Materials and Methods
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The fish, *Heteropneustes fossilis* commonly called as 'Singhi' is taken for experimental project because they are easily available throughout the year, economic and has a high percentage of survivability in laboratory condition due to the presence of paired sac like pharyngeal lungs as accessory respiratory organs (Das 1927). Singhi is a bottom dweller fish and found in fresh water bodies of Sind, India, Ceylon, Burma and China. The fish is reported to be mixed feeder i.e. heteroomnivorous food habit. The fish was evolved in the Eosine epoch and still continued up to the recent period of the cenozoic era. The body of the fish is tappered uniformly in the horizontal plane from the gills to the caudal fin. The head is flattented and covered with a very thin skin and with transverse mouth opening. Eyes are provided with a free circular margin. Barbels are 8 in number, the maxillary barbel extends to the commencement of the ventral fin. Gills are covered with operculam. Teeths are present in the jaws and on the vomer and the vomer teeth are in a pyriform patch on either side, covering anteriorly and widely divergent posteriorly. The dorsal fin is with six rays situated under the dorsal fin. The anal fin is long and separated from the caudal by a notch. Pectoral spines are serrated intranally also usually with a few serration externally. The body is scaleless with two longitudinal yellowish band. The youngs are
occasionally reddish. The systematic position of the fish is:

Chordata

Craniata (Vertebrata)

Gnathostomata

Pisces

Ostcichthyes

Actinopterygii or Teleostomi

Cypriniformes

: **Phylum** (Dorsal tubulated nervechord).

: **Sub phylum** (Internal skeleton of cartilage or bone, Spinal chord forming main axis and composed of overlapping vertebrae nervous system dorsal to digestive tract, complex brain and red blood).

: **Division** (Presence of Paired jaws).

: **Super Class** (Paired and unpaired fins supported by fin rays).

: **Class** (skeleton consists of true bone only, one pair of gill opening).

: **Sub Class** (gill openings may be jointed as single ventral slit or may be two separate slit.

: **Order** (abdomen is not keeled, barbels may present or absent).
Saccobranchidae: Family (anal fin long, barbels are 8 in number, nasal barbels are present)

Heteropneustes fossilis: Type (7 branchiostegal, dorsal fin small and spinless, gill opening wide the membranes not being confluent with the skin of the isthmus and secreted by a deep notch).

The fish Heteropneustes fossilis (Singhi) were procured locally and reared in the laboratory. The healthy fishes of the size (appro. 13.882 ± 1.60 cm.) were separated out and kept in an aquarium for acclimatization in the laboratory condition. During this period the fishes were regularly fed with minced goat liver, earthworm and dry food (powder) and kept under constant observation. The water of the aquarium was changed on every alternate day so as to remove the debris and excretory products accumulated in the medium.

After one month of acclimatization the fishes were washed in 0.1% KMnO₄ and transferred into two aquaria (Size 60.5 cm. X 30.5 cm. X 29.5 cm.) of which one served as control, containing only the solvent, while the other contain pesticide in sublethal concentrations, dissolved in acetone.
(solvent). The experiment was conducted from 0 hour to 90 days for different observations and during this period regular feeding of fishes and renewal of water on every alternate day was done. The fishes were handled very carefully, so as to offer minimum stress during the period of experimentation. The temperature and pH of water was maintained at 28.81 ± 1.73° and 6.77 ± 0.07 respectively throughout the acclimatization and experimental period.

For the experimental purpose the pesticide, Benzene hexachloride (BHC) was chosen. BHC, the most widely used pesticide in India (Jales 1980) and more particularly in Assam is continuously washed away from the agricultural fields and accumulated in the nearly water bodies, hampering generally the aquatic life, specially the fishes. Moreover, BHC preparations are also used as medicines for certain skin diseases. Due to its extensive use, persistency and limited report of effect on fish fauna the BHC was selected for the present investigation.

Benzene hexachloride (BHC) more correctly known as hexachlorocyclohexane was first synthesised by Micheal Faraday in 1825 (Metcalf, 1976) and later discovered independently by Dupire of France (1941) and Thomas of England (1942), Martin and Worthing (1974) as a simple effective insecticide. The crude BHC is a complex mixture of closely related materials
(isomers) and formed by the addition of chlorine to benzene in presence of peroxides or photochemically by irradiation with ultra violet light catalyst (Benn and McAuliffe, 1975).

\[
\text{Benzene} + 3\text{Cl}_2 \rightarrow \text{BHC}
\]

Hexachlorocyclohexane can theoretically exist as eight different stereoisomers of which five actually found in the crude product. The different isomers of BHC are \( \alpha, \beta, \gamma, \delta, \kappa, \epsilon \) of which only the \( \gamma \) isomer, called lindane has significant insecticidal properties and some of the other materials in the mixture affect the central nervous system. (Assuming for simplicity that cyclohexane ring is planner than these five isomers may be depicted as follows):
HOC (70%)  

HI Cl Cl

\( \alpha \) (70%)  

\( \beta \) (6%)  

H

CI

Cl

\( \gamma \) (6%)  

\( \epsilon \) (traces only)  

\( \alpha = \frac{1:2:4}{5:5:6} \)

\( \beta = \frac{1:3:5}{2:4:6} \)

\( \gamma = \frac{1:4}{2:3:5:6} \)

\( \delta = \frac{1:3}{2:4:5:6} \)

\( \epsilon = \frac{1:2:3}{4:5:6} \)
The cost of production of this insecticide is very cheap due to low raw materials cost and therefore used widely in agriculture and public health.

During the present investigation a single dose of sublethal concentration was used after determining the LC$_{50}$ value, to evaluate the effect of sublethal BHC on different parameters of fishes.

**Determination of LC$_{50}$**

For determining the LC$_{50}$ concentration a series of glass aquarium was set up containing different concentrations of BHC viz. 50 ppm, 40 ppm, 38 ppm, 36 ppm, 34 ppm, 30 ppm, 28 ppm, 26 ppm, 24 ppm, 20 ppm, in 15 litres of tap water. A batch of 10 fishes were then liberated in each aquarium (set). The experiment is selected concentrations of BHC was repeated 5 times noting every time the number of fish killed in each set at 48 hours, 72 hours, 96 hours. The average mortality in each concentration was chosen to determine the lethal concentration and LC$_{50}$ value.

Exposure of fish to BHC for 48 hours showed '0' mortality in 24 and 26 ppm, 22% mortality in 28 ppm, 48% mortality in 30 ppm, 64% mortality in 32 ppm, 86% mortality in 34 ppm, 98% mortality in 36 ppm, and 100% mortality in 38 ppm. Whereas 72 hours exposure exhibited '0' mortality in 24
Fig. 1: LC$_{50}$ of BHC (Direct method).
ppm, 8% in 26 ppm, 52% in 28 ppm, 74% in 30 ppm, 90% in 32 ppm and 100% in 34 ppm. With 96 hours of treatment percentage of mortality observed was 6, 24, 58, 92 and 100 in 24 ppm, 26 ppm, 28 ppm, 30 ppm and 32 ppm respectively.

Reed - Muench Method (Ipsen & Feigl 1970) showed mortality percentage as 10% in 28 ppm, 41% in 30 ppm, 74% in 32 ppm, 92% in 34 ppm and 100% in 36 ppm at 48 hours, 5% in 26 ppm, 43% in 28 ppm, 72% in 30 ppm, 96% in 30 ppm and 100% in 34 ppm, at 72 hours and 4% in 24 ppm, 19% in 26 ppm, 64% in 28 ppm, 94% in 30 ppm and 100% in 32 ppm, at 96 hours.

The LC₅₀ was obtained by -
1) plotting the mortality percentage against the BHC concentration (direct method Fig. - 1 ).
2) plotting Ds and Sg (Table - 1 ) on the same set of axis.

The LC₅₀ value, as estimated from the above mentioned methods, was found to be 30.25 ppm at 48 hours, 27.91 ppm at 72 hours and 27.70 ppm at 96 hours (direct method) and 30.49 ppm, at 48 hours, 28.32 ppm at 72 hours and 27.35 ppm at 96 hours (Reed Muench Method) (Fig.- 2 ).

Fujiya (1964) reported that any given period of exposure, a harmful dose as far as physiological and
Fig. 2: Calculation of LC$_{50}$ by the graphic method where $Sg$ = No. of animals surviving this and greater doses and $DS$ = No. of animals dying at this and smaller doses.
**TABLE - I**

Sg, DS and percentage of mortality for calculation of LC\textsubscript{50} of BHC for *Heteropneustes fossilis* at 48 hours, 72 hours and 96 hours.

<table>
<thead>
<tr>
<th>BHC concentration in ppm</th>
<th>Survived at this and greater dose (Sg)</th>
<th>Died at this and smaller dose (DS)</th>
<th>% mortality</th>
<th>Sg + DS</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 hours</td>
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<td></td>
</tr>
<tr>
<td>28</td>
<td>18</td>
<td>2</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>10</td>
<td>7</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>5</td>
<td>14</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>2</td>
<td>22</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>0</td>
<td>32</td>
<td>100</td>
<td></td>
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<tr>
<td>72 hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>18</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>9</td>
<td>6</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>3</td>
<td>13</td>
<td>77</td>
<td></td>
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<tr>
<td>32</td>
<td>0</td>
<td>32</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>0</td>
<td>32</td>
<td>100</td>
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</tr>
<tr>
<td>96 hours</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>22</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>13</td>
<td>3</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>5</td>
<td>9</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>1</td>
<td>18</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>0</td>
<td>28</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
biochemical aspects are concerned, is much lower than $LC_{50}$ value. Therefore, for the present experiment, a sublethal concentration of 15 ppm was chosen by diluting the $LC_{50}$ concentration of 96 hours. The selected dose was the $LC_{25}$ value at 48 hours.

For the experimental purpose a control batch (1 ml acetone in 15 litres of water was also setup and 5 fishes from the each set (Control and 15 ppm Test set) were sacrificed at 0 hour, 15 days, 30 days, 45 days, 60 days, 75 days and 90 days of treatment for analysis of different parameters.

Biochemical Estimations

Estimation of protein

Principle

*Total* protein of testis, ovary and kidney were estimated according to the method of Lowry et al., (1951). Protein was first precipitated with a strong acid, made fat free and brought into solution with NaOH. In presence of alkaline Na$_2$CO$_3$ and rochelle salt CuSO$_4$.5H$_2$O reacted with protein to form protein copper compound which reduced the phosphomolybdic - phosphotungstic reagent to give a final colour, which was measured in colorometer (Systronic Spectro-Colorimeter 103).
Reagent required

(a) For extraction

(1) 10% trichloroacetic acid (TCA)
(2) Absolute alcohol
(3) Ether
(4) 1N NaOH

(b) For estimation

Reagent A -- 2% Na₂CO₃
Reagent B -- 2% Na₂CO₃ in 0.1N NaOH
Reagent C -- 0.5% CuSO₄, 5H₂O in 1% sodium potassium tartarate (Rochelle salt)
Reagent D -- 50 ml. of reagent A was added to 1 ml. of reagent C.
Reagent E -- 50 ml. of reagent B was added to 1 ml. of reagent C.

Both the reagent D and reagent E are unstable and therefore prepared freshly before the estimation.

Reagent F -- Folin ciocalteu phenol reagent, IN in acid (Sisco Research lab B. No. 30215, analar grade).
Procedure

(a) **Extraction** :

The testis, ovary and kidney were quickly dissected out from the living fish, weighed and homogenised in ice cold redistilled water (1 : 20). To 5 ml. of filtrate 1 ml. of 10% TCA was added for precipitation of protein. The precipitated protein was then washed three times with ethanol and twice with ether. The protein was brought into solution by adding 1 ml. of IN NaOH and keeping the solution at 73°C for 16 to 20 hours. The protein solution was diluted 20 times with redistilled water and was ready for estimation.

(b) **Estimation** :

To 1 ml. of protein solution 5 ml. of reagent E was added and mixed well. After 10 minutes 0.5 ml. of reagent F was added, mixed immediately by shaking and kept for 30 minutes. The blue colour was read against a redistilled water blank at 760 μm (red filter) and compared with reading of standard solution.

**Preparation of standard solution** :

**Stock** : 10 mg. of analar grade bovine serum albumin (Sigma chemical company B. NO. 51 F-0321) was dissolved in 100ml. of all glass redistilled water to give a solution of 0.1%.
**Working** : From the standard stock solution a series of working solutions, containing 100 µg, 200 µg, 400 µg, 500 µg, and 600 µg, of albumine were prepared by diluting the stock solution as shown below (Table - II) and were used for the preparation of standard calibration curve.

### Table - II

<table>
<thead>
<tr>
<th>No.</th>
<th>Strength of standard working solution µg albumin/ml</th>
<th>Volume of standard stock solution (ml)</th>
<th>Volume of redistilled water (ml)</th>
<th>Total volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>1.00</td>
<td>9.00</td>
<td>10.00</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>2.00</td>
<td>8.00</td>
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</tr>
<tr>
<td>3</td>
<td>300</td>
<td>3.00</td>
<td>7.00</td>
<td>10.00</td>
</tr>
<tr>
<td>4</td>
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<td>5</td>
<td>500</td>
<td>5.00</td>
<td>5.00</td>
<td>10.00</td>
</tr>
<tr>
<td>6</td>
<td>600</td>
<td>4.00</td>
<td>6.00</td>
<td>10.00</td>
</tr>
</tbody>
</table>

**Preparation of standard calibration curve** :

To 1 ml. of the standard working solution 5 ml. of reagent D was added, mixed and kept for 10 minutes, 0.5ml. of reagent F was added, mixed by shaking and allowed to stand at room temperature for 30 minutes. The blue colour was read against a redistilled water, blank treated in the same manner, at 660 mµ (red filter) in a colorimeter.
Fig. 3(a): Calibration curve of protein.

Fig. 3(b): Regression Curve of protein.

\[ Y = -0.5 + 0.143X \]
The mean optical density of five replicates of each standard working solution was plotted against the strength curve was obtained which was found to fit the regression curve and was judged reliable (Fig. - 3a, 3b).

Total protein was calculated by plotting the unknown value (optical density) on the calibration curve and also from the following formula.

\[
\frac{\text{Concentration of Standard}}{\text{Optical density of standard}} = \frac{\text{Concentration of unknown}}{\text{Optical density of unknown}}
\]

Total protein content in the present experiment was expressed in mg/g of testis, ovary and kidney.

Estimation of nucleic acid (DNA and RNA)

Principal:

The DNA and RNA was extracted, separated and estimated by the method of Schmidt-Thaunhauser-Schnider (1946). The method is based on the alkali lability of RNA and acid lability of both RNA and DNA, which permits the use of sugar assay as the measure of nucleic acid.

Reagents required:

(a) For extraction and separation

(I) 10% trichloroacetic acid (TCA)
(II) 5% trichloroacetic acid (TCA)
(III) 95% Ethanol
(IV) 100% Ethanol (absolute alcohol)
(V) IN NaOH
(VI) Ether
(VII) 6 N Hcl

(b) For estimation
Reagent A -- Con. Hcl, sp. gr. 1.19 (analar grade)
Reagent B -- 0.4% Indole reagent
Reagent C -- Chloroform.
Reagent D -- Standard DNA solution.

(I) Stock solution :
100 mg of DNA powder (calf thymus DNA Na salt 1492t, Koch - light lab., B. No. 53092) was dissolved in 100 ml. of redistilled water with little NaOH (0.1%).

(II) Working standard solution :
From the standard stock solution a series of working standard solution containing 6 μg, 8 μg, 10 μg, 12 μg, and 14 μg per ml. was prepared by diluting in the manner shown in Table.

Reagent E
Colouring reagent for RNA : 0.1% Fe cl₃, 6H₂O and 0.1% orcinol in conc. Hcl. The solution was prepared freshly during the time of estimation.
Reagent F — Standard RNA solution.

(I) Stock solution:
The stock solution was prepared by dissolving 100 mg. RNA powder (sigma chemical company, B. No. 112F - 8160) in redistilled water and diluted to 100 ml.

