CHAPTER III

NEUROSECRETION
INTRODUCTION

Pollutants are known to affect the normal environmental as well as the physiological processes of the animal. The failure of physiological adaptations with the altered environment can lead to death of the animals. It is a well established fact that the neuroendocrine centres are known to regulate the physiological functions of the animal. Pollutant of any nature can act directly or indirectly, bringing out the physiological and behavioural alterations endogenously. Pollutant may manifest its impacts directly on the different physiological processes or it may act on the neuroendocrine centres, which govern the physiological processes. The ability of an organism to react to the environmental signals essentially involves three integrated units of afferent pathways, integrative centres and efferent pathways (Scharrer, 1965).

Neurosecretory cells which are located throughout the nervous system of Crustacea produce physiologically active substances. The neurosecretory system is specifically designed for the production of neurohormonal
materials or neurotransmitters like acetylcholine and adrenaline or nonadrenaline and is an essential concommitant of the impulse transmission, which is of millisecond duration and travels across a distance of several milliseconds. Bern and Hagedorn (1965) and Cabe (1966) have provided excellent reviews of neurosecretion in Crustacea. Nagabhushanam and Vasantha (1972) and Nagabhushanam et al. (1980) made a detailed study of the neurosecretory system in Caridina weberi and demonstrated that a number of physiological processes are under the endocrine control in decapod crustaceans.

Highnam (1965) illustrated the relationship which exists in all neurosecretory cells between release, transport and synthesis, when all the three parts are in balance, the amount of hormone released will equal the amount being synthesized and may vary over a wide range, limited only by the physical and chemical dynamics of the specific cell. If the release from perikaryon into the axon, or rate of transport down the axon, or release from the axon terminal to the vascular system is reduced, then the possibility of storage in the entire system, or proximal to the point of stoppage becomes real.
The neurosecretory cells have been shown to be present in several decapod crustaceans (Enami, 1951, Deshmukh and Rangnekar, 1965; Nagabhushanam and RangaRao, 1966; Nagabhushanam et al., 1972, 1980). Morphologically these neurosecretory cells have been solely classified on the basis of their size and staining properties. Diwan (1971) has studied the neurosecretory system of the freshwater crab, *Barytelphusa cunicularia* and classified neurosecretory cells present in the brain and thoracic ganglion as cell type A, B and C on the basis of size and staining properties.

Odum et al. (1969) observed that DDT exposure results in maladaptive behaviour which is caused from abnormal neurophysiological functions in the fiddler crab, *Uca pugilator*. Schneider (1975) proposed a mechanism for DDT, inhibition of Na, K-ATPase in which the pollutant decreases its fluidity and thus affect the ion activated allosteric transitions required for enzyme activity. Electrophysiological evidence shows that DDT affects Na$^+$ and K$^+$ inactivation and Ca$^{2+}$ regulatory mechanism in axons (Narahashi and Haas, 1967; Matsumura and Narahashi, 1971).
Gundevia (1972) was the first to observe various histopathological changes in neurosecretory cells and retrocerebral endocrine complex of *Hydromedusa* olivaceous after treatment with dimecron, diazinon and dieldrex. Meagre work has been carried out on the crustacean neurosecretory cytopathological changes in relation to pollutants. Doherty and Matsumura (1975) have observed the effect of DDT on ATPase system and the functions of nerve cells in peripheral nervous system of the lobster, *Homarus americanus*. Nimmo et al. (1979) have studied the effect of diflubenzuron on an estuarine crustacean, *Mysidopsis bahia*. Nagabhushanam et al. (1979) described the cytological evidence that Aroclor 1242 produces an increase in the quantity of neurosecretory material in the medulla terminalis X-organ of the fiddler crab, *Uca pugilator* which indicates that the Aroclor inhibits release, but not synthesis of neurohormones. Bodkhe (1983) in freshwater crab, *Barytelphusa cunicularis* and Manikumar (1986) in marine prawn, *Penaeus merguiensis* observed the cytological changes in the neurosecretory cells in relation to pesticides. The role of neurosecretory hormones in relation to pesticides was worked in freshwater prawn, *Macrobrachium lamerii*.
(Gyananath, 1982; Mary, 1984) and Macrobrachium kistnensis (Mirajkar and Sarojini, 1985). Gangshettiwar (1986) also observed the cytological changes in the neurosecretory cells in the freshwater prawn, M. lamerrii after exposure to phenol. Recently Subhash (1988) noticed the cytological alterations in brain and thoracic ganglion of the marine crab, Scylla serrata after benzene treatment.

