitoes and cockroaches.

Formerly corpuscles of Stannius (CS) were
considered to be unique to teleostean and holo-
stean fishes. Recently, stanniocalcin (the hypo-
calcemic hormone secreted by CS) has been
immunocytochemically identified in the kid-
ney, ovary, pancreas (alpha cells) and bladder
of human and rat (Olsen et al.1996, de Niu et
al. 1998, Moore et al. 1999, Worthington et al.
2008). Different cell types have been noticed
within the CS of few fish species on the basis of
the staining affinity of their cytoplasm. These
cell types were considered as two ultrastruc-
turally distinct cell types and considered as
type-1 (AF-positive) and type-2 cells (AF-nega-
tive) (Wendelaar Bonga & Pang 1991, Singh &
Srivastav 1996).

There is extensive literature on the impact
of environmental toxicants on fish e.g. beha-
vioural and toxicological responses, disturb-
ances in carbohydrate metabolism, hematolo-
gical anomalies and histopathology of vital
organs (Soengas et al. 1997, Begum &
Vijayaraghavan 1999, Poleksic & Karan 1999,
Moore & Waring 2001, Saha & Kaviraj 2003,
2009, Marigoudar et al. 2009, Sarikaya 2009,
Solati et al. 2010, Suvetha et al. 2010); but very
little information is available regarding the
effect of these toxicants on the endocrine regu-
lation of calcium homeostasis in fish. Spe-
cifically, a single paper by Srivastav et al.
(2009) investigated the effect of toxicants on the
corpuscles of Stannius of teleosts. This study
investigated the toxic effect of a pyrethroid –
cypermethrin (trade name “Basathrin”) on
plasma calcium levels and the histological
changes in the corpuscles of Stannius of a
freshwater catfish Heteropneustes fossilis.

Materials and Methods

Freshwater catfish, Heteropneustes fossilis (both sexes;
body wt 37-42 g) were procured and acclimatized for two
weeks in plastic pools under laboratory conditions
(natural photoperiod – 11.28-12.06 and temperature 26.72 ±
2.14°C). The physicochemical conditions of water have
been reported by Mishra et al. (2002). The fish were fed
daily 2-3 times with wheat flour pellets and ground-dried
shrimps.

After acclimation, the experiments were performed
for short-term and long-term duration. The fish were
subjected to 5.76 μg/L (0.8 of 96 h LC50) and 1.44 μg/L
(0.2 of 96 h LC50) solution of cypermethrin (96 h LC50
value for cypermethrin for the fish H. fossilis is 7.20 μg/L;
Mishra et al. 2002) for short-term (96 h) and long-term (28
days), respectively. A control group was also run
concurrently. The media (both control and experimental)
were renewed every 24 h. Food was withheld 24 h prior
to the start of the experiment and during the experiment.
The fish were sacrificed after 24, 48, 72 and 96 h in the
short-term experiment and after 7, 14, 21 and 28 days in
the long-term experiment. Blood was collected in
heparinized tubes after sectioning the caudal peduncle
on these intervals and plasma calcium levels were
determined using a Sigma kit (#587 A). After collection of
the blood samples, the corpuscles of Stannius were fixed
in aqueous Bouin’s fluid. Fixed tissues were routinely
processed in graded series of alcohol, cleared in xylene
and then embedded in paraffin. Serial sections were cut
at 6 μm and stained with hematoxylin-eosin and
aldehyde fuchsin (AF).

The nuclear indices (maximal length and maximal
width) of corpuscles of Stannius were determined (fifty
nuclei were measured per specimen, thus 300 nuclei were
measured from six specimens) with the aid of an ocular
micrometer and then the nuclear volume was calculated
as: volume = 4/3 π ab2; where ‘a’ is the major semiaxis
and ‘b’ is the minor semiaxis.

Student’s t test was used to analyze the statistical
significance between the control and cypermethrin-
treated fish.