(II) Working Standard solution:
A series of standard working solution between 6 to 14 ug was prepared by diluting the stock solution with redistilled water as shown in Table - III.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Strength of the working stock solution (ug.)</th>
<th>Volume of the stock solution (ml.)</th>
<th>Volume of redistilled water (ml.)</th>
<th>Final Volume (ml.)</th>
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</thead>
<tbody>
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<td>DNA</td>
<td>RNA</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>0.06</td>
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</tr>
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<td>9.92</td>
<td>10.00</td>
</tr>
<tr>
<td>3.</td>
<td>10</td>
<td>0.10</td>
<td>9.90</td>
<td>10.00</td>
</tr>
<tr>
<td>4.</td>
<td>12</td>
<td>0.12</td>
<td>9.98</td>
<td>10.00</td>
</tr>
<tr>
<td>5.</td>
<td>14</td>
<td>0.14</td>
<td>9.86</td>
<td>10.00</td>
</tr>
</tbody>
</table>

Procedure
Preparation of Testis, ovary and Kidney homogenate
The testis, ovary and kidney were dissected out quickly, weighed and were homogenised in ice cold redistilled water (1 : 20).
Removal of acid-soluble compounds:

1 ml. of tissue homogenate was mixed with 2.5 ml. cold 10% TCA and centrifuged. The precipitate was resuspended in 2.5 ml. of cold 10% TCA and centrifuged. The combined extracts constitute the acid soluble phosphorous compounds.

Removal of Phospholipids:

The tissue residue was suspended in 1.0 ml. water and 4.0 ml. 95% ethanol and centrifuged. The residue was resuspended in 5.0 ml. of absolute alcohol and centrifuged. It was then extracted two times with two portions of 3:1 alcohol (100%): either mixture of centrifuged. The residue was finally suspended in ether brief stirring and centrifuged. The combined extracts (supernatant) constitute the phospholipid phosphorous fraction.

(a) Extraction of DNA and RNA

The residue was treated with 1 N NaOH for 16 to 20 hours at 37°C at a volume of 1 ml. per 100 mg. fresh tissue to make a solution of the tissue residue. The solution was neutralized with 6N HCl and the DNA was precipitated by adding 1 volume of 5% TCA. The centrifuged precipitated was washed with 5 ml. of 5% TCA. The combined extract contained RNA is solution was diluted to 50 ml. double distilled water. The residue (contain DNA) was dissolved in 5 ml. of 1 N NaOH and diluted to 50 ml. by redistilled water.
(b) Estimation of DNA:

The estimation of DNA was made by treating DNA solution with indole as described by Ceriotti (1952). The reaction of DNA with indole was first described by Dische (1929) and was developed by Ceriotti (1952).

To 2.0 ml. of DNA extract solution 1 ml. of Reagent B and 1.0 ml. of Reagent B was added to make a final volume of 4.0 ml. The mixture was boiled for 10 minute at 100°C in a boiling water bath, cooled in room temperature and extracted three times with 4 volumes of Reagent C (Chloroform) using a pasture pipette. The chloroform layer was discarded after each washing and the intensity of the yellow colour, developed in the aqueous phase was measured at 490 m\( \mu \) against a redistilled water blank and compared with reading of the standard solution of standard calibration curve.

Preparation of standard calibration curve of DNA:

The DNA standard calibration curve was prepared by plotting colour measurement against known concentration. The working standard (Reagent D) were treated in the same manner as described above to obtain the colour measurement. For each concentration (working standard) five readings were taken and the mean value of the optical densities were plotted against the concentration. The calibration curve (Fig -4a ) thus obtained was a linear one and found to fit with the regression curve (Fig 4b ) and was judged reliable.
Fig. 4(a) : Calibration curve of DNA

Fig. 4(b) : Regression curve of DNA.

\[ Y = -4.2 + 3.85X \]
Fig. 5(a): Calibration curve of RNA

Fig. 5(b): Regression curve of RNA.

\[ Y = -10.8 + 2X \]
(c) **Estimation of RNA**

The RNA content was estimated according to the method of Majbaun (1939). To 2 ml. of diluted RNA extract solution equal volume of reagent E was added and was heated for 20 minutes in a boiling water bath. The mixture was then cooled at room temperature and the green colour was measured against a distilled water blank at 670 μμ. The reading was compared with the standard calibration curve to obtain the RNA content.

**Preparation of Standard calibration curve of RNA :**

The RNA standard calibration curve was prepared by plotting colour measurement against known concentration. The working standard solution (Reagent F-II) was treated with Reagent E as described above and the mean colour measurement of 5 replicates of each working standard concentration was plotted against concentration. The calibration curve (Fig 5a) was found to fit with the regression curve (Fig 5b).

Both the DNA, RNA were expressed in mg/100gm of wet testis, ovary and kidney homogenate.

**Estimation of Total Ascorbic Acid :**

**Principle :**

The method was determined demonstrated by Roe and Kuther (1943) in which ascorbic acid is converted to dehydroascorbic acid by shaking with norit and this is then
coupled with 2, 4 dinitrophenylhydrazine in presence of thiourea as a mild reducing agent. Sulphuric acid then converts the dintrophenylhydrazine into a red compound which is assayed colorimetrically.

Reagent required:
(a) For Extraction:
(I) 6% Trichloroacetic acid (TCA) 60 gm of TCA was dissolved in 1 litre of distilled water.
(II) 4% Trichloroacetic acid (TCA) 40 gm of TCA was dissolved in 1 litre of distilled water.
(III) Acid washed norit (200 gm of norit was added with one litre of 10% Hcl, heated to boil and filtrate with suction. The norit cake was added with one litre of distilled water and filtered then kept at 110°C overnight for drying).

(b) For Estimation:
Reagent A - 85% Sulphuric Acid (900 ml. of conc. sulphuric acid is added with 100 ml. of distilled water).
Reagent B - 2, 4 dinitrophenylhydrazine (2 gm. of 2, 4 dinitrophenylhydrazine was dissolved in 100 ml. of 9 N sulphuric acid and added 4 gm of thiourea and filtered).
Reagent C - Standard ascorbic acid solution.

(I) Stock solution:
Standard stock ascorbic acid was prepared by dissolving 50 mg. of analar grade ascorbic acid (Sigma B. No.) in 100 ml. of 4% TCA.

(II) Standard working solution:
For working standard 2 ml. of the stock solution as added with 100 ml. 6% TCA.

Table IV

<table>
<thead>
<tr>
<th>Solution No.</th>
<th>Strength (mg. ascorbic acid/100 ml.)</th>
<th>Volume of Standard Stock Solution (ml.)</th>
<th>Volume of tri Chloroacetic acid (ml.)</th>
<th>Total Volume (ml.)</th>
</tr>
</thead>
<tbody>
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<td>98.7</td>
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</table>
Procedure:

Extraction:
The testis, ovary and kidney were dissected out quickly, weighed and homogenised in ice cold 6% TCA.

6 ml. of tissue homogenate was added with 0.3 gm. of acid washed norit, corked and shaken vigorously and filtered. This converts the whole of the ascorbic acid present into dehydroascorbic acid.

Estimation:
2 ml. of the filtrate was added with reagent B, test tubes were closed with stopper and placed in water bath at 37°C for exactly 3 hours. Removed after 3 hour and place in a ice cold water and added slowly 2.5 ml. of Reagent A drop by drop. Mixed well and kept in ice cold water for 30 minutes and read in colorimeter at 540 nm (Yellow filter).

The total ascorbic acid was calculated by the following formula.

\[
\text{Mg ascorbic acid per 100 ml.} = \frac{\text{Reading of unknown}}{\text{Reading of standard}}
\]

Preparation of standard calibration curve:
The ascorbic acid standard calibration curve was prepared by plotting colour measurement against known concentration (fig. - 6a ). The working standard solution C
Fig. 6(a) Calibration curve of Ascorbic Acid

Fig. 6(b) Regression curve of Ascorbic Acid

\[ Y = 10.4 + 151X \]
(II) was treated with reagent B as described earlier and mean colour measurement of 5 replicates of each working standard concentration was plotted against concentration. The calibration curve was found to fit with the regression curve and was judged reliable (Fig. - 6b ).

Ascorbic acid was expressed in mg/100 gm of wet testis ovary and kidney homogenate.

Estimation of total adrenaline and Noradrenaline :

Principle :

The method was demonstrated by U.S.V. Euler (1950). The tissues were treated with trichloracetic acid and washed with ether at $P^H 4$. It was also purified with fuller's earth and filtered. With the filtrate acetate buffer and iodine was added with sodium thiosulphate and measured in colorimeter.

Reagent required :

a) For extraction :
   I) 10% Trichloroacetic acid
   II) Ether
b) For purification :
   I) 20% of fuller's earth
(c) For estimation :

Reagent A -- : Acetate buffer, $P^H 4$ (Acetic acid and sodium acetate).
Reagent B -- : Acetate buffer $P^H 6$ (Acetic acid and sodium acetate).
Reagent C - - : 0.1 N iodine (12.7 gm. is added with 1000 ml. of water slightly potassium iodide is also added).

Reagent D - - : 0.05 Sodium thiosulphate (0.989 sodium thiosulphate in 1000 ml of water).

Reagent E - - : Standard Adrenaline and Noradrenaline solution.

(I) Stock solution :

Stock solution of adrenaline and noradrenaline was prepared by dissolving 10 mg. of adrenaline and noradrenaline separately in 50ml of 10% TCA.

(II) Standard working solution :

For working standard 1 ml. stock solution was added with 10 ml. of 10% TCA.

Procedure :

Extraction :

The testis, ovary and kidney were weighed and were homogenised with 5 ml. cold 10% trichloroacetic acid (TCA). The volume was made 10 times and filtered. The filtrate was washed with ether repeatedly until the reaction was about pH 4-5.

Purification :

10 ml. of the extract was added with 0.25 ml. of 20% fuller's earth and filtered.
Estimation : 

For Adrenaline :

1 ml. of filtrate was added with 1 ml. of acetate buffer (pH 4) and then 0.2 ml. of 0.1 N iodine was added. Just after one and half minute 0.5 ml. 0.5 N sodium thiosulphate was added and brought the volume up to 5 ml. with the help of acetate buffer of pH 4, and measured at 529 μm after 5 minutes against a blank which was without iodine.

For Noradrenaline :

1 ml. of filtrate was added with 1 ml. of acetate buffer pH 6 and then 0.2 ml. of 0.1 N iodine was added. After 3 minutes 0.5 ml of 0.05 N sodium thiosulphate was added and brought the volume upto 5 ml. with the help of acetate buffer having pH 6. After 5 minutes it was measured at 529 μm against a blank which was without iodine.

The Adrenaline and Noradrenaline were calculated by the following formula :-

\[
\text{Standard Adrenaline factor} = \frac{100}{\text{OD of Adrenaline standard}}
\]

\[
\text{Standard Noradrenaline factor} = \frac{100}{\text{OD of noradrenaline standard}}
\]

\[
\text{Standard adrenaline factor} = \frac{(\text{OD of adrenaline} - (0.1 \times \text{OD of Noradrenaline})}{0.9} = \mu g/ml. \text{adrenaline}.
\]
Standard Noradrenaline factor (OD of Noradrenaline - OD of adrenaline) = \( \mu g/ml \) Noradrenaline

Preparation of standard Calibration Curve:

The Adrenaline and Noradrenaline standard calibration curve was prepared by plotting colour measurement against known concentration. The working standard solution E (II) was treated with reagent C and mean colour measurement of 5 replicates of each working standard concentration was plotted against concentration. The calibration curve was (Fig-7a, 8a) found to fit with the regression curve (Fig-7b, 8b) and judged reliable.
Fig. 7(a) : Calibration curve of Adrenaline

\[ Y = 0.9 + 207.9 \times X \]

Fig. 7(b) : Regression curve of Adrenaline
Fig. 8(a) : Calibration curve of Noradrenaline

\[ y = -2.96 + 292.9x \]

Fig. 8(b) : Regression curve of Noradrenaline
Specimen Preparation for SEM Study

Preparatory techniques :-

(1) Fixation :-

The first step in the preparation of the specimens for scanning election microscopy is the fixation. The purpose of the fixative is to preserve the surface morphology in detail. The fixative also has to protect the specimen against alterations during subsequent treatment such as dehydration, drying etc.

The commonly used fixative for SEM are :-

(a) 1.5 to 3% glutaraldehyde in 0.1 M of either sodium phosphate or sodium cacodylate buffer (pH 7.2 to 7.4).
(b) 1 or 2% osmium tetroxide in 0.1 M Sodium Phosphate or sodium cacodylate buffer.

Preparation of buffers :-

1) 0.2 Phosphate buffer

Sodium dihydrogen phosphate = 6.41 g.
Di - sodium hydrogen phosphate = 41.3 gm.

\[
\begin{align*}
\text{Na}_2\text{HPO}_4 & \quad 7\text{H}_2\text{O} \\
\text{Na}_2\text{HPO}_4 & \quad 12\text{H}_2\text{O} = 55.2 \text{ gm}
\end{align*}
\]

Double (glass) distilled water is used to make the volume to 1000 ml.
ii) 0.2M Cacodylate buffer

Sodium cacodylate $\text{Na}(\text{CH}_3)_2\text{AsO}_2\text{OH} = 42.3 \text{ gm}$

$\text{N. Hcl} = 6.9 \text{ ml.}$

Doubled distilled water is used to make the Vol. to 1000 ml.

**Preparation of Primary fixative :-**

(3% glutaraldehyde in 0.1 M phosphate or Cacodylate buffer)

To 30 ml of 50% or 60 ml of 25% glutaraldehyde, double distilled water (glass distilled) is added and the volume is made up to 250 ml. To this, 250 ml of either 0.2M phosphate or Cacodylate buffer is added. When Cacodylate buffer is used, 12.5 mg. of anhydrous calcium chloride is added to the solution. The solution can be stored at 4°C.

**Preparation for secondary fixative :-**

1% Osmium tetroxide : In 0.1 M Buffer 1gm. of $\text{os}_4$ is dissolved in 50 ml. of double distilled water, and is kept overnight to dissolve. It can be stored at 4°C, but always in tightly stoppered brown coloured bottle.

To a part of this 2% osmium tetroxide equal part of 0.2 M Sodium Cacodylate is added to obtain 1% $\text{os}_4$ in 0.1 M buffer solution. (It was prepared fresh before use).
Procedure for fixation: -

Tissues are fixed by immersion or vascular perfusion. When the tissues are fixed by immersion, the size of the tissue should be 1 to 2 mm. The small size of the tissue is taken because rate of penetration of fixative is very low.

To increase the rate of preparation, some additive like acrolein (0.1%) is added to the primary fixative.

Fixative by vascular perfusion is carried out using a transfusion set.

Fixation in the primary fixative is carried at 4°C generally for 2 to 3 hours.

However, in glutaraldehyde, fixation is carried out often for 24-48 hours and the fixative is changed at least twice during that period.

(2) Washing:

After the treatment of the tissue with primary fixative, the tissue are washed in 0.1 M buffer. The buffer used for the preparation of the fixative can be used. After washing, the tissues may be post fixed by secondary fixative Osmium tetraxide (os0₄ 1% solution) for 2 hours.
(3) **Dehydration** :

After post fixative of the tissue, it has to be dehydrated. Dehydrating agent used are either distilled ethanol or acetone (Analar). When acetone is used as dehydrating agent then it should be in the anhydrous state. Dry acetone is prepared by adding copper sulphate to absolute acetone. It is shaken well and kept for sometime. It has to be refilled before use.

**Procedure for dehydration** :

The size of the specimen should be 2 to 3 mm. Dehydration is to be carried out in gradual steps, because rapid dehydration will result in shrinkage of the specimen. Following steps has to be followed.

Specimen (2 to 3 mm)  

\[ \downarrow \]

30% acetone (15 mins.)  

\[ \downarrow \]

50% acetone (15 mins.)  

\[ \downarrow \]

70% acetone (15 mins.)  

\[ \downarrow \]

80% acetone (15 mins.)  

\[ \downarrow \]

90% acetone (15 mins)  

\[ \downarrow \]

95% acetone (25 mins.)
I. 100% acetone (15 mins.)

II. 100% acetone (15 mins)

**Critical Point drying** :

After the tissues are dehydrated, they are to be dried but it was observed that air (evaporative) drying cause some distortions and disruption on the surface of the specimen. The major cause of damage on drying is the surface tension of the liquid medium on which the specimen is placed. The medium subjects the specimen to crushing and tearing forces while evaporating through and around soft hydrated specimens. Hence comes the importance of critical point drying.