The present investigation was designed to determine the effect of benzene on cytology of neurosecretory cells (NSC) of brain and thoracic ganglion of the freshwater prawn, Macrobrachium lamerrii.
MATERIAL AND METHODS

The freshwater prawns, *Macrobrachium lamerrii* were collected from Kham river near Aurangabad and acclimatized to laboratory conditions for 3-4 days in aquaria with aeration. During acclimatization, water was changed daily and they were fed with algae and starved one day before the experiment.

Adult intermoult (stage C) laboratory acclimatized prawns were selected for this study. The prawns were divided into nine groups, the first group served as base control and remaining eight groups were exposed separately to 1.09, 1.0, 0.95, and 0.90 ppm of benzene for 24, 48, 72 and 96 hours and 0.1 ppm (1/10 of 48 hrs. LC50) for 7, 14, 21 and 30 days respectively. The water media was changed daily and requisite amount of benzene was added. The prawns were not fed during the course of experiment but those exposed to chronic treatment were fed twice a week. At the end of the exposure period the experimental and control prawns were sacrificed.
The tissues i.e. brain and thoracic ganglion of the prawns were removed and fixed in Bouin's fixative. The fixed tissues were dehydrated in graded series of alcohol, embedded in paraffin wax and sections were cut 4-5 μ and stained with Chrome Alum Haematoxylin Phloxin (CHP). The intensity of neurosecretory material in cell perikarya and in neurohaemal area was measured by visual arbitrary scale as follows:

0-1 : No neurosecretory material;
2-3 : Moderate;
4-8 : Accumulation;
5 : Heavy accumulation
OBSERVATIONS AND RESULTS

The neurosecretory cells of the brain and thoracic ganglion of *Macrobrachium lamerrii* showed marked changes in their structure and secretory activity after benzene treatment.

**Effect of lethal and sublethal concentration of benzene on brain neurosecretory cells of prawn, M. lamerrii**

**Neurosecretory cells in brain :**

**Control :**

Histological observations indicate the presence of four types of neurosecretory cells (viz., A, B, C and D) in the central nervous system depending upon the size, shape and stainable secretory material in the cell perikaryon (Fig. 1).

**'A' cells :**

These cells are oval or round in shape with or without axons. Nucleus is round with 2-3 nucleoli. Vacuoles are present in the cytoplasm. Neurosecretory material intensity is 3.5.
Effect of benzene on the brain neurosecretory cells of freshwater prawn, *M. lamarrhii*

**Fig. 1:** T.S. of brain of control freshwater prawn, *M. lamarrhii* showing different types of neurosecretory cells. Chrome alum haematoxylin Phloxin (CHP) X 150.

**Fig. 2:** T.S. of brain of freshwater prawn, *M. lamarrhii* exposed to 1.09 ppm of benzene for 24 hrs. CHP X 250. 
**Note:** Increase in the synthesis of neurosecretory material

**Fig. 3:** T.S. of brain of freshwater prawn, *M. lamarrhii* exposed to 1.0 ppm of benzene for 48 hours. CHP X 250. 
**Note:** Damage to cell wall.

A - 'A' type cell  
B - 'B' type cell  
C - 'C' type cell  
D - 'D' type cell  
NP - Neuroplile area  
NSM - Neurosecretory material
'B' cells:

B-cells are oval and medium in size. The nucleus is prominent and cells are similar to A type cells showing distinct staining characteristics. Neurosecretory material intensity is 3.0.