Results

After short-term cypermethrin exposure, the
plasma calcium levels of Heteropneustes fossilis
exhibited no change at 24 h. The levels
indicated a decrease at 48 h. This response
persisted until the end of experiment (96 h)
(Fig. 1). In the chronically exposed fish there
was a decrease in the plasma calcium level 7
days after exposure to cypermethrin. This
decrease persisted progressively up to 28 days
(Fig. 2).

In control fish, two cell types – AF-positive
and AF-negative have been noticed after
aldehyde fuchsni staining (Fig. 3). Up to 72 h
following cypermethrin exposure there was no
Corpuscles of Stannius of a teleost *H. fossilis* following intoxication with cypermethrin

In cypermethrin-treated fish the CS remained unaffected up to 7 days. The nuclear volume of AF-positive cells decreased at 14 days (Fig. 2). Increased granulation in the AF-positive cells was observed following 21 days exposure to cypermethrin (Fig. 5). The nuclear volume of these cells further decreased (Fig. 2). These changes were exaggerated and few de-

change in the AF-positive and AF-negative cells of CS. Increased granulation in the AF-positive cells of CS was noticed following exposure to cypermethrin for 96 h (Fig. 4). The nuclear volume of AF-positive cells was unaffected (Fig. 1). The AF-negative cells of CS increased in nuclear volume only after 96 h exposure to cypermethrin (Fig. 1).

Figure 1. Plasma calcium levels (mg/100ml) and nuclear volume of AF-positive and AF-negative cells (μm³) of short term cypermethrin treated *Heteropneustes fossilis*. Values represent mean ± S.E. of six specimens. Asterisk indicates significant differences (P<0.05) from control.

Figure 2. Plasma calcium levels (mg/100ml) and nuclear volume of AF-positive and AF-negative cells (μm³) of long term cypermethrin treated *Heteropneustes fossilis*. Values area mean ± S.E. of six specimens. Asterisk indicates significant differences (P<0.05) from control.
Figure 3. Corpuscles of Stannius of control fish exhibiting AF-positive and AF-negative cells. AF x 200.

Figure 4. Corpuscles of Stannius of 96 hr cypermethrin treated *H. fossilis* exhibiting increased granulation in AF-positive cells. AF x 200.

Figure 5. Corpuscles of Stannius of 21-day cypermethrin treated *H. fossilis* exhibiting increased granulation in AF-positive cells. AF x 200.

Figure 6. Corpuscles of Stannius of 28-day cypermethrin treated *H. fossilis* exhibiting degenerating cells. AF x 200.
generating cells were encountered (Fig. 6) after 28 days. No change was observed in the AF-negative cells of CS until 14 days of cypermethrin exposure. These cells exhibited an increase in their nuclear volume after 21 and 28 days treatment (Fig. 2).

Discussion

Accumulation of granules and decreased nuclear volume of AF-positive cells have been noticed in the corpuscles of Stannius of cypermethrin exposed fish. Similar responses of the CS have been reported by Srivastav et al. (2009) after exposure of the fish to the deltamethrin (a pyrethroid). AF-positive cells (type 1-cells) of the CS secrete Stanniocalcin (a hypocalcemic hormone) which regulates branchial calcium uptake in the fish (Meats et al. 1978, Wendelaar Bonga 1980, Srivastav et al. 1989, Singh 1990, Tiwari 1993). The increased granulation in the AF-positive cells observed in the present study can be attributed to the prolonged hypocalcemia caused by cypermethrin exposure. Singh (1990) and Tiwari (1993) have reported accumulation of AF-positive granules in CS of fishes in response to experimentally induced hypocalcemia provoked by maintaining them in acacic freshwater. Accumulation of secretory granules among calcitonin cells (responsible for the secretion of a hypocalcemic factor) of mammals has also been noticed in response to hypocalcemia (Gittes et al. 1968, Leitz & Donath 1970, Biddulph & Metbenco 1972, Swarup et al. 1980). The accumulation of granules may possibly be due to the inhibition of release of granules and/or continuation of its biosynthesis.

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References


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