**Principale** :

Critical point dryer consists of sturdy container (Bomb), which is approximately half filled with a very volatile liquid, eg. liquid CO₂ under pressure. The container has valved orifices for filling and venting. When the container is warmed, the liquid expands and evaporates. If the 'Bomb' is approximately half filled evaporation just balances expansion and surface of the liquid remains about in the centre. However, the density of the expanding but nearly incompressible liquid is reduced. But the density of the compressible gas phase increases. Thus the total density remains constant. The interface (meniscus) between the two
phases becomes less distinct, and the interfacial tension (surface tension) declines as the densities and other properties of the two fluid approach each other.

At a specific temperature, the critical temperature ($T_c$), the densities and all other properties are identical and the miniscus and the surface tension vanish. This is the critical point. The critical surface tension is always zero.

The valve is then opened slightly, when the temperature was elevated, the gas streams out until atmospheric pressure is reached and the dry specimen may be removed.

**Metal coating**:

The dry specimens are fixed on brass stub, by electroconductive point, and are kept on the specimen stand of the ionsputter, and are coated with a thin layer of gold vapour.

The specimen is now ready to be observed under SEM and photographed on 125 ASA/22 DIN 112 m.m. black and white film.
Statistical Analysis:

Statistical analysis of the experimental data were performed following the method of Goon et al. (1979) to determine the significance of the impact of BHC treatment on some specific biochemical parameters. The statistical methods undertaken in the present experimental project were as follows:

A. Regression Curve:

The regression curve was prepared to get the accuracy of the methods of biochemical estimation by using the linear regression equation.

\[ Y = a + bx \]

Where

\[ b = \frac{\sum (X_i - \bar{X})(Y_i - \bar{Y})}{\sum (X_i - \bar{X})^2} \]

\[ a = \bar{Y} - bx \]

B. Standard deviation:

Mean standard deviation (SD) was calculated out by the following formula.

\[ SD = \sqrt{\frac{(X_i - \bar{X})^2}{(n - 1)}} \]
C. Standard Error for mean:

Mean standard error ($SE_m$) were determined by the following formula.

$$SE_m = \frac{SD}{\sqrt{n}}$$

D.

One way analysis of variance (ANOVA) was performed for significant test. If there was significant ($t$) variation among the treatment groups, Fisher's significant test for pairing were undertaken.

$$CD = t \text{ of } 5\% \text{ & } 1\% \sqrt{\text{Error M.S.}(\frac{1}{k} + \frac{1}{k})}$$

$$K = \text{No. of observations in each group.}$$
Effect of BHC on Protein, DNA and RNA of Testis, Ovary and Kidney of *H. fossilis*. 
Introduction:

Indiscriminate use of BHC is causing serious environmental pollution problem in India. Aquatic ecosystem is more sensitive to organic pollutants. (Edwards 1973). The residues of insecticide ultimately find their way into the aquatic ecosystem and accumulate in the fauna. The specific properties of BHC slow degradation, high lipophilicity and low water solubility may result in their prolonged persistence.

Because of bioconcentration and biomagnification phenomenon of organochlorine pesticides, their residues increase the concentration at each higher step of food chain. Fishes are very much susceptible to the residues of organochlorine pesticide as they occupy the higher tropic level of organisation and form the highest bulk of the aquatic fauna. Hence the fish can be taken as the indicator group of the organic pollution in the aquatic environment in general and in particular the reproductive failure in fish is one of the indicators of the pollution in the environment.

Studies on the toxic effects of pollutants on the reproductive organs of fresh water teleost are few (Ahsan and Ahsan 1974; Beconil 1975; Shastry and Agarwal 1980; Shukla et al., 1983; Pandey and Shukla 1985; Singh et al., 1985; Stephan et al., 1986; Srivastava 1989). Though they are least affected...
organs but presence of any pollutant affects their normal metabolism, fecundity and hence their population (Pandey and Shukla 1980; Kaur and Virk 1983; Saxena and Garg 1978). In teleost the gonadal function with reference to the insecticide pollutant has been described by few workers for variety of species. (Singh and Singh 1980; Pandey and Shukla 1985; Bhattachary and Pandey 1989; Behura and Bhunya 1991).

Gonads are the vital organ of reproductive function and their protein and nucleic acid pattern shows different abnormalities after exposure to the toxic substances. It has been noted that toxicity brings the changes in the gonadal cells. It has also been observed that the effect of pesticide slightly reduce the size and deformities also occurred in gonadal cells (Behura and Bhuyan 1991).

Although kidney is an excretory organ and it eliminates the excretory product yet the kidney of fishes renders haemopoietic and endocrine function. The effect of pesticide will produce adverse effect on kidney. As a result the kidney is also very much at the threshold level to be affected by the insecticide toxicity. Sharma et al., (1991) observed necrosis of the kidney tubules and shrinkage of glomerular tuft occurs in pesticide exposed fish.

A wide variety of chemicals generally interfere with the function or metabolism of protein and nucleic acids. Ecobichon and Comeau (1974) treated male wister rat with
biphenyl observed increase of RNA and protein content of liver with decreased DNA value. Carbon tetrachloride (ccl₄) has been reported to cause DNA inhibition in *Heteropneustes fossilis* (Shastry and Agarwala 1976). Sharma et al., (1979) recorded reduction of serum protein due to congo red treatment in *H.fossilis*. Giurgea et al., (1989) showed that intoxication of lead lowered the DNA content with concomitant enhancement of protein and RNA. The effect of malathion on *Channa punctatus* results in the significant fall of protein and RNA in liver (Jyoti et al., 1989). Bursch and Herman (1983) noted increased synthesis of DNA due to BHC in rat. However, few reports are available on the effect of chlorinated pesticides on the nucleic acid and protein of the gonads and the kidney of teleost. Since the synthesis of DNA, RNA and protein are interrelated process therefore effect on anyone of them could bring alteration at fish population level. The present investigation was designed to explore the possible hazards of dilute concentration of 15 ppm. BHC on the protein and nucleic acid profile of the gonads and the kidney of *Heteropneustes fossilis*.

**Methods and Materials** : The fish weighing 5 - 10 gms. bodyweight were reared for experimental purpose as detailed in the chapter-II alongwith the methodology employed in this experiment.

**Results** : The results of protein, DNA and RNA of 15 ppm. BHC exposed testis, ovary and kidney are depicted
in the table v to xiii. The values at control, 15th day, 30th day, 45th day, 60th day, 75th day and 90th day were recorded and values of five observations are presented.

**Effect of BHC on the Protein content of Testis, Ovary and Kidney:**

**Testis:** The protein concentration of the testis (Table v) was noted in between 14.92 to 16.58 mg/gm of tissue in the control group. Significantly reduced ($P<0.01$; ANOVA-I) protein concentration was noted compared to control except on 60th day. Comparison among the treated groups a significant difference was observed except between the group F & C.

**Ovary:** The protein concentration of the control group was recorded in between 6.41 to 8.35 mg/gm of wet tissue. With the treatment of BHC the protein content of the ovary was gradually increasing from the control to 45th day (7.43 mg/gm to 47.40 mg/gm); but gradual fall of protein was recorded after 45th day of BHC treatment compared to their previous test group ($P<0.01$; ANOVA-2). Enhanced value was recorded in all the test group compared to their respective control (Table-vi). C.D. of means showed significant difference amongst the protein values of different groups (Table xiv).
**TABLE V** Protein concentration in control and 15ppm BHC exposed testis of *H.fossilis* in different days interval. Values are replica of 5 fresh fish tissues ± SEM, expressed in mg/gm.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>15-days</th>
<th>30-days</th>
<th>45-days</th>
<th>60-days</th>
<th>75-days</th>
<th>90-days</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A)</td>
<td>15.92</td>
<td>24.86</td>
<td>10.76</td>
<td>21.28</td>
<td>16.27</td>
<td>9.17</td>
<td>4.90</td>
</tr>
<tr>
<td>(B)</td>
<td>14.92</td>
<td>23.62</td>
<td>10.15</td>
<td>20.87</td>
<td>16.71</td>
<td>8.25</td>
<td>4.45</td>
</tr>
<tr>
<td>(C)</td>
<td>15.92</td>
<td>21.31</td>
<td>8.15</td>
<td>22.23</td>
<td>14.20</td>
<td>9.56</td>
<td>5.94</td>
</tr>
<tr>
<td>(D)</td>
<td>16.58</td>
<td>24.86</td>
<td>9.23</td>
<td>20.87</td>
<td>15.68</td>
<td>9.17</td>
<td>5.34</td>
</tr>
<tr>
<td>(E)</td>
<td>15.92</td>
<td>23.62</td>
<td>10.15</td>
<td>22.23</td>
<td>15.36</td>
<td>9.17</td>
<td>5.94</td>
</tr>
<tr>
<td>Mean</td>
<td>15.85</td>
<td>23.65ab</td>
<td>9.78a</td>
<td>21.49ab</td>
<td>15.64ab</td>
<td>9.06ab</td>
<td>5.31ab</td>
</tr>
<tr>
<td>±0.24</td>
<td>±0.59</td>
<td>±0.34</td>
<td>±0.28</td>
<td>±0.39</td>
<td>±0.19</td>
<td>±0.26</td>
<td></td>
</tr>
</tbody>
</table>

*a* = Significantly differ from the control groups  
*b* = Significantly differ from the treated groups

**ANOVA 1** Analysis of variance for one way classified data with 5 observations in each group

<table>
<thead>
<tr>
<th>Sources of variance</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F.</th>
<th>Tabulated value of F at 0.05</th>
<th>Inferene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among animal groups</td>
<td>6</td>
<td>1360.09</td>
<td>226.68</td>
<td></td>
<td>303.04</td>
<td>2.45</td>
</tr>
<tr>
<td>Error (within the animal groups)</td>
<td>28</td>
<td>20.96</td>
<td>0.748</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant, ** Highly Significant

(CD (P<0.05) = 1.12 ; CD (P<0.01) = 1.50)
### Table VI

Protein concentration in Control and 15 ppm BHC exposed ovary of *H. fossilis* in different days interval. Values are replica of 5 fish tissues + SEM, expressed in mg/gm.

<table>
<thead>
<tr>
<th>Control (A)</th>
<th>15-days (B)</th>
<th>30-days (C)</th>
<th>45-days (D)</th>
<th>60-days (E)</th>
<th>75-days (F)</th>
<th>90-days (G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.46</td>
<td>33.92</td>
<td>38.20</td>
<td>47.80</td>
<td>25.56</td>
<td>19.58</td>
<td>15.23</td>
</tr>
<tr>
<td>6.41</td>
<td>34.46</td>
<td>37.31</td>
<td>48.60</td>
<td>26.56</td>
<td>18.21</td>
<td>14.28</td>
</tr>
<tr>
<td>7.47</td>
<td>36.25</td>
<td>35.82</td>
<td>45.15</td>
<td>26.93</td>
<td>20.95</td>
<td>15.87</td>
</tr>
<tr>
<td>8.35</td>
<td>34.46</td>
<td>37.76</td>
<td>48.20</td>
<td>26.56</td>
<td>18.63</td>
<td>14.28</td>
</tr>
<tr>
<td>7.46</td>
<td>36.25</td>
<td>35.82</td>
<td>47.27</td>
<td>26.56</td>
<td>20.54</td>
<td>14.28</td>
</tr>
</tbody>
</table>

Mean

<table>
<thead>
<tr>
<th>Control (A)</th>
<th>15-days (B)</th>
<th>30-days (C)</th>
<th>45-days (D)</th>
<th>60-days (E)</th>
<th>75-days (F)</th>
<th>90-days (G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.43</td>
<td>35.07ab</td>
<td>36.99ab</td>
<td>47.40ab</td>
<td>26.43ab</td>
<td>19.59ab</td>
<td>14.79ab</td>
</tr>
<tr>
<td>±0.28</td>
<td>±0.44</td>
<td>±0.54</td>
<td>±0.20</td>
<td>±0.48</td>
<td>±0.30</td>
<td></td>
</tr>
</tbody>
</table>

a = Significantly differ from the control groups
b = Significantly differ from the treated groups

**ANOVA 2** Analysis of variance for one way classified data with 5 observations in each group.

<table>
<thead>
<tr>
<th>Sources of variance</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F</th>
<th>Tabulated value</th>
<th>Inferene of F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among animal groups</td>
<td>6</td>
<td>5841.38</td>
<td>973.57</td>
<td>2.45</td>
<td>3.58 **</td>
<td></td>
</tr>
<tr>
<td>Error (within the animal group)</td>
<td>28</td>
<td>27.73</td>
<td>0.990</td>
<td>0.990</td>
<td>CD(P&lt;0.05) = 1.28; CI(P&lt;0.01) = 1.73.</td>
<td></td>
</tr>
</tbody>
</table>

* Significant, ** Highly Significant
Table VII: Protein concentration in control and 15 ppm BHC exposed kidney of H. fossilis in different days interval. Values are replica of 5 fresh fish tissues ± SEM, expressed in mg/gm.

<table>
<thead>
<tr>
<th>Control (A)</th>
<th>15-days (B)</th>
<th>30-days (C)</th>
<th>45-days (D)</th>
<th>60-days (E)</th>
<th>75-days (F)</th>
<th>90-days (G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.15</td>
<td>17.66</td>
<td>11.85</td>
<td>7.32</td>
<td>16.12</td>
<td>16.18</td>
<td>12.25</td>
</tr>
<tr>
<td>8.62</td>
<td>18.86</td>
<td>11.42</td>
<td>7.75</td>
<td>17.09</td>
<td>15.78</td>
<td>11.29</td>
</tr>
<tr>
<td>6.61</td>
<td>17.15</td>
<td>10.85</td>
<td>7.32</td>
<td>13.87</td>
<td>16.18</td>
<td>10.16</td>
</tr>
<tr>
<td>8.15</td>
<td>15.95</td>
<td>12.28</td>
<td>7.75</td>
<td>16.12</td>
<td>13.94</td>
<td>11.77</td>
</tr>
<tr>
<td>6.61</td>
<td>17.66</td>
<td>10.42</td>
<td>6.29</td>
<td>13.87</td>
<td>16.18</td>
<td>10.16</td>
</tr>
</tbody>
</table>

Mean

7.62 ± 0.38

17.45ab ± 0.42

11.36a ± 0.30

7.28b ± 0.24

15.41a ± 0.59

15.65ab ± 0.39

11.12ab ± 0.38

a = Significantly differ from the control groups
b = Significantly differ from the treated groups

ANOVA: Analysis of variance for one way classified data with 5 observations in each group.

<table>
<thead>
<tr>
<th>Sources of variance</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F.</th>
<th>Tabulated value of F</th>
<th>Inferene of F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among animal groups</td>
<td>6</td>
<td>483.76</td>
<td>80.62</td>
<td>81.84</td>
<td>2.45</td>
<td>3.58**</td>
</tr>
<tr>
<td>Error (within the animal group)</td>
<td>28</td>
<td>27.58</td>
<td>0.985</td>
<td>0.05</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

* Significant, ** Highly significant

CD(P<0.05) = 1.28; CD (P<0.01) + 1.73
Kidney: The protein concentration ranged between 6.61 to 8.62 mg/gm wet tissue in control group. The protein content significantly enhanced (P<0.01; ANOVA-3) in all the BHC exposed groups over their respective control, except on 45th day (Group-D); the highest being recorded on 15th day (17.45 + 0.42; Table-vii). On comparison among the treated groups showed significant difference except between the group E & F.

Effect of BHC on the DNA content of Testis, Ovary and Kidney:

Testis: The DNA in control group of testis was observed in between 0.099 to 0.115 mg/100 gm. The DNA content of the BHC exposed testis was noted to be significantly decreased on 30th day (0.033 + 0.003 mg/100 gm); but increased on 45th day (0.186 + 0.002 mg/100 gm of tissue) compared to control set (Table-viii). The DNA value was also recorded at declining trend on 75th day (0.066 + 0.001 mg/100 gm). However, the enhanced value was observed on 60th day (0.148 + 0.002 mg/100 gm) and 90th day (0.152 + 0.001 mg/100 gm). The values differed significantly from the control value (P<0.01; ANOVA-4).