'C' cells:

These cells are round, nucleus of the cells is comparatively large and cytoplasm scanty. Neurosecretory material intensity is 2.5.

'D' cells:

D-cells are smallest of all the cell types and occur in groups. Neurosecretory material intensity is 1.5.

Effect of lethal concentration of benzene on brain neurosecretory cells of freshwater, *M. lamerrii*

On exposure to 1.09 ppm of benzene for 24 hours there was an increased synthesis of neurosecretory material. A change in cell shape and vacuolisation in nucleus was observed. The intensity of neurosecretory material of cells was 5 (Fig. 2).
48 hours exposure to 1.0 ppm of benzene caused irregular distribution of neurosecretory material, changes in cell shape and undulated cell wall. The intensity of neurosecretory material of cells was 4 (Fig. 3). 72 hours exposure to 0.95 ppm of benzene caused depletion of neurosecretory material in cells. Irregular shape of cells and nuclear material in the nucleus become clumped. The intensity of neurosecretory material of A-cells was 3 and B and C cells was 2.5 (Fig. 4). 96 hours exposure to 0.90 ppm of benzene caused disintegration of cell wall and nuclear wall. The B cells were highly damaged. A cells were showing undulated cell wall, pycnotic nuclei and vacuolisation in cell perikaryon. The intensity of neurosecretory material of cells was 2.5 (Fig. 5).

Effect of sublethal concentration (0.1 ppm) of benzene on brain neurosecretory cells of freshwater prawn, *M. lamerrill*:

After 7 days exposure, the neurosecretory material took dark stain as they were loaded with neurosecretory material. The cell wall was intact but vacuolisation in nucleus and clumped nuclear
Fig. 4: T.S. of brain of freshwater prawn, *M. lamertii* exposed to 0.95 ppm of benzene for 72 hours.
CHP X 250.
Note: Clumped nuclear material.

Fig. 5: T.S. of brain of freshwater prawn, *M. lamertii* exposed to 0.90 ppm of benzene for 96 hours.
CHP X 250.
Note: Pyknotic nuclei, vacuolisation in cell perikaryon and degeneration in the neuropile area.

Fig. 6: T.S. of brain of freshwater prawn, *M. lamertii* exposed to 0.1 ppm of benzene for 7 days.
CHP X 250.
Note: Clumped nuclear material and vacuolisation in nucleus.

A : 'A' type cell
B : 'B' type cell
C : 'C' type cell
NP : Neuropile area
NSM : Neurosecretory material
NSC : Neurosecretory cells
material was noticed. The intensity of A, B cells was 5 and C-cells was 4.5 (Fig. 6). 14 days exposure caused shrinkage in neurosecretory cells, vacuolisation in nucleus and undulated cell wall due to shrinkage of cell. The intensity of neurosecretory material of cells was 3 (Fig. 7). After 21 days exposure, depletion in neurosecretory material synthesis, shrinkage of cytoplasm from perikaryon, disintegration of cell and nuclei and more damage in neuropile area was noticed. The intensity of neurosecretory material of cells was 2.5 (Fig. 8). At the end of 30 days exposure, extensive damage to neurosecretory cells and degeneration of neuropile area was observed. The intensity of neurosecretory material of cells was 2 (Fig. 9).

Effect of lethal and sublethal concentration of benzene on thoracic ganglion neurosecretory cells of freshwater prawn, M. lamerrii:

Neurosecretory cells in thoracic ganglion:

Control:

Four A, B, C and D types of neurosecretory cells as in brain were observed in thoracic ganglion (Fig. 10).
Fig. 7: T.S. of brain of freshwater prawn, M. lamerrii exposed to 0.1 ppm of benzene for 14 days.
CHP X 250.
Note: Shrinkage in neurosecretory cells

Fig. 8: T.S. of brain of freshwater prawn, M. lamerrii exposed to 0.1 ppm of benzene for 21 days.
CHP X 250.
Note: Disintegration of cell and nuclei as well as depletion in neurosecretory material.