Ovary: The DNA of the control group of ovary valued in between 0.137 to 0.141 mg/100 gm. The DNA content of ovary significantly increased over the control on 15th day (0.155 + 0.001
Table VIII  DNA Concentration in control and 15ppm. BHC exposed Testis of H.fossilis in different days interval. values are replica of 5 fresh fish tissues + SEM, expressed in mg/100gm.

<table>
<thead>
<tr>
<th></th>
<th>Control (A)</th>
<th>15-days (B)</th>
<th>30-days (C)</th>
<th>45-days (D)</th>
<th>60-days (E)</th>
<th>75-days (F)</th>
<th>90-days (G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.105</td>
<td>0.109b</td>
<td>0.033 ab</td>
<td>0.186 ab</td>
<td>0.148 ab</td>
<td>0.066 ab</td>
<td>0.152</td>
</tr>
<tr>
<td></td>
<td>+0.002</td>
<td>+ 0.001</td>
<td>+ 0.001</td>
<td>+ 0.002</td>
<td>+0.002</td>
<td>+0.001</td>
<td>+0.001</td>
</tr>
</tbody>
</table>

*a* = Significantly differ from the control group  
*b* = Significantly differ from the treated groups.

ANOVA-4  Analysis of variance for one way classified data with 5 observations in each group.

<table>
<thead>
<tr>
<th>Sources of variance</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F</th>
<th>Tabulated value of F</th>
<th>Inferene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among animal groups</td>
<td>6</td>
<td>0.084</td>
<td>0.014</td>
<td></td>
<td>466.67 2.45 3.58</td>
<td>**</td>
</tr>
<tr>
<td>Error (within the animal groups)</td>
<td>28</td>
<td>0.001</td>
<td>0.0003</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant ; ** Highly Significant

\[ CD \text{ (P<0.05)} = 0.006; \ CD \text{ (P<0.01)} = 0.008 \]
Table IX  DNA concentration in control and 15 ppm BHC exposed ovary of *H.fossilis* in different days interval. Values are replica of 5 fresh fish tissues + SEM, expressed in mg/100gm.

<table>
<thead>
<tr>
<th></th>
<th>Control (A)</th>
<th>15 days</th>
<th>30 days</th>
<th>45 days</th>
<th>60 days</th>
<th>75 days</th>
<th>90 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.139</td>
<td>0.154</td>
<td>0.127</td>
<td>0.434</td>
<td>0.345</td>
<td>0.260</td>
<td>0.282</td>
</tr>
<tr>
<td></td>
<td>0.141</td>
<td>0.160</td>
<td>0.130</td>
<td>0.423</td>
<td>0.336</td>
<td>0.252</td>
<td>0.288</td>
</tr>
<tr>
<td></td>
<td>0.138</td>
<td>0.154</td>
<td>0.132</td>
<td>0.434</td>
<td>0.345</td>
<td>0.243</td>
<td>0.282</td>
</tr>
<tr>
<td></td>
<td>0.137</td>
<td>0.150</td>
<td>0.120</td>
<td>0.432</td>
<td>0.327</td>
<td>0.257</td>
<td>0.273</td>
</tr>
<tr>
<td></td>
<td>0.140</td>
<td>0.160</td>
<td>0.120</td>
<td>0.447</td>
<td>0.345</td>
<td>0.243</td>
<td>0.276</td>
</tr>
</tbody>
</table>

Mean

|          | 0.139   | 0.155₂⁺ | 0.125₂⁺ | 0.434₂⁺ | 0.339₂⁺ | 0.251₂⁺ | 0.280₂⁺ |
|          | +0.001  | +0.001  | +0.003  | +0.003  | +0.003  | +0.002  |

*a* = Significantly differ from the control group.

*b* = significantly differ from the treated groups.

ANOVA - 5 Analysis of variance for one way classified data with 5 observations in each group.

<table>
<thead>
<tr>
<th>Sources of variance</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F.</th>
<th>Tabulated value</th>
<th>Inference of F.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among animal groups</td>
<td>6</td>
<td>0.397</td>
<td>0.066</td>
<td>1320</td>
<td>2.45 3.58</td>
<td>**</td>
</tr>
<tr>
<td>Error (within animal groups)</td>
<td>28</td>
<td>0.001</td>
<td>0.00005</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant; ** Highly Significant

(CD *(P< 0.05) = 0.008 ; CD *(P< 0.01) = 0.011)
Table X DNA concentration in control and 15ppm. BHC exposed kidney of H. fossilis in different days interval. Values are replica of 5 fresh fish tissues + SEM, expressed in mg/100 gm.

<table>
<thead>
<tr>
<th></th>
<th>Control (A)</th>
<th>15-days (B)</th>
<th>30-days (C)</th>
<th>45-days (D)</th>
<th>60-days (E)</th>
<th>75-days (F)</th>
<th>90-days (G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.057</td>
<td>0.082</td>
<td>0.056</td>
<td>0.161</td>
<td>0.266</td>
<td>0.085</td>
<td>0.189</td>
<td></td>
</tr>
<tr>
<td>0.062</td>
<td>0.085</td>
<td>0.054</td>
<td>0.160</td>
<td>0.269</td>
<td>0.087</td>
<td>0.186</td>
<td></td>
</tr>
<tr>
<td>0.058</td>
<td>0.087</td>
<td>0.059</td>
<td>0.158</td>
<td>0.265</td>
<td>0.089</td>
<td>0.185</td>
<td></td>
</tr>
<tr>
<td>0.057</td>
<td>0.084</td>
<td>0.055</td>
<td>0.157</td>
<td>0.261</td>
<td>0.083</td>
<td>0.187</td>
<td></td>
</tr>
<tr>
<td>0.056</td>
<td>0.082</td>
<td>0.056</td>
<td>0.159</td>
<td>0.264</td>
<td>0.081</td>
<td>0.188</td>
<td></td>
</tr>
</tbody>
</table>

Mean

0.058 0.084ab 0.056b 0.159ab 0.265ab 0.085a 0.187ab
+ 0.001 +0.001 +0.001 +0.001 +0.001 +0.001 +0.001

a = Significantly differ from the Control group
b = Significantly differ from the treated groups.

ANOVA Analysis of variance for one way classified data with 5 observations in each group.

<table>
<thead>
<tr>
<th>Sources of variance</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F.</th>
<th>Tabulated value of F</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among animal groups</td>
<td>6</td>
<td>0.185</td>
<td>0.030</td>
<td>3.58</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Error (within the animal group)</td>
<td>28</td>
<td>0.001</td>
<td>0.00003</td>
<td>2.45</td>
<td>1026.67</td>
<td>**</td>
</tr>
</tbody>
</table>

*Significant ; ** Highly Significant

CD (P < 0.05) = 0.006 ; CD (P < 0.01) = 0.008
mg/100 gm), but it was found to be declined on 30th day (0.125 \pm 0.001 mg/100 gm; P<0.01) compared to control (Table-ix). The DNA content of the ovary of 45th day significantly increased over its control (0.434 \pm 0.003 mg/100 gm; ANOVA-5). The fall of DNA on 75th day of (0.251 \pm 0.003 mg/100 gm) was recorded; but enhanced over the control. However, 90th day test group ovarian DNA (0.280 \pm 0.002 mg/100 gm) was noted to be significantly enhanced over the 75th day test group and still maintained higher value compared to control (Table-ix).

Kidney: The DNA content of the kidney was observed in between 0.056 to 0.062 mg/100 gm of tissue in control set of fish. Significant increase (P<0.01) of DNA was recorded in the BHC treated kidney of 15th day (0.084 \pm 0.057 mg/100 gm of tissue; Table-x). The DNA content of kidney was found to be increased on 45th day (0.159 \pm 0.001 mg/100 gm) and continued up to 60th day (0.265 \pm 0.001 mg/100 gm of tissue); but on 75th day it was noted to be significantly reduced (0.085 \pm 0.001 mg/100 gm of tissue). On 90th day the DNA content significantly increased over 75th day test group and over its respective control (ANOVA-5).

Effect of BHC on the RNA content of Testis, Ovary and Kidney:

Testis: The RNA quantity of testis in control fish was recorded in between 0.331 to 0.337 mg/100 gm of fresh tissue. With the exposure to sublethal concentration of BHC the RNA of testis
significantly decreased on 75th day (0.185 ± 0.007 mg/100 gm of tissue) compared to other test group as well as control group of fish (Table-xi). But the highest quantity of RNA was noted on 45th day (0.99 ± 0.001 mg/100gm; P<0.01) over its control (ANOVA-7).

Ovary : The RNA value of control ovary varied from 0.456 to 0.464 mg/100 gm (Table-xii). The RNA of the BHC exposed ovary progressively increased upto 45th day over its control as well as previous test group (P<0.01; ANOVA-8). But on 60th day it was noted to be significantly decreased compared to that immediate previous group. The RNA content of the ovary on 90th day was significantly increased over the 75th day BHC treated ovary as well as control group.

Kidney : The RNA value of the kidney was observed in between 0.19 to 0.20 mg/100 gm of fresh tissue in control fish (Table-xiii). With the exposure to 15 ppm. BHC the kidney exhibited significantly increased of RNA from the 15th day (P<0.05) to 45th day with marked higher value on 45th day (P<0.01). Although, the RNA value insignificantly decreased on 60th and 75th day, yet on 90th day was significantly higher than control(P<0.05; ANOVA-9).
Table XI  RNA concentration in control and 15 ppm. BHC exposed testis of H.fossilis in different days interval. values are replica of 5 fresh fish tissues + SEM, expressed in mg/100 gm.

<table>
<thead>
<tr>
<th></th>
<th>Control (A)</th>
<th>15-days (B)</th>
<th>30-days (C)</th>
<th>45-days (D)</th>
<th>60-days (E)</th>
<th>75-days (F)</th>
<th>90-days (G)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.335</td>
<td>0.337</td>
<td>0.333</td>
<td>0.334</td>
<td>0.331</td>
<td>0.334</td>
<td>0.330</td>
</tr>
<tr>
<td></td>
<td>0.435</td>
<td>0.422</td>
<td>0.413</td>
<td>0.428</td>
<td>0.435</td>
<td>0.426</td>
<td>0.406</td>
</tr>
<tr>
<td></td>
<td>0.400</td>
<td>0.391</td>
<td>0.362</td>
<td>0.400</td>
<td>0.406</td>
<td>0.391</td>
<td>0.980</td>
</tr>
<tr>
<td></td>
<td>0.980</td>
<td>0.984</td>
<td>0.981</td>
<td>0.979</td>
<td>0.976</td>
<td>0.980</td>
<td>0.976</td>
</tr>
<tr>
<td></td>
<td>0.569</td>
<td>0.573</td>
<td>0.571</td>
<td>0.570</td>
<td>0.572</td>
<td>0.571</td>
<td>0.570</td>
</tr>
<tr>
<td></td>
<td>0.171</td>
<td>0.208</td>
<td>0.200</td>
<td>0.160</td>
<td>0.188</td>
<td>0.185</td>
<td>0.248</td>
</tr>
<tr>
<td></td>
<td>0.248</td>
<td>0.240</td>
<td>0.240</td>
<td>0.272</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean

|            | 0.334       | 0.426       | 0.391       | 0.980_{\text{ab}} | 0.571_{\text{ab}} | 0.185_{\text{ab}} | 0.248       |
|            | +0.001      | +0.003      | +0.006      | +0.001          | +0.001          | +0.007          | +0.005      |

\( a \) = Significantly differ from the control group
\( b \) = Significantly differ from the treated groups

ANOVA - 7 Analysis of variance for one way classified data with 5 observations in each group.

<table>
<thead>
<tr>
<th>Sources of variance</th>
<th>D.F.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F.</th>
<th>Tabulated value</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among animal groups</td>
<td>6</td>
<td>1.743</td>
<td>0.290</td>
<td>22.307</td>
<td>2.45</td>
<td>3.58 **</td>
</tr>
<tr>
<td>Error (within the animal groups)</td>
<td>28</td>
<td>0.377</td>
<td>0.013</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant; ** Highly Significant

\((\text{CD} (P<0.05)) = 0.147 \); \((\text{CD} (P<0.01)) = 0.198 \)
Table XII RNA concentration in control and 15 ppm BHC exposed ovary of H. fossilis in different days interval. Values are replica of 5 fresh fish tissues + SEM, expressed in mg/100 gm.

<table>
<thead>
<tr>
<th></th>
<th>Control (A)</th>
<th>15-days (B)</th>
<th>30-days (C)</th>
<th>45-days (D)</th>
<th>60-days (E)</th>
<th>75-days (F)</th>
<th>90-days (G)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.459</td>
<td>0.634</td>
<td>0.750</td>
<td>0.980</td>
<td>0.560</td>
<td>0.407</td>
<td>0.698</td>
</tr>
<tr>
<td></td>
<td>0.454</td>
<td>0.611</td>
<td>0.740</td>
<td>0.971</td>
<td>0.594</td>
<td>0.415</td>
<td>0.676</td>
</tr>
<tr>
<td></td>
<td>0.462</td>
<td>0.627</td>
<td>0.700</td>
<td>0.980</td>
<td>0.560</td>
<td>0.425</td>
<td>0.717</td>
</tr>
<tr>
<td></td>
<td>0.464</td>
<td>0.641</td>
<td>0.740</td>
<td>0.971</td>
<td>0.578</td>
<td>0.407</td>
<td>0.685</td>
</tr>
<tr>
<td></td>
<td>0.456</td>
<td>0.658</td>
<td>0.715</td>
<td>1.00</td>
<td>0.568</td>
<td>0.450</td>
<td>0.707</td>
</tr>
</tbody>
</table>

Mean

|        | 0.459       | 0.634<sup>ab</sup> | 0.729<sup>ab</sup> | 0.980<sup>ab</sup> | 0.572<sup>ab</sup> | 0.420<sup>ab</sup> | 0.696<sup>ab</sup> |
|        | ±0.001      | ±0.006        | ±0.008        | ±0.004        | ±0.005        | ±0.007        | ±0.006        |

* = Significantly differ from the control group
b = Significantly differ from the treated groups.

AVONA - 8 Analysis of variance for one way classified data with 5 observations in each group.

<table>
<thead>
<tr>
<th>Sources of variance</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F.</th>
<th>Tabulated value</th>
<th>Inference of F.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among animal groups</td>
<td>6</td>
<td>1.063</td>
<td>0.177</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error (within the animal groups)</td>
<td>28</td>
<td>0.003</td>
<td>0.0001</td>
<td>1770</td>
<td>2.45</td>
<td>3.58 **</td>
</tr>
</tbody>
</table>

* Significant; ** Highly Significant

CD (P < 0.05) = 0.012; CD (P < 0.01) = 0.016
**Table XIII**  RNA concentration in control and 15 ppm. BHC exposed kidney of *H. fossilis* in different days interval. Values are replica of 5 fresh fish tissues $\pm$ SEM, expressed in mg/100 gm.

<table>
<thead>
<tr>
<th>Control (A)</th>
<th>15-days (B)</th>
<th>30-days (C)</th>
<th>45-days (D)</th>
<th>60-days (E)</th>
<th>75-days (F)</th>
<th>90-days (G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.197</td>
<td>0.586</td>
<td>0.749</td>
<td>0.899</td>
<td>0.384</td>
<td>0.290</td>
<td>0.529</td>
</tr>
<tr>
<td>0.200</td>
<td>0.581</td>
<td>0.746</td>
<td>0.902</td>
<td>0.379</td>
<td>0.300</td>
<td>0.530</td>
</tr>
<tr>
<td>0.190</td>
<td>0.583</td>
<td>0.744</td>
<td>0.904</td>
<td>0.376</td>
<td>0.294</td>
<td>0.528</td>
</tr>
<tr>
<td>0.186</td>
<td>0.582</td>
<td>0.747</td>
<td>0.897</td>
<td>0.380</td>
<td>0.296</td>
<td>0.525</td>
</tr>
<tr>
<td>0.192</td>
<td>0.578</td>
<td>0.749</td>
<td>0.893</td>
<td>0.376</td>
<td>0.295</td>
<td>0.528</td>
</tr>
</tbody>
</table>

Mean

| 0.193      | 0.582$^a$  | 0.747$^a$  | 0.899$^a$  | 0.379       | 0.295       | 0.528$^a$  |
| $\pm$ 0.001 | $\pm$0.001 | $\pm$0.001 | $\pm$0.001 | $\pm$0.001  | $\pm$0.001  | $\pm$0.001  |

$a$ = Significantly differ from the control

$\text{ANOVA} \quad$ Analysis of variance for one way classified data with 5 observations in each group.

<table>
<thead>
<tr>
<th>Sources of variance</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F.</th>
<th>Tabulated value of F</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among animal groups</td>
<td>6</td>
<td>0.206</td>
<td>0.034</td>
<td></td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>Error (within the animal groups)</td>
<td>28</td>
<td>1.675</td>
<td>0.059</td>
<td></td>
<td>0.576 2.45 3.58</td>
<td></td>
</tr>
</tbody>
</table>

CD($\text{P} < 0.05$) $= 0.313$ ; CD ($\text{P} < 0.01$) $= 0.422$
Discussion:

Metabolic disturbances leading to the disruption of gonadal parameters of *Heteropneustes fossilis* due to pesticide toxicity have been observed in this investigation. It has also been observed that the protein concentration of the treated testis was recorded at higher level on 15th day, however, declined significantly on 75th and 90th day of BHC exposure compared to their respective control.

The BHC exposed ovarian protein registered an overall enhancement over their respective control. The maximum quantity of protein was recorded on 45th day, however, further depleted on 75th and 90th day experimentation (Fig. 9b). Similarly kidney protein also maintained higher value compared to their control. The kidney protein on 45th day was significantly lower (A<0.01 ANOVA; Fig.9c) than the other test group fish. It has been postulated that the decrease of protein and RNA in certain stages of test group fishes are associated with the increased lysosomal enzymatic activity. This could be due to the adverse effect of BHC on the lysosomal membrane which releases nucleases and proteases affecting RNA and protein metabolism (Dubale & Awasthi, 1982).
Fig. 9(a): Effect of 15 PPM BHC on total Protein Content of Testis.
This view has been confirmed by the recorded fall of protein in the 30th day, 75th day and 90th day BHC exposed testis of *H. fossilis*. Reduction of protein are considered probably due to formation of protein complexes on the cell membrane of tissues since BHC shows same affinity towards SH group of protein (Agarwal 1992). This results in the inhibition of active transport through cells, blocked enzymatic activity and change conformation and solubility of the protein. (Valle and Ulmer, 1982; Sahana et al., 1986). Thus BHC disrupts the fundamental physiological and biochemical mechanism. However, the erratic pattern of the protein concentration of the 15th, 45th and 60th day BHC exposed testis cannot be explained with this view. The fall of aminoacid pools as well as the aminoacid content in the melathion exposed *lamellidens marginalis* was brought about by aminotransferase activites corroborated with the decrease in protein content due to proteolysis possibly to compensate the decrease in the aminoacid pool and to overcome the impending energy demand under toxicant stress.

Awasthi et al., (1984) exposed *H. fossilis* in a sublethal concentration of melathion and dimethoate and noted initial decrease of protein and thereafter gradual enhancement observed in the latter stage of experiment. Drozdz et al.,(1976), Sanker and Seth (1986) noticed decreased total protein content of blood and liver in nitrogen dioxide exposed guineapig. Further the reduced value of protein was noted in the brain of *Periplaneta americana* after 60 minutes exposure to 2% melathion in acetone fream unresoled. (Saxena and Saxena 1991). Decreased protein content could have resulted in
Fig. 9(b) Effect of 15 PPM BHC on total Protein Content of ovary
Protein (mg/gm)
Control
Treated
DAYS

Fig. 9(c) Effect of 15 PPM BHC on total Protein Content of Kidney.

Protein (mg/gm)

Control
Treated

DAYS

Fig. 9(c) Effect of 15 PPM BHC on total Protein Content of Kidney.
atrophy of the testis and reduced the activity at resting stage of muscles which would have led to the accumulation of glycogen and lipid (Piska et al., 1992). The reduction of total protein content in the testis appeared to be related to the action of BHC on nucleic acid also suggested by Haqqi and Adhami (1979). This may effect protein content of a tissue either by (a) inhibiting RNA transcription (not observed in this investigation) or (b) inhibition of uptake of aminoacid into the polypeptide chain or (c) increasing the rate of degradation of protein in the cell. While in case of (a) both RNA and protein are reduced and (b) and (c) only the protein value is reduced which was evident in this investigation. fig. 10

Protein constitutes the basic material of all cell are utilised in the formation of cellular products and can also supply energy in need. The enhanced value of protein in the ovary and kidney of BHC exposed fish may be due to the hypertrophy of the organs. However, only marginal enhancement of protein over their respective control after prolonged period of chronic exposure to BHC may be due to the development of pesticide tolerance. The enhancement of protein synthesis in normal embryonic growth and differentiation of dividing cell is a general phenomenon. Choudhuri and Mandal (1980) observed increased quantity of RNA and protein by two to three orders of magnitude in oocytes of mature unfertilised eggs of H. fossilis. Vanosten and Gibson (1975) reported the increased protein content in the rat liver after 32 hours of paraquate administration. Moreover, Saxena and Saxena (1991) noted the
Fig. 10.: Effect of 15 PPM BHC on Protein content of Testis, Ovary and Kidney in (three dimension)
increase in protein content on treatment with 2% melathion in benzene of P. americana which might be either an induction phenomenon or due to blocking the synthesis of messenger for the protein repressor which in turn would stimulate protein synthesis by depression. The increased value of protein synthesis in the ovary and kidney of BHC exposed H. fossils over the control reflects the stimulated protein synthesis of detoxicating enzymes, at the expense of glycogen to meet the additional energy requirement for the synthetic activity of the tissues. Balakrishnan et al. (1985) observed that protein content of the albino rat liver was increased when treated with BHC. Shivanandappa and Krishnakumari (1981) also recorded enhanced value of protein in the liver of rat fed with 750 and 1500 ppm dietary BHC.

Since protein synthesis seems to be enhanced and homeostatic mitochondrial conformations are present until very severe damage is observed and a direct action of the toxicant on enzyme activity is unfeasible (Marigomez et al., 1990). Either an increased excretory activity or an enhancement of protein synthesis might account for the mitochondria mainly involved in substance accumulation and therefore are less energetic. The general thought is that most divalent cations cause the impairment of oxidative phosphorylation (Crespo and Sala 1986); but this is true when the organism suffer from acute toxicity (Marigomez et al., 1990).

The DNA of the BHC exposed testis and kidney enhanced
Fig. 11(a): Effect of 15 PPM BHC on DNA content of Testis.
on 15th day but declined on 30th day while in case of ovary the rise of DNA continued up to the 45th day of experimentation (Fig. 11b, 11c). The significantly (P<0.01) increased DNA content at the initial stages of BHC exposed testis and kidney (Fig.10) might be responsible for the enhanced protein synthesis. In support of this view Sharma & Goswami (1984) recorded higher quantity of DNA at the early stages of chick embryonic carcinogeneses and suggested higher protein synthesis. However, the fall of DNA in these two organs might be due to the tissues attempt to repair the damage. Similarly the ovary after pesticide exposure exhibited significant rise of DNA upto 45th day (Table-ix) might be "unusual DNA" as explained; but thereafter the DNA value significantly lowered up to 90 days, however, higher than the control. It has been suggested that organochlorine pesticides are reported to inhibit the synthesis of DNA, RNA and Protein in various biological systems including Tetrahymena pyriformis (Saxena et al, 1981); although Choudhuri & Mandal (1980) suggested several fold increased DNA content per oocyte during the development of H. fossilis while protein and RNA increased only in the metabolic stage.

The enhanced quantity of DNA over their control in the pesticide exposed fish organs of this experiment from the 45th day might be due to the active metabolism brought about by the BHC induction. Nasiell et al, (1978) suggested that enhanced DNA under toxicant stress is necessary for the survival of the cells. These works further stated that the enhanced DNA is of unusual nature
Fig. 11(b) Effect of 15 PPM BHC on DNA content of ovary.
Fig. 11(c) : Effect of 15 PPm BHC on DNA content of Kidney.
and helps in the transformation of normal cell to the neoplastic cells.

Suzuki et al (1980) suggested that the increased DNA may enhance survivability and growth ability of the cells under adverse condition. But our experimental findings and subsequent SEM images suggest a tendency towards normalisation of the tissues after prolonged exposure to sublethal dose of BHC. This may be due to the development of pesticide tolerance of fish after long term exposure to BHC. Sharasin and Michele (1976) suggested that all lesion in cellular DNA caused by chemical carcinogen/mutagen are repaired by DNA repair system. In respective regeneration of tissues the surviving cells are stimulated to divide faster to compensate for damage. During mitosis the DNA strand is first replicated, therefore the content in the particular cell is duplicated as evident from the work of Saxena et al (1981). They observed the recovery within 3 days from the effect of toxicant stress after transferring the ciliates from DDT to normal medium. The high DNA content of the ovary and kidney even after BHC exposure might be attributable to high frequency of rejection. Because the DNA of higher organism contains repeated nucleotide sequence frequency (Britten and Kohne 1969). The fall of DNA in the BHC exposed testis on 30th day may be due to the instability in this macromolecules. The increase in DNA of the BHC exposed testis thus indicates that the majority of the surviving cells are engaged in active mitosis; but this does not last long due to the stress of the pesticide as a result of which the DNA content falls significantly on 90th day (Fig.11a)
Fig. 12(a): Effect of 15 PPM BHC on RNA content of Testis.
Shastry and Agarwal (1976) studied the effect of CCl_4 on the DNA content of liver of H. fossilis and found that at initial stage the DNA content increased; but with the increasing time of exposure the DNA content decreased. The fall of DNA of the BHC exposed gonads and kidney may be inferred as a hinderance in growth. Walter et al (1980) noted reduced DNA and RNA fraction of lymphocytes in vitro due to melathion treatment. Gantayat and Patnaik (1975) noted reduction of relative protein content, DNA:RNA ratio and protein content in testis and liver of rat due to heavy metal treatment. Increased protein content and decreased DNA and RNA level in the kidney and ovary of pesticide exposed rat was demonstrated and suggested the disturbances in the synthesis of these macromolecules (Rip and Cherry 1976; Vanosten and Gibson 1975) are in conformity with the present findings.

The highest value of RNA was recorded in all these three organs after 15 ppm BHC exposure on the 45th day (Fig.15) and the fall of RNA was noted on the 75th day testis and ovary compared to control; but the kidney RNA maintained higher value compared to control throughout the period of experimentation (Fig.12c). The enhanced quantity of RNA might be associated with the quantity of DNA and protein synthesis of the BHC exposed organs particularly in the synthesis of the detoxicating enzymes or with the gonadotrophin steroids. Higher quantity of RNA in the liver of rat fed with dietary BHC was attributable to the hypertrophy of the gonads suggested by Shivanandappa and Krishnakumari.
Fig. 12(b): Effect of 15 PPM BHC on RNA content of ovary.
Fig. 12(c) Effect of 15 PPM BHC on RNA content of Kidney.
However, it appears to be difficult to explain the enhanced value of RNA in this experiment with the available literature. The higher values of RNA in all these organs suggest co-ordinated activities bringing the higher rate of protein synthesis. Fig. 13, 14, 15

Protein and RNA content of liver of Cyprinus carpio is reported to be declined after lead nitrate exposure (Narbonne et al. 1975). Similar observations after treatment with various pollutants in the liver and kidney were also made by several workers (Shukla et al. 1976; Panigrahi and Mishra 1978; Sharma et al. 1979; Nammalwar 1984).

The presently available literature and the findings of the present investigation suggest that the effect of BHC on the biochemical structure of the cell is marked in the pathway of protein metabolism. It has been observed that the BHC inhibited/altered the cellular synthesis by binding with DNA and produced a delayed decrease of protein synthesis. The decrease of protein synthesis in later part of the experiment compared to that of the previous test group may be due to the blockage of m-RNA production. The erratic pattern on DNA synthesis may be explained that the BHC interfere with the DNA metabolism at the onset of the experiment, probably by binding structurally with DNA and deviating from the usual synthesis. This pesticide covalently binds to the macromolecular structure of protein, DNA and RNA as a result the DNA has been observed to be enhanced. The enhanced DNA is an
Fig. 13 Showing protein content (mg/gm) in testis, ovary and kidney of *H. fossilis* after treatment with BHC.
important factor in the development and maintenance of the cells in terms of local growth ability of the testis, ovary and the renal cells even under an adverse environment. It may be the protein part which is utilised to meet the additional energy demand of these cells by way of gluconeogenesis. It is however, interesting to note that atleast the protein was built upto normal or near normal level. After chronic exposure the fish did succeed in raising the energy metabolism to a certain extent.

The kidney and ovarian protein and nucleic acid presented enhanced value compared to their respective control (Table-xiv). The variation of the concentration of protein and nucleic acid in these tissues after BHC treatment might be associated with the tissues proliferative activity. For e.g. kidney of fish is a haemopoietic organ and exhibits fast dividing rate of cells (Matty 1985) hence the higher quantity of protein, DNA and RNA was noticeable on 90th day of this experiment. However, to substantiate this explanation more evidences based on labelled study both in vivo and in vitro are necessary. In an attempt Choudhuri and Mandal (1980) explained that the presence of extra-chromosomal DNA might be responsible for enhanced protein, DNA and RNA quantity in ovary. But this explanation could not be extended to the testis under BHC treatment although testis is also a proliferative organ.
Table XIV  Summary of the mean values of Protein, DNA and RNA concentration of the gonads and Kidney of *H. fossilis* both at control and after 15ppm of BHC treatment, values are expressed mg/gm for Protein & mg/100gm for DNA & RNA + SEM.

<table>
<thead>
<tr>
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<th>Control (A)</th>
<th>15-days (B)</th>
<th>30-days (C)</th>
<th>45-days (D)</th>
<th>60-days (E)</th>
<th>75-days (F)</th>
<th>90-days (G)</th>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Protein</td>
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<td>23.65ab</td>
<td>9.78a</td>
<td>21.49ab</td>
<td>15.64b</td>
<td>9.06ab</td>
<td>5.31ab</td>
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<tr>
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<td>+0.59</td>
<td>+0.34</td>
<td>+0.27</td>
<td>+0.39</td>
<td>+0.19</td>
<td>+0.27</td>
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<tr>
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<td>0.109b</td>
<td>0.033ab</td>
<td>0.186ab</td>
<td>0.184ab</td>
<td>0.066ab</td>
<td>0.152a</td>
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<tr>
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<td>+0.003</td>
<td>+0.002</td>
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<td>0.426</td>
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<td>0.980ab</td>
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<tr>
<td>Protein</td>
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<td>35.06 ab</td>
<td>36.98 ab</td>
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<tr>
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<tr>
<td></td>
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<td>+0.005</td>
<td>+0.007</td>
<td>+0.006</td>
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<td><strong>Kidney</strong></td>
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<td></td>
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<td>+ 0.30</td>
<td>+0.24</td>
<td>+ 0.59</td>
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<td>+ 0.38</td>
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<tr>
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<td>+0.001</td>
<td>+0.001</td>
<td>+0.001</td>
</tr>
</tbody>
</table>

a: Significantly differ from its control
b: Significantly differ from its treated groups.
Figure 14: Showing DNA content (mg/100gm) in testis, ovary, and kidney of *H. fossilis* after treatment with BHC.
Fig. 15: Showing RNA Content (mg/100 gm) in testis, ovary, kidney of H. fossilis after treatment with BHC.
It thus appears that BHC exerts some functional toxicity on the gonads and kidney of *H. fossilis* when exposed chronically for a period of 90 days at the dose level of 15 ppm. But treatment for a longer duration i.e. after 45 days leads to the development of resistance to the pesticide. A similar notion was drawn by Bhatnagar and Soni (1990) on the male gonads of Swiss mice administered with phosphamidon sub-chronically for a period of 60 days. Moreover, the male gonads are susceptible to the BHC toxicant stress compared to that of the female counterpart and BHC at dilute concentration and at longer period appeared to be relatively less harmful for the gonads than that for shorter period.