Fig. 9: T.S. of brain of freshwater prawn, M. lamerrii exposed to 0.1 ppm of benzene for 30 days.
CHP X 250.
Note: Damage to neurosecretory cells. Degeneration of neuropile area.

B : 'B' type cell
C : 'C' type cell
NP : Neuropile area
NSM : Neurosecretory material
NSC : Neurosecretory cells
**A cells:**

A cells are larger in size. The intensity of neurosecretory material of cell was 2.5.

**B cells:**

B cells are medium in size. The intensity of neurosecretory material of cell was 2.5.

**C cells:**

These type of cells are round. The intensity of neurosecretory material of cell was 2.0.

**D cells:**

D - cells are smallest in size. The intensity of neurosecretory material of cells was 1.5.

**Effect of lethal concentration of benzene on thoracic ganglion neurosecretory cells of freshwater prawn, M. lamertii:**

On exposure to 1.09 ppm of benzene for 24 hours increased neurosecretory material accumulation in A and B cells was observed. The intensity of neurosecretory material of A, B cells was 5 and C cells was 4 (Fig. 11). 48 hours exposure to 1.0 ppm of benzene caused shrinkage in neurosecretory cells.
Effect of benzene on the thoracic ganglion neurosecretory cells of freshwater prawn, *M. lamereii*.

Fig. 10 : T.S. of control thoracic ganglion of freshwater prawn, *M. lamereii* showing different types of neurosecretory cells.
CHP X 250.

Fig. 11 : T.S. of thoracic ganglion of freshwater prawn, *M. lamereii* exposed to 1.09 ppm of benzene for 24 hours.
CHP X 250.
Note: Enhanced synthesis of neurosecretory material.

Fig. 12 : T.S. of thoracic ganglion of freshwater prawn, *M. lamereii* exposed to 1.0 ppm of benzene for 48 hours.
CHP X 250.
Note: Shrinkage in neurosecretory cells
A : 'A' type cell
B : 'B' type cell
C : 'C' type cell
D : 'D' type cell
NP : Neuropile area
NSM : Neurosecretory material
Undulated cell wall due to increased rate of transport of neurosecretory material. Highly affected smaller C cells was noticed. The intensity of neurosecretory material of cells was 3 (Fig. 12). After 72 hours exposure to 0.95 ppm of benzene, irregular distribution of neurosecretory material, degeneration of neuropile area, vacuolisation in neurosecretory cells and depleted neurosecretory activity was observed. The intensity of neurosecretory material of cells was 2 (Fig. 13). 96 hours exposure brought about extensive damage to neurosecretory cells and neuropile area. The neurosecretory material intensity of cells was 1.5 (Fig. 14).

Effect of sublethal concentrations (0.1 ppm) of benzene on thoracic ganglion neurosecretory cells of freshwater prawn, M. lamereii.

After 7 days exposure, darkly stained neurosecretory material, change in cell shape and undulated cell wall was noticed. The intensity of A cell was 5 and B, C cell was 4 (Fig. 15).
Fig. 13: T.S. of thoracic ganglion of freshwater prawn, *M. lamerrii* exposed to 0.95 ppm of benzene for 72 hours.
CHP X 250.
Note: Degeneration of neuropile area.
Vacuolisation in the neurosecretory cells.

Fig. 14: T.S. of thoracic ganglion of freshwater prawn *M. lamerrii* exposed to 0.90 ppm of benzene for 96 hours.
CHP X 250.
Note: Extensive damage to neurosecretory cells.