Summary:

The nucleic acids of BHC exposed gonad and kidney tissues of *H. fossilis* was estimated by the method of Schmidt Thaunhauser - Schnider (1946) and the protein was estimated by Lowry et al (1951).

(1) The protein content of the testis either increased up to 45th day or decreased on the 75th and 90th day of BHC exposure compared to control. Protein content of the ovary was gradually increasing up to 45th day. The kidney and the ovarian protein concentration registered an overall enhancement over the control throughout the period of experimentation.
(2) The protein in certain stages decreased as a result of BHC toxicity. Reduction of protein might be the result of protein complex formation.

(3) The DNA concentration of the testis of BHC treated H. fossilis exhibited erratic values. However, significant fall of DNA on 75th day with subsequent rise on 90th day was recorded. The ovarian DNA of treated fish was recorded at the highest level on the 45th day. However, it maintained a comparatively higher DNA value over its control. The DNA of BHC treated kidney registered its highest concentration on 60th day thereafter fall of DNA was recorded.

(4) The RNA concentration of BHC treated fish exhibited sharp elevation on 45th day. A tendency of being revived to normal or near normal on 90th day was noticed in the testis and ovary. The kidney also followed the same trend except on 90th day where RNA was significantly higher over its control.

(5) The present findings suggestive of the alteration of DNA synthesis induced by BHC probably be associated with the development and maintenance of local growth under adverse condition.
Effect of BHC on Ascorbic acid of Testis, Ovary and Kidney of *H. fossilis*. 
Ascorbic acid is a water soluble vitamin nutrient. Because of its availability in abundance in natural fresh foods, neither was it felt nor there were any manifestation of signs and symptoms of lack of it because more complicated so did its disturbance of metabolism, resulting from the deficiency or absence of ascorbic acid from the diet. Ascorbic acid promotes hydroxylation reactions in many biosynthetic process. Since Primates (including man), guineapig, flying mammals and fishes can not synthesis the ascorbic acid of their own, therefore, they are solely dependent on dietary source of ascorbic acid (Stone 1965). The dependency on ascorbic acid is due to loss of enzyme L-gulonolactone oxidase that catalyses the last step in the conversation of glucose to ascorbic acid (Young and Newberne 1981).

\[
\begin{align*}
\text{Ascorbic acid} \quad & \quad \text{Sepiaptenin} \\
\text{D - Glucose} \quad & \quad \text{Guanosine Triphosphate} \\
\downarrow \quad & \quad \downarrow \\
\text{D - Gulonolactone} \quad & \quad \text{Dihydronopte r in} \\
\downarrow \quad & \quad \downarrow \\
\text{Enzymeblock} \quad & \quad \text{L-Sepiaptenin} \\
\downarrow \quad & \quad \downarrow \\
\text{Ascorbic acid} \quad & \quad \text{L-erythro 5,6,7,8} \\
\quad & \quad \text{tetra hydrobiopterin}
\end{align*}
\]

Fig. 16 : Deletion of an enzyme, as a result of genetic factors in a metabolic pathway leads to the evolution of vitamin requirement.
Ascorbic acid accumulates intensely in the gonads in significant amount particularly in the testis. Ascorbic acid is present in high concentration in the interstitial cells of the testis and ovary. Gonadal ascorbate showed significant increase during breeding season in both sexes of the frog *R. tigrina* (Kumari et al, 1991). The role of ascorbic acid in reproductive physiology has been investigated by different workers. Stubbs and McKernan (1967) noted sex difference in tissue concentration of vitamin C in rats. The tissue ascorbate concentrations were shown to be significantly higher in all the tissue of male rats with the exception of adrenal and bone. The activities of ascorbic acid synthesizing enzyme were also found to be higher in male rats.

Salmon and Stubbs (1961) has demonstrated a marked hypophysial influence on ascorbic acid biosynthesis in rats. Hypophysectomy caused no significant change in the activities of the enzymes of female rats with the exception of a considerable decrease in gulonolactone hydrolase (Stubbs and McKernan 1967) in both sexes. On the other hand, the enzymatic activities of the gulonate NADP oxidoreductase, glucuronolactone hydrolase and gulonolactone, oxidoreducase in hypophysectomized males were significantly diminished to
the level characteristic of female rat.

Stimulation of the rabbit ovary by gonadotropin causes a rapid and marked decrease in ovarian interstitial cell ascorbate content suggesting a correlation between ascorbate level and estrogenic hormone production. Jennings (1970) showed that gonadotropic stimulation of the corpus luteum in pregnancy causes a rapid decrease in ascorbate content. Deb and Chatterjee (1963) found that "alloxan diabetes" produced disturbance in the estrous cycle of rats which could be corrected by injection of ascorbic acid.

Ascorbic acid is also known to possess antioxidant activity and its function in animal metabolism may in part relate to this property. The antioxidant activity of ascorbic acid is partially due to the fact that it enhances the activity of Vitamin E (Niki et al., 1984 and Bendich et al., 1986). However, in relation to normal physiology, the main electron transfer role of vitamin C appears to be that of reducing metals so that the associated enzyme system can act in the transport of molecular oxygen as in hydroxylation of proline during collagen synthesis (Bates et al., 1972) and in the formation of noradrenaline.

Staudinger et al., (1961) studied the system in animal tissue and found the presence of ascorbic acid dependent NADH oxidation in the rat liver and kidney microsomes. This microsomal ascorbic acid dependent oxidase is highly specific for NADH.
The relation between the gonadal ascorbic acid and the pesticide toxicity is still obscure not only in fish but also in other animals. Moreover, ascorbate supplementation can reduce the toxic effect of certain chemicals like carbon tetrachloride (Kunert & Tappel 1983) cyclophosphamide (Kola et al, 1989). Vitamin C reduces tumour incidence in mice exposed to ultraviolet light and in vitro studies have shown inhibition of growth of human melanoma, neuroblastoma and leukaemia cells (Gardiner and Duncan 1989). Thus ascorbic acid has been shown to have antimutagenic/antitoxic effects in some systems. Therefore, it has been attempted to evaluate the effect of pesticide on the gonadal and renal ascorbic acid in fish.

Methods and Materials: Two sets of fishes were considered in this investigation. The control group exposed to 1 ml. of acetone in 15 litre of water while the test groups were exposed to 15 ppm BHC as described under materials and methodology (Chapter-II).

Results: The results of this investigation are presented in the table from xv to xvii.

Effect of BHC on the Ascorbic acid content of Testis, Ovary & Kidney:

Testis: The ascorbic acid concentration of testis was noted in between 0.762 to 0.820 mg/100 gm of tissue in the control
Table XV  Ascorbic acid concentration in control and 15 ppm BHC exposed Testis of *H. fossilis* in different days interval. Values are replica of 5 fresh fish tissues + SEM, expressed in mg/100 gm.

<table>
<thead>
<tr>
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<th>Control (A)</th>
<th>15-days (B)</th>
<th>30-days (C)</th>
<th>45-days (D)</th>
<th>60-days (E)</th>
<th>75-days (F)</th>
<th>90-days (G)</th>
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<td>0.425</td>
<td>0.727</td>
<td>0.401</td>
<td>0.857</td>
</tr>
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</table>

Mean  
0.801  1.019<sup>ab</sup>  0.649<sup>ab</sup>  0.435<sup>ab</sup>  0.706<sup>ab</sup>  0.374<sup>ab</sup>  0.884<sup>ab</sup>  
+0.008  +0.007  +0.008  +0.014  +0.008  +0.007  +0.006

a = Significantly differ from the control.  
b = Significantly differ from the treated groups.

**ANOVA** 10 Analysis of variance for one way classified data with 5 observations in each group.

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<th>M.S.</th>
<th>F</th>
<th>Tabulated value of F at 0.05</th>
<th>Inference</th>
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<tr>
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</tbody>
</table>

* Significant; ** Highly significant  
CD<sub>P<0.05</sub> = 0.024; CD<sub>P<0.01</sub> = 0.033
group, significant increase of ascorbic acid was noted on 15th day BHC treated testis (1.01 ± 0.007 mg/100 gm) compared to control (0.80 ± 0.008 mg/100 gm), (P<0.01; ANOVA-10). However, significantly depleted value of ascorbic acid was recorded upto 75 days of BHC exposure (Table-xv), while the 90th day of exposure again showed significantly higher ascorbic acid compared to control(A). Comparison amongst different test groups revealed significant variation of mean values (CD at 0.01 = 0.033).

Ovary: The ascorbic acid concentration of ovary ranged between 0.043 to 0.501 mg/100gm in control group. Exposure to BHC the ascorbic acid registered an erratic rise and fall in the ovary (Fig. 18). The 30th day and 45th day ascorbic acid significantly depleted (P<0.01; ANOVA-11). However, significantly increased ascorbic acid was detected from 60 days to 90 days. Comparison amongst the different treated groups showed significant difference of means (CD at 0.01 level 0.0002).

Kidney: The kidney ascorbic acid estimated in different days of exposure registered a declining tendency throughout the period of experimentation after 30 days of exposure. Maximum fall of ascorbic acid was recorded on 45th day (0.148 ± 0.007 mg/100gm). The ascorbic acid concentration was attempted to elevate over the previous test groups of kidney from 60th day to 90th day, yet below the control level. Significant difference of means were recorded amongst the test group except in F & C (Table-xvii).
Table XVI  Ascorbic acid concentration in control and 15ppm. BHC exposed ovary of *H. fossilis* in different days interval. values are replica of 5 fresh fish tissues + SEM, expressed in mg/100gm.

<table>
<thead>
<tr>
<th></th>
<th>Control (A)</th>
<th>15-days (B)</th>
<th>30-days (C)</th>
<th>45-days (D)</th>
<th>60-days (E)</th>
<th>75-days (F)</th>
<th>90-days (G)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.490</td>
<td>0.877</td>
<td>0.449</td>
<td>0.315</td>
<td>0.500</td>
<td>0.657</td>
<td>0.651</td>
</tr>
<tr>
<td></td>
<td>0.501</td>
<td>0.930</td>
<td>0.480</td>
<td>0.326</td>
<td>0.471</td>
<td>0.630</td>
<td>0.638</td>
</tr>
<tr>
<td></td>
<td>0.438</td>
<td>0.917</td>
<td>0.440</td>
<td>0.334</td>
<td>0.454</td>
<td>0.657</td>
<td>0.651</td>
</tr>
<tr>
<td></td>
<td>0.478</td>
<td>0.941</td>
<td>0.480</td>
<td>0.315</td>
<td>0.490</td>
<td>0.638</td>
<td>0.661</td>
</tr>
<tr>
<td></td>
<td>0.480</td>
<td>0.837</td>
<td>0.440</td>
<td>0.334</td>
<td>0.519</td>
<td>0.646</td>
<td>0.651</td>
</tr>
</tbody>
</table>

Mean

|        | 0.48        | 0.90         | 0.45         | 0.32         | 0.49         | 0.64         | 0.65         |
|        | +0.009      | +0.017       | +0.008       | +0.003       | +0.009       | +0.004       | +0.003       |

a = Significantly differ from the control  
b = Significantly differ from the treated groups.

ANOVA 11 Analysis of variance for one way classified data with observations in each group.

<table>
<thead>
<tr>
<th>Sources of variance</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F. Tabulated value</th>
<th>Inference of F at</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>among animal groups</td>
<td>6</td>
<td>1.046</td>
<td>0.174</td>
<td></td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>Error (within the animal groups)</td>
<td>28</td>
<td>0.011</td>
<td>0.0003</td>
<td></td>
<td>580</td>
<td>2.45 3.58**</td>
</tr>
</tbody>
</table>

* Significant ; ** Highly Significant

CD (P<0.05) = 0.0002; CD (P<0.01) = 0.0002
Table XVII  Ascorbic acid concentration in control and 15 ppm. BHC exposed kidney of *H.fossilis* in different days interval. Values are replica of 5 fresh fish tissues + SEM, expressed in mg/100gm.

<table>
<thead>
<tr>
<th>Control (A)</th>
<th>15-days (B)</th>
<th>30-days (C)</th>
<th>45-days (D)</th>
<th>60-days (E)</th>
<th>75-days (F)</th>
<th>90-days (G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.571</td>
<td>0.629</td>
<td>0.279</td>
<td>0.143</td>
<td>0.267</td>
<td>0.352</td>
<td>0.417</td>
</tr>
<tr>
<td>0.560</td>
<td>0.574</td>
<td>0.288</td>
<td>0.124</td>
<td>0.287</td>
<td>0.325</td>
<td>0.450</td>
</tr>
<tr>
<td>0.561</td>
<td>0.534</td>
<td>0.279</td>
<td>0.170</td>
<td>0.296</td>
<td>0.297</td>
<td>0.417</td>
</tr>
<tr>
<td>0.558</td>
<td>0.574</td>
<td>0.310</td>
<td>0.162</td>
<td>0.287</td>
<td>0.313</td>
<td>0.431</td>
</tr>
<tr>
<td>0.556</td>
<td>0.574</td>
<td>0.310</td>
<td>0.143</td>
<td>0.304</td>
<td>0.336</td>
<td>0.417</td>
</tr>
</tbody>
</table>

Mean

<table>
<thead>
<tr>
<th>Control (A)</th>
<th>15-days (B)</th>
<th>30-days (C)</th>
<th>45-days (D)</th>
<th>60-days (E)</th>
<th>75-days (F)</th>
<th>90-days (G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.56</td>
<td>0.58b</td>
<td>0.29a</td>
<td>0.15ab</td>
<td>0.29ab</td>
<td>0.32ab</td>
<td>0.42ab</td>
</tr>
</tbody>
</table>

+0.002      +0.013      +0.006      +0.007      +0.005      +0.005

a = Significantly differ from the control
b = significantly differ from the treated groups.

**ANOVA - 12**  Analysis of variance for one way classified data with 5 observations in each group.

<table>
<thead>
<tr>
<th>Sources of variance</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F.</th>
<th>Tabulated value of F at 0.05</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among animal groups</td>
<td>6</td>
<td>0.731</td>
<td>0.121</td>
<td></td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>Error (within the animal group)</td>
<td>28</td>
<td>0.007</td>
<td>0.0002</td>
<td></td>
<td>605</td>
<td>2.45</td>
</tr>
</tbody>
</table>

*Significant ; ** Highly Significant.

CD(P<0.05) = 0.016 ; CD (P<0.01) = 0.022
Discussions: Benzene hexachloride causes deficiency of the gonadal ascorbic acid on 30th and 45th day and kidney ascorbic acid throughout the period of experimentation. Rudrapal et al., (1975) stated that lead poisoning causes the alteration of ascorbic acid in rats. The role of ascorbic acid in the cellular process in general and more particularly in the gonads of fishes are very much untraced. However, ascorbic acid deficiency is usually associated with microcytic hypochromic anaemia by interfering with the absorption of iron from the gastro-intestinal (G-I) tract and also by its action of folic acid. Poikilocytosis and aminocytosis are also common in anaemia due to ascorbic acid deficiency in fish and in other animals (Baker 1967). Dhawan et al., (1988) suggested that supplementation of ascorbic acid reduces the pb toxicity in rats.

Ascorbic acid is related with the synthesis of different vitamins within the body. Several vitamins such as thiamin, riboflavin, pantothenic acid and biotin are related to the proper functioning of ascorbic acid and vice versa. (Chatterjee 1961, Herbert 1974, Campbell 1975). Due to deficiency of these vitamins there is a decrease in the tissue distribution and urinary excretion of ascorbic acid which may be due to the inhibition of certain enzymes associated with ascorbic acid biosynthesis (Basu et al., 1974). Deficiency of ascorbic acid most probably causes the alteration of
Fig: 17: Effect of 15 PPm BHC on Ascorbic acid content of Testis.
the microsomal structure (Staudinger et al., 1961) rather than biosynthetic process directly. The reason for the deficiency of ascorbic acid perhaps due to enzyme inhibition triggered by the pesticide, thus the interstitial cells were destroyed as revealed by histopathological study of testis. Moreover, the loss of ascorbic acid results the impairment of immune response (Levine 1986). In order to give an explanation for the mechanism of the decrease of ascorbic acid content in the tissues in infection, Czina (1959) suggested the increase expenditure of glucoronic acid. The glucoronic acid is the precursor of ascorbic acid and one of the normal detoxicating agents.

Although the deficient value of ascorbic acid in kidney was observed throughout the experiment, yet gradual enhancement was also noticed over the previous test groups from the 45th day of experiment and continued up to 90th day (Fig. 19) which was still lower than the respective control. The deficiency of the ascorbic acid might be due to the increased metabolic need for ascorbate undoubtedly occurs in areas such as adrenals producing more adrenaline and corticoids and the immune system producing more antibodies (Siegel, 1975; Siegel & Morton 1977) and other substances to overcome the toxicant stress. The macrophages utilizing more ascorbate with their increased activity and the production and protection of C-AMP and C-GMP with the subsequent increased activity of other endocrine gland (Lewin 1976). Cathcart (1981) has suggested that there must be a tremendous draw on ascorbate,
Fig. 18: Effect of 15 PPM BHC on Ascorbic acid content of Ovary.
locally by increased metabolic rates in the primarily infected tissues and the infecting organisms themselves liberate toxins which are neutralised by ascorbate, but in the process destroys ascorbate. Moreover, under stress the adrenals are capable of utilising large amounts of ascorbate. The ascorbic acid seems to reduce the systematic toxicity considerably but does not eliminate the primary infection (Cathcart 1981).

Significant increase of ascorbic acid after BHC treatment was recorded in the testis and ovary of 15th day BHC exposed *H. fossilis*. The 90th day pesticide treated testis showed significant rise of ascorbic acid (fig. 17) may be associated with the restoration of normal cellular function. Similar tendency have also been recorded in case of ovarian ascorbic acid (fig 18). Several investigators (Goyer and Cherian 1979, Flora and Tandon 1986, Flora et al., 1986, Tandon et al., 1987, Tandon et al., 1988) suggested that thiamine, folic acid, pyridoxine and ascorbic acid either individually or in combination proved to be effective in reducing the toxic manifestation of lead.

The ascorbic acid acts as an antitoxicant (Bendich 1986) and prevented the initiation of 712 DBA induced skin cancer and the dehydroascorbate inhibits the sarcoma growth. Further, Cameron and Pauling (1979) suggested that vitamin C rendered the cellular matrix more resistant to the invasiveness of tumour enzymes or otherwise it increases the physiological hyaluronidase (PHI) activity to retard or resist the tumour
Fig. 19: Effect of 15 PPM BBC on Ascorbic acid content of Kidney.
growth (Sigel and Morton 1977). Dunham et al. (1982) noted a pronounced effect of L-ascorbic acid in decreasing the incidence and delaying the onset of malignant lesion induced in mice by uv irradiation, once again suggests the anticarcinogenic/antimutagenic potency of ascorbic acid (Norkus and Kuenzig 1985).

Recently ascorbic acid has been shown to inhibit "in vivo" mutagenecity of di-iodinehydroxy-chlorine (Ghaskadbi and Vaidya 1989). Ascorbic acid reduces the mutagenecity of Aflatoxin B1 (Bhattacharya et al., 1987) and different vitamins modify the mutagenic action of N-methyl N-nitro n-nitrosoguanidine (Shetty et al., 1988) and ethylmethane sulphate (Kuroda 1988). In mice treated with Ccl4 the ascorbic acid protects cells from lipid peroxidation (Kunert and Tappel 1983). The in vivo antioxidant activity of ascorbic acid is partially due to the fact that it enhances the activity of vitamin E (Niki et al., 1984, Bendich et al., 1986). Kola et al. (1989) suggested that vitamin C administered with cyclophosphamide decrease the chromosomal aberrations but not sister chromatid exchanges. Thus ascorbic acid has been shown to antimutagenic and antitoxicant agent (Leboulanger 1981).

The ascorbic acid is present in high concentration in the interstitial cells of the testis and ovary. Stimulation of the rabbit ovary by gonadotropin causes rapid and marked decrease in ovarin interstitial cell ascorbate content
suggesting a correlation between ascorbate level and estrogenic hormone production. Jennings (1970) showed that gonadotropic stimulation of the corpus luteum in pregnancy caused rapid decrease of ascorbate content. Singh et al (1991) described that a significant increase in the hepatic ascorbic acid of the female frog during breeding season indicated its involvement in the process of reproduction.

The present results indicate that restoration of the normal cellular activity of the kidney and gonads after sublethal dose of BHC exposure dependent on the ascorbic concentration apart from the enhancement of adrenaline and noradrenaline. The development of tolerance to the pesticide may be directly related to the ascorbic acid biosynthesis however, needs detail investigation.

SUMMARY :

1) The ascorbic acid of gonad and the kidney of BHC exposed fish i.e. *Heteropneustes fossilis* was estimated by the method of Roe and Kuther (1943).

2) Ascorbic acid was present in high concentration in the testis and ovary. The ascorbic acid of gonad were found to be decreased after BHC exposure on 30th and 45th day. But significantly increase of ascorbic acid was
recorded in 15th day and 90th day test group testis and in ovary of 15th or 60th and 90th day BHC treated might be associated with the restoration of normal cellular status.

3) The kidney ascorbic acid was found to be decreased throughout the period of experimentation. However, enhanced ascorbic acid value was noticed from 60 days to 90 days in the BHC exposed H. fossilis, still lower than their respective control. The deficiency of ascorbic acid in kidney might be due to increased metabolic need for ascorbate associated with the release of adrenalin and noradrenalin.

4) The present findings suggest that BHC toxicity causes the alteration of ascorbic acid in the gonads and kidney. The ascorbic acid is related with the reproduction and adrenaline synthesis in fish however, needs further details.
Effect of BHC on Adrenaline and Noradrenaline of Testis, Ovary and Kidney of *H. fossilis*. 
Effect of BHC on Adrenaline and Noradrenaline of Testis, Ovary and Kidney

Adrenaline and Noradrenaline are two hormones which are secreted by adrenal gland. Though these two hormones are different in nature, yet they have some close relationship with each other. Adrenaline causes powerful contraction of many arterioles resulting in the rise of blood pressure throughout the body. Practically all the smooth muscles of the body are affected by this hormone, some being stimulated to contract whereas some others are inhibited. The muscles of stomach, intestine and urinary bladder are inhibited by adrenaline. Adrenaline enhances the process of liver glycogenolysis and causes the rise of sugar level in the blood and hepatocytes (Birnbaum et al, 1976). It does play a role in the release of free fattyacids from adipose tissue. Adrenaline has both the dilator as well as the constrictor effect but the noradrenaline has only the constrictor effect as a result of which the latter causes a great rise in the blood pressure. Its main function is to maintain the normal control of circulation.

The cells of the adrenal medulla elaborate adrenaline and noradrenaline, often referred to collectively as catecholamines. Both hormone molecules contain an asymmetric carbon atom and therefore can exist in two optically active forms. The hormones secreted by the gland are levorotatory whereas those produced by laboratory synthesis are racemic. The two hormones are very similar in chemical properties but
the presence of an additional methyl group in adrenaline changes the side chain from a primary amine (noradrenaline) to a secondary amine (adrenaline).

Adrenal extracts contain both adrenaline and noradrenaline, the proportion of which is fairly constant and characteristic for the species. Dopamine, a precursor of noradrenaline is also found in medullary extracts.

It has been known that catecholamine containing nerve exist in the gonads of birds and mammals that possibly and exert influences in the regulatory the functional state of the organs. A remarkable gametokinetic disturbances in the gonad following administration of epinephrine has been well documented in both birds and mammals (Kar and Ghosh 1951, Vandemark and Boyel, 1956, Chatterjee, 1968). Although controversial interpretation, however, arises confusion regarding the nature of epinephrine action on the gonad (Cross & Silver, 1962, Chatterjee 1968, Setchell, 1970), yet sufficient information concerning the effect of norepinephrine on gonads is still lacking. Moreover, no information is available about the origin of synthesis and time of the initial secretion of these hormones in fish.

In fish the adrenal gland is situated in the anterior part of the kidney which is characterised by the presence of chromaffin tissue. However, patches of chromaffin tissues are generally found in region of the post cardinal venous.
drainage of the kidney. The chromaffin tissues are associated with renal tissue (Nandi type I and type II) or associated with inter-renal tissues (Nandi type-III). Presence of adrenaline and noradrenaline have been shown histologically in the anterior kidney. Matty (1985) suggested that stressful condition invariably causes and increases the catecholamine in fish.

There have been extensive study of adrenaline and noradrenaline (epinephrine and norepinephrine) in various mammalian testicular and ovarian tissues but very little is known in fish. In fish it has been shown that the level of circulating catecholamine increases in response to hypoxia (Butler et al., 1978, Butler et al., 1979) and physical stress (Nakano and Tomlinson 1967). These are due to effect of the adrenaline and noradrenaline upon the circulation of fish which have received most attention in the recent years. Therefore, the present experiment is designed to evaluate the effect of BHC, a chlorinated pesticide, at sublethal concentration on the adrenaline and noradrenaline activity of kidney and gondas of fresh water Indian cat fish, Heteropneustes fossilis.

Methods and Materials: The fish of body weight 5-10 gms. were reared for experimental purpose are detailed in Chapter - II along with the methodology employed in this experiment.
RESULTS:

Results of Adrenaline and Noradrenaline of 15 ppm BHC exposed testis, ovary and kidney are depicted in the table from xvii to xxii. The values of control, 15 days, 30 days, 45 days, 60 days, 75 days and 90 days are described below. The values are expressed in ug/gm of tissue.

Effect of BHC on the Adrenaline content of Testis, Ovary and Kidney:

Testis: The adrenaline concentration of testis was recorded in between 190.5 to 196.6 ug/gm in control group. BHC exposed testis was significantly increased upto 30 days (Table xviii), however, a marked significant fall was noted on 45th day followed by gradual elevation as recorded on 60th & 90th (E & G) day. A significant difference was observed amongst the treated groups.

Ovary: The adrenaline concentration of ovary was ranged between 219 to 224 ug/gm, in the control group. Comparison of treated groups showed significant difference with control. The adrenaline concentration of ovary was significantly increased upto 30 days, while significant depletion was noted on 45th day (Table-xix). Adrenaline content again depleted on 75th day (142.24 ± 0.78 ug/gm) compared to control.
<table>
<thead>
<tr>
<th>Control (A)</th>
<th>15-days (B)</th>
<th>30-days (C)</th>
<th>45-days (D)</th>
<th>60-days (E)</th>
<th>75-days (F)</th>
<th>90-days (G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>196.6</td>
<td>227</td>
<td>314.4</td>
<td>82.4</td>
<td>110.2</td>
<td>112.5</td>
<td>225.8</td>
</tr>
<tr>
<td>190.5</td>
<td>229</td>
<td>308.5</td>
<td>95.9</td>
<td>108.3</td>
<td>104.6</td>
<td>224.7</td>
</tr>
<tr>
<td>192.3</td>
<td>226</td>
<td>312.3</td>
<td>90.7</td>
<td>113.4</td>
<td>100.3</td>
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<td>191.3</td>
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<td>305.6</td>
<td>94.4</td>
<td>115</td>
<td>101.2</td>
<td>212.3</td>
</tr>
<tr>
<td>191.9</td>
<td>228</td>
<td>310.3</td>
<td>98.6</td>
<td>116.1</td>
<td>99.0</td>
<td>218.3</td>
</tr>
</tbody>
</table>

Mean
192.52 ± 0.95  
228 ± 0.63  
310.22 ± 1.36  
92.4 ± 2.51  
112.6 ± 1.31  
103.5 ± 2.18  
221 ± 2.26

a = Significantly differ from the control
b = Significantly differ from the treated groups

**ANOVA**

Analysis of variance for one way classified data with 5 observations in each group.

<table>
<thead>
<tr>
<th>Sources of variance</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F.</th>
<th>Tabulated value</th>
<th>Inference</th>
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<tr>
<td>Among animal groups</td>
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<td>32637.25</td>
<td></td>
<td>1739.7</td>
<td>**</td>
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</tr>
<tr>
<td>Error (within the animal group)</td>
<td>28</td>
<td>525.4</td>
<td>18.76</td>
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<td></td>
</tr>
</tbody>
</table>

* Significant; ** Highly significant

CD (P<0.05) = 5.59;   CD(P<0.01) = 7.53
Table XIX  Adrenaline concentration in control and 15ppm BHC exposed ovary of H.fossilis in different days interval. Values are replica of 5 fresh fish tissues ± SEM, expressed in µg/gm.

<table>
<thead>
<tr>
<th></th>
<th>Control (A)</th>
<th>15-days (B)</th>
<th>30-days (C)</th>
<th>45-days (D)</th>
<th>60-days (E)</th>
<th>75-days (F)</th>
<th>90-days (G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>224</td>
<td>264.5</td>
<td>278.2</td>
<td>126.2</td>
<td>191.6</td>
<td>144.3</td>
<td>275</td>
<td></td>
</tr>
<tr>
<td>221</td>
<td>261.9</td>
<td>279.3</td>
<td>128.4</td>
<td>192.4</td>
<td>143.9</td>
<td>277.4</td>
<td></td>
</tr>
<tr>
<td>220</td>
<td>260.8</td>
<td>280.1</td>
<td>126.5</td>
<td>193.3</td>
<td>140.2</td>
<td>276.6</td>
<td></td>
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<tr>
<td>219</td>
<td>262.3</td>
<td>280.4</td>
<td>128.7</td>
<td>196.7</td>
<td>142.5</td>
<td>273.2</td>
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<tr>
<td>221</td>
<td>257.5</td>
<td>270</td>
<td>125.5</td>
<td>194.5</td>
<td>140.3</td>
<td>275.6</td>
<td></td>
</tr>
</tbody>
</table>

Mean  
221  261.4_{ab}  277.6_{a}   127.06_{ab}   193.7_{ab}   142.24_{ab}   275.56_{ab}
+0.75 ± 1.03 ± 1.74 ± 0.57 ± 0.80 ± 0.78 ± 0.64

a = Significantly differ from the control  
b = Significantly differ from the treated groups.

ANOVA 14  Analysis of variance for one way classified data with 5 observations in each group.

| Sources of variance | d.f. | S.S.  | M.S.  | F. Tabulated value | Inference of F at  
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Among animal groups</td>
<td>6</td>
<td>11625.32</td>
<td>1937.55</td>
<td>329.69 2.45</td>
<td>3.58</td>
</tr>
<tr>
<td>Error (within the animal groups)</td>
<td>28</td>
<td>164.58</td>
<td>5.88</td>
<td>**</td>
<td></td>
</tr>
</tbody>
</table>

* Significant; ** Highly significant  
CD(P<0.05) = 3.14; CD(P<0.01) = 4.23
Kidney: The adrenaline content of kidney was recorded in between 111 to 117 µg/gm in control group. A significant difference was noted between the control and the treated groups (ANOVA-15). The adrenaline concentration of kidney was significantly enhanced up to 30 days (C) (245.58 ± 0.71 µg/gm) however, on 45th day (D) it was recorded to be declined (97.34 ± 0.28 µg/gm). But gradually adrenaline content was significantly increasing up to 90 days (G) (Table-xx). Compared mean differences of the treated groups were significant (CD = 2.92 : P<0.01).

Effect of BHC on the Noradrenaline content of Testis, Ovary and Kidney:

Testis: The noradrenaline concentration of testis was observed in between 6.3 to 10 µg/gm in the control group. The values were significantly enhanced in the treated groups over the control (Table-xxi, ANOVA-16). Comparison of means difference of the test groups displayed significant variation except between F & C.

Ovary: The noradrenaline concentration of ovary was noted in between 5 to 8 µg/gm in the control group. Significantly higher quantities of noradrenaline was observed in all the treated groups. The highest being the 45 days BHC exposed ovary (27.8 ± 0.39 µg/gm). Significant difference of means of treated groups were observed.
**Table XX** Adrenaline concentration in control & 15 ppm BHC exposed kidney of *H. fossilis* in different days interval. Values are replica of 5 fresh fish tissues + SEM, expressed in μg/gm.