Fig. 15: T.S. of thoracic ganglion of freshwater prawn, *M. lamerrii* exposed to 0.1 ppm of benzene for 7 days.
CHP X 250.
Note: Darkly stained neurosecretory material in A cell

A : 'A' type cell
B : 'B' type cell
C : 'C' type cell
NP : Neuropile area
NSM : Neurosecretory material
After 14 days exposure, neurosecretory cells showed disintegration and cells took faint stain due to depletion of neurosecretory material. The intensity of neurosecretory material of cells was 2.5 (Fig. 16). 21 days exposure caused disintegration of neurosecretory cells. The intensity of neurosecretory material of cells was 2 (Fig. 17). 30 days exposure brought about extensive damage to neuropile area and small cells are badly affected. The intensity of neurosecretory material of cells was 1.5 (Fig. 18).
Fig. 16: T.S. of thoracic ganglion of freshwater prawn, *M. lamerrii* exposed to 0.1 ppm of benzene for 14 days.
CHP X 250.
Note: Depletion in neurosecretory material.

Fig. 17: T.S. of thoracic ganglion of freshwater prawn, *M. lamerrii* exposed to 0.1 ppm of benzene for 21 days.
CHPX 250.
Note: Disintegration of neurosecretory cells.

Fig. 18: T.S. of thoracic ganglion of freshwater prawn, *M. lamerrii* exposed to 0.1 ppm of benzene for 30 days.
CHP X 250.
Note: Extensive damage to neuropile area and small cells are badly affected.

B: 'B' type cell
C: 'C' type cell
D: 'D' type cell
NP: Neuropile area
DISCUSSION

In the present study, it was observed that benzene altered the histological architecture of neurosecretory cells of brain, thoracic ganglion and in *Macrobrachium lamerrii*. Initial exposure showed an increase in synthesis of neurosecretory material and prolonged exposure caused decrease in synthetic activity and damage to cells. In acute and chronic treatment the general changes observed were loss in compactness of cells, undulation of cell wall, irregular distribution of neurosecretory material, clumping of chromatin material, pycnosis, depletion in nuclear material, vacuolisation and shrinkage of cells. Nanda (1974) poisoned cockroach, *Periplaneta americana* with endrin and sumithion, and observed dramatic impairment in both inter and intra-cellular structural features of neurosecretory cells, accompanied by heavy vacuolisation within perikarya. Similar changes were observed by Hanumante et al. (1979) with molluscicide in the snail *Indoplanorbis exustus*, neurosecretory cell cytology. In the crab, *Uca pugilator* (Nagabhushanam et al., 1979) medulla terminalis X-organ neurosecretory material quantity is increased after exposure to
the pollutant Aroclor 1242 and PCB. In the light of the above findings, Hanumante et al. (1979) concluded that there is no uniformity in the responses of the neurosecretory cells of invertebrates to the pollutants and the nature of their response is probably linked to the chemical structure of the pollutant. The impact of pollutants on neurosecretory cell morphology has been studied in a few cases (Gundevi, 1972 and Nanda, 1974).

Sabesan and Ramalingam (1979) observed in Odontopus varieornis after treatment with the endosulfan, a stimulation of neurosecretory material synthesis in the early phase of treatment which diminished in the later stage. They also reported the vacuolisation and shrinkage in the neurosecretory cells.

Acute and chronic exposure of M. lamerrii to benzene caused enhanced synthetic activity of brain and thoracic ganglion neurosecretory cells as well as release of neurohormones to counteract the stress condition but during subsequent exposure period the synthetic activity appeared to be decreased which is evident from the decreased
neurosecretory material intensity. This might be due to intra nuclear changes and so crabs could not withstand the prolonged exposure to benzene. The vacuolisation increased with the increase in incubation period and at times it become so much that the entire cell perikarya gave a foamy appearance. The undulation of cell wall is due to vigorous release of secretory material. The loss in compactness of neurosecretory cells and decrease in number is perhaps due to necrosis of neuropilar tissue and degeneration of cells respectively. Richards and Cutkomp (1945) though did not observe any structural changes in the nerve cell of Periplaneta americana after DDT treatment, yet concluded that nerve cell in general underwent vacuolisation due to action of toxins and prolonged action of insecticides caused chromatolysis.

Cours et al. (1953) in Pedicular nuncus, observed striking hyperchromatolysis in the nerve cells after parathion and BHC treatment. The brain neurosecretory cells of M. lamerrui after pesticidal treatments showed peripheral
and central vacuolisation, loss in compactness and changes in cell shape. Prolonged incubation caused inhibition of synthesis thereby showing depletion of the neurosecretory material and decrease in number of neurosecretory cells (Gyananath, 1982). Reddy (1982) observed damage to the brain neurosecretory cells in the form of decrease in the amount of neurosecretory material and reduction in the number of neurosecretory cells in *Caridina weberi* after treatment with organophosphates. Sarojini and Mirajkar (1982) observed decrease in neurosecretory activity and degeneration of neurosecretory cells in the brain of *Macrobrachium kistnensis* after chronic exposure to dimecron. Bodkhe (1983) made similar observation in the freshwater crab, *Barytelphusa cunicularis* after treatment with carbamate, sevin.

Lethal concentration of organochlorines brought about accumulation and sublethal exposure depleted the neurosecretory material in the prawn, *M. lamerrii* (Mary, 1984). She also reported cytoarchitectural alterations such as vacuolisation in the cytoplasm, distortion of cell wall and changes in
the cell shape. Mirajkar (1984) noticed enhancement in synthetic activity of neurosecretory cells in the early phase of treatment but decline in synthesis and clumping of chromatin material, vacuolisation, undulation of cell wall, necrosis of neuropile during prolonged exposure of M. kistnensis to insecticides. Dimecron and endocel caused acute cellular degeneration, vacuolisation in the brain neurosecretory cells of Scylla serrata, and the thoracic ganglion cells showed shrinkage, vacuolisation in the cytoplasm and degeneration of cell wall (SambasivaRao, 1984).

Mirajkar and Sarojini (1985) observed during chronic exposure of M. kistnensis to endosulphan, the B cell of the brain was affected to a great extent than A cells showing clumping of chromatin material. Formation of vacuoles in cells as well as undulation and distortion of cell wall was noticed in A as well as B cells but the A cells showed many big vacuoles due to highly increased rate of transport. DeCaraman and Fingerman (1985) observed the changes in neurosecretory cells of the brain of fiddler crab, Uca pugilator on exposure to WSP of crude oil and concluded that the
accumulation of the neurosecretory substances of the cytoplasm of the A and B types of cells may be due to the exposure to the WSF of the crude oil on the neurosecretory cells of the brain. Manikumar (1986) in marine shrimp, Penaeus merguiensis after acute treatment to organophosphate for 24 hours observed changes in neurosecretory cells like, shrinkage, increased neurosecretory material intensity and irregular distribution of neurosecretory cells. 96 hours exposure brought about depletion of neurosecretory material, appearance of vacuoles, changes in cell shape, loss in compactness and undulation of cell wall. The changes observed after chronic exposure were damage to the neurosecretory cells of brain and thoracic ganglion and complete depletion of neurosecretory material. All these reports are in agreement with the observations of neurosecretory cells in M. lamertii on exposure to benzene.

The structural changes in the form of clumping and disappearance of nuclear material, vacuolisation in the cytoplasm and undulation of cell wall may have some correlation with the alterations in the nucleic acid constituent of neurosecretory elements.
(Ghosh et al., 1968). The nuclear material in the nucleii of neurosecretory cells treated with benzene become so immobilized after clumping that it is unable to act with other cellular constituents and it is possible that in this state the nucleii may not be able to produce optimal amount of RNA. The DNA content of such nucleii become quite diminished leading to a loss in the production of an optimal amount of RNA. In this way the inhibition of synthetic activity after prolonged incubation period may be assumed as failure of the RNA synthetic machinery which inhibits further synthesis of secretory material.