<table>
<thead>
<tr>
<th></th>
<th>Control (A)</th>
<th>15-days (B)</th>
<th>30-days (C)</th>
<th>45-days (D)</th>
<th>60-days (E)</th>
<th>75-days (F)</th>
<th>90-days (G)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>115</td>
<td>158.5</td>
<td>245.7</td>
<td>97.6</td>
<td>119</td>
<td>130</td>
<td>154</td>
</tr>
<tr>
<td></td>
<td>117</td>
<td>159.2</td>
<td>244.4</td>
<td>98</td>
<td>117</td>
<td>134</td>
<td>156.7</td>
</tr>
<tr>
<td></td>
<td>113</td>
<td>158.3</td>
<td>247.9</td>
<td>96.6</td>
<td>120</td>
<td>131</td>
<td>155.5</td>
</tr>
<tr>
<td></td>
<td>111</td>
<td>157.6</td>
<td>246.5</td>
<td>97.9</td>
<td>118</td>
<td>133</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>114</td>
<td>161.4</td>
<td>243.4</td>
<td>96.9</td>
<td>116</td>
<td>132</td>
<td>152.4</td>
</tr>
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</table>

Mean

<table>
<thead>
<tr>
<th></th>
<th>114</th>
<th>159&lt;sup&gt;ab&lt;/sup&gt;</th>
<th>245.58&lt;sup&gt;ab&lt;/sup&gt;</th>
<th>97.34&lt;sup&gt;ab&lt;/sup&gt;</th>
<th>116&lt;sup&gt;ab&lt;/sup&gt;</th>
<th>132&lt;sup&gt;ab&lt;/sup&gt;</th>
<th>154.12&lt;sup&gt;ab&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>+0.9</td>
<td>+0.58</td>
<td>+0.71</td>
<td>+0.28</td>
<td>+0.63</td>
<td>+0.63</td>
<td>+0.80</td>
<td></td>
</tr>
</tbody>
</table>

a = Significantly differ from the control
b = Significantly differ from the treated groups

ANOVA - 15 Analysis of variance for one way classified data with 5 observations in each group.

<table>
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<tr>
<th>Sources of variance</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F</th>
<th>Tabulated value of F at 0.05</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among animal groups</td>
<td>6</td>
<td>473332.11</td>
<td>78888.69</td>
<td>28024.4</td>
<td>2.45</td>
<td>3.58</td>
</tr>
<tr>
<td>Error (within the animal groups)</td>
<td>28</td>
<td>78.83</td>
<td>2.81</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* Significant ; ** Highly Significant
CD (P<0.05) = 2.17; CD (P<0.01) = 2.92
Table XXI  Noradrenaline concentration in control & 15ppm. BHC exposed testis of *H.fossilis* in different days interval. Values are replica of 5 fresh fish tissues ± SEM, expressed in μg/gm.

<table>
<thead>
<tr>
<th>Control (A)</th>
<th>15-days (B)</th>
<th>30-days (C)</th>
<th>45-days (D)</th>
<th>60-days (E)</th>
<th>75-days (F)</th>
<th>90-days (G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.1</td>
<td>16.1</td>
<td>18.1</td>
<td>14.1</td>
<td>23.3</td>
<td>17.3</td>
<td>28.1</td>
</tr>
<tr>
<td>7.0</td>
<td>17.2</td>
<td>17.4</td>
<td>15.6</td>
<td>24.1</td>
<td>18.0</td>
<td>29.4</td>
</tr>
<tr>
<td>10.0</td>
<td>15.1</td>
<td>19.5</td>
<td>13.3</td>
<td>22.2</td>
<td>16.4</td>
<td>27.3</td>
</tr>
<tr>
<td>6.3</td>
<td>16.4</td>
<td>18.2</td>
<td>14.4</td>
<td>23.2</td>
<td>17.2</td>
<td>27.4</td>
</tr>
<tr>
<td>8.1</td>
<td>16.0</td>
<td>17.8</td>
<td>13.6</td>
<td>23</td>
<td>17.8</td>
<td>28.3</td>
</tr>
</tbody>
</table>

Mean

| 7.9         | 16.16<sup>ab</sup> | 18.2<sup>a</sup> | 14.2<sup>ab</sup> | 23.16<sup>ab</sup> | 17.34<sup>ab</sup> | 28.1<sup>ab</sup> |
| ± 0.56      | ±0.30         | ±0.32        | ±0.36         | ±0.27         | ±0.25         | ±0.34        |

a = Significantly differ from the control
b = Significantly differ from the treated groups.

ANOVA - Analysis of variance for one way classified data with 5 observations in each group.

<table>
<thead>
<tr>
<th>Sources of variance</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F. Tabulated value</th>
<th>Inference of F at</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among animal groups</td>
<td>6</td>
<td>1244.10</td>
<td>207.35</td>
<td>262.80 2.45</td>
<td>3.58 **</td>
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<tr>
<td>Error (within the animal groups)</td>
<td>28</td>
<td>22.09</td>
<td>0.79</td>
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<td></td>
</tr>
</tbody>
</table>

* Significant ; ** Highly Significant
CD(P<0.05) = 1.51; CD (P<0.01) = 1.54
**Table XXII** Noradrenaline concentration in control and 15ppm. BHC exposed ovary of *H. fossilis* in different days interval. Values are replica of 5 fresh fish tissues ± SEM, expressed in μg/gm.

<table>
<thead>
<tr>
<th></th>
<th>Control (A)</th>
<th>15-days (B)</th>
<th>30-days (C)</th>
<th>45-days (D)</th>
<th>60-days (E)</th>
<th>75-days (F)</th>
<th>90-days (G)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>6.0</td>
<td>22.5</td>
<td>27.2</td>
<td>13.1</td>
<td>15.3</td>
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<td></td>
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<td>14.2</td>
<td>12.1</td>
<td>19.1</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>21.2</td>
<td>27.2</td>
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<td>17.2</td>
<td>11.4</td>
<td>18.0</td>
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<td>20.0</td>
<td>27.1</td>
<td>14.3</td>
<td>16.2</td>
<td>11</td>
<td>19</td>
</tr>
</tbody>
</table>

Mean

6.6 22.3 ab 27.8 ab 13.6 ab 15.8 ab 11.8 ab 18.4 ab

+ 0.47 +0.69 +0.39 +0.49 +0.45 +0.38 +0.24

a = Significantly differ from the control
b = Significantly differ from the treated groups

**ANOVA - 17** Analysis of variance for one way classified data with 5 observations in each group.

<table>
<thead>
<tr>
<th>Sources of variance</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F. Tabulated value</th>
<th>Inference of F at 0.05</th>
<th>0.01</th>
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<tbody>
<tr>
<td>Among animal groups</td>
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<td>1469.24</td>
<td>244.88</td>
<td>187.07</td>
<td>2.45</td>
<td>3.58</td>
</tr>
<tr>
<td>Error (Within the animal groups)</td>
<td>28</td>
<td>36.66</td>
<td>1.31</td>
<td></td>
<td></td>
<td>**</td>
</tr>
</tbody>
</table>

* Significant; ** Highly Significant

CD(P<0.05) = 1.48; CD(P<0.01) = 1.99
Kidney: The noradrenaline concentration of kidney was observed in between 9.2 to 11 μg/gm in control group. Comparison amongst treated groups showed a significant difference with control (ANOVA-18). The highest concentration of noradrenaline was detected in the 90th day BHC exposed kidney. Comparison of means difference displayed a significant variation amongst the treated groups.

Discussion:

Benzene hexachloride at 15 ppm concentration has changed the adrenaline and noradrenaline concentration of the gonads and kidney of Heteropneustes fossilis. Although, these two medullary hormones are similar in some of their biologic action, there are important differences in the nature and degree of their effect. The results indicated a direct toxic action of the 15 ppm BHC on the cells of the adrenal gland (kidney) and in the testis. Probably this is related with the increased secretion of corticosterone as have been documented in the several avian species (Manna and Ghosh 1979, Silverin 1979) and in mammals. Several workers (Inaba and Kamata 1975, Dallman and Jones 1979), reported that excess corticosterone released by a feed back mechanism enhances ACTH secretion and this has inhibitory effect.
Table XXIII  Noradrenaline concentration in control and 15ppm. BHC exposed kidney of H.fossilis in different days interval. Values one replia of 5 fresh fish tissues ± SEM expressed in μg/gm.

<table>
<thead>
<tr>
<th></th>
<th>Control (A)</th>
<th>15-days (B)</th>
<th>30-days (C)</th>
<th>45-days (D)</th>
<th>60-days (E)</th>
<th>75-days (F)</th>
<th>90-days (G)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>17.2</td>
<td>31.3</td>
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</tr>
<tr>
<td>12.0</td>
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<td>18.4</td>
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<td>17.4</td>
<td>12.0</td>
<td>21.8</td>
<td>16.8</td>
<td>33.3</td>
<td></td>
</tr>
<tr>
<td>9.2</td>
<td>14.2</td>
<td>16.1</td>
<td>13.2</td>
<td>20.5</td>
<td>19.0</td>
<td>31.1</td>
<td></td>
</tr>
<tr>
<td>11.0</td>
<td>14.1</td>
<td>15.2</td>
<td>15.0</td>
<td>23.3</td>
<td>16.1</td>
<td>31.2</td>
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</tbody>
</table>

Mean

<table>
<thead>
<tr>
<th></th>
<th>10.44</th>
<th>14.22a</th>
<th>16.0ab</th>
<th>13.5ab</th>
<th>22.3ab</th>
<th>17.5ab</th>
<th>31.8ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>± 0.43</td>
<td>±0.28</td>
<td>±0.35</td>
<td>±0.45</td>
<td>±0.48</td>
<td>±0.47</td>
<td>±0.37</td>
<td></td>
</tr>
</tbody>
</table>

*a = Significantly differ from the control
b = Significantly differ from the treated groups

ANOVA - 18 Analysis of variance for one way classified data with 5 observations in each group.

<table>
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<tr>
<th>Sources of variance</th>
<th>d.f.</th>
<th>S.S.</th>
<th>M.S.</th>
<th>F.</th>
<th>Tabulated value of F at</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among animal groups</td>
<td>6</td>
<td>1524.32</td>
<td>254.05</td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>Error (within the animal groups)</td>
<td>28</td>
<td>29.6</td>
<td>1.057</td>
<td></td>
<td>**</td>
</tr>
</tbody>
</table>

* significant; ** Highly significant

CD(P<0.05) = 1.33 ; CD (P<0.01) = 1.79
on the turnover rate of gonadatrophin from adenohypophysis under the toxicant stress. Liu et al. (1990) reported that after exposure to heavy metals the cells of Leydig of testis and the adrenal decapsular cells were adversely affected and regression of testis was recorded with the exogenous administration of catecholamine in weaver bird (Mukherjee 1985).

The enhanced adrenaline due to BHC toxic stress of this investigation could be substantiated by the work of Matty (1985) that stressful conditions invariably cause an increase of catecholamines but in some species it is adrenaline while in other species it is noradrenaline i.e. produced in larger amounts following a stressful stimulus. Hepatocytes isolated from goldfish, Carassius auratus respond to adrenaline treatment by increasing glucose release to the medium via the glycogenolytic pathway (Birnbaum et al., 1976). Mazeand and Mazeand (1981) noted the enhanced blood glucose within two hours of adrenaline administration and free fatty acids appeared might be essential to meet the energy demand under toxic stress.
Therefore, this stress might be responsible to induce glycogenolysis and hyperglycaemia along with the inhibition of mitosis in the gonad.

Adrenaline and noradrenaline may pass into the bloodstream either by direct secretion from the chromaffin tissue of the kidney or by diffusion from adrenergic nerve endings. However, there is good evidence that baclofen (4 amino 3,4-chlorophenyl butanoic acid) decreases the release of norepinephrine from central and peripheral neurons by action at a presynaptically located gamma aminobutyric acid (GABA) receptor. The inhibition of neurotransmitter release is thought to be due to decrease in influx of calcium into the nerve terminal which is noradrenergic or adrenergic neuron may reduce tyrosine hydroxylase action as well as impair the release of catecholamine or otherwise inhibits the enzyme phenylethanolamine N-methyl transferase (PNMT) (Wurtman et al., 1968). However, there is no such evidence that the pesticide directly inhibits any of the enzyme of catecholamine biosynthesis. It is evident from the work of different workers that adrenal gland plays a cardinal role in adjustment to stress (Christian, 1980, Ghosh and Banerjee, 1983).

Adrenaline and noradrenaline hormones have their effect upon heart and other tissues by combining with specialised region on the membranes of effector cells which in turn become active and this process in fish is not yet known.
However, the direct type of catecholamine receptor in the circulatory system namely \( \alpha, \beta_1, \beta_2 \) have been identified in fish (Stenelarsen 1981).

Gill (1974), Srivastava and Mishra (1983), reported hepatic and muscular glycogenolysis with concomitant hyperglycaemia in Indian fresh water fish after treatment with melathion. Further Sengupta et al. (1982) observed significant depletion of liver glycogen and elevation of blood glucose level in 5 ppm BHC exposed H.fossilis. Ottolenghi et al., (1985, 1986), found that catfish liver slices incubated in the presence of epinephrine revealed a glycogen phosphorylase activity exceeding that of the controls, with a decrease in glycogen content accompanied by an increase in glucose output into the incubation medium. Brighenti et al., (1987) reported that in the catfish hepatocyte system the epinephrine had the greatest effect on phosphorylase activity and on glucose output: norepinephrine, Phenylalanine and isoproterenol followed closely. This descending order of potency in the activity of catecholamine could indicate that in Catfish the classical \( \beta \) adrenergic action does not exist. Moreover, it is difficult to explain why norepinephrine had the greatest effect only in the case of hepatocyte glycogen.

Although it is suggested that the kidney adrenaline and noradrenaline do not change appreciably under disturbed condition (Matty 1985) yet, the present findings revealed a significant change of both the hormones under toxic stress.
Fig. 20: Effect of 15 PPM BHC on Adrenaline content of Testis
The epinephrine decreased markedly on 45th day (fig.20) while norepinephrine maintained a rise throughout the period of experimentation (fig. 23). It is beyond doubt that the 45th day appeared to be somewhat critical period and thereafter the fish exhibited normalisation/resistance towards toxicant stress. The physiological changes brought about by the adrenaline and noradrenaline are associated with hyperglycaemia. It has been reported (Gill 1974, Srivastava and Mishra, 1983), that BHC causes glycogen degradation and elevated the blood glucose level mediated through gamma amylolytic pathway (Sengupta et al., 1990). It has been reported that BHC severely influenced the activity of adrenal hormone (Evodokimov and Klochkova, 1974) and the adrenaline enhances the catalysing effect of gamma amylase (Sengupta et al., 1990). Moreover, it bears the evidence that pesticide decreases the blood pH towards acidity due to the accumulation of metabolic products (Jafri and Shaffi 1975, Dhillon and Gupta 1983) and in turn provides a favourable environment for the gamma amylase activity. The hormone has a tendency to increase its concentration in the kidney and gonad except 45th day of experiment (Table xvi to x). In addition to catecholamine, the thyroid hormone influences the reproduction in teleost and that possibly by enhancing the effect of gonadotrophin hormone on gonad. (Ball 1960, Sage 1973, Ichikawa et al., 1974, Dodd 1975, Hurlburt 1977, Dettlaff and Davydova 1979, Sen and Bhattacharya 1981, Bhattacharya et al., 1982). Pesut - Mikincic et al., (1987) concluded that the
Fig. 21: Effect of 15 PPM BHC on Adrenaline content of Ovary.
Fig. 22: Effect of 15 PPM BHC ON Adrenaline content of kidney
inhibition of testosterone metabolism through 5β-DHT and 5β-diol formation provides more substrate (testosterone) for the other metabolic pathway leading to andostenedione and 5β-dione formation. This implies a hazardous effect of lindane on the hormonal balance of the neuroendocrine level in birds.

It is known that pesticides are considered as an antispermatogenic and antifertility agents which directly affect the germinal epithelium while affecting Leydig cells and gonadotrophin regulation hormones (Balash et al, 1987).

The enhanced epinephrine has been observed to equip the organism to meet certain kinds of emergency situation under toxic stress. It is also able to prevent hypoglycaemia by stimulating metabolism and mobilising glycogen as glucose through gamma amylase activity. Contrary to this norepinephrine generally produces general vasconstriction and stimulate the heart but is relatively impotent in its metabolic action. Increased value of epinephrine is responsible for the increase of blood sugar and is one of the most important factors to bring the toxicant stressed fish towards normalisation on or after 45th day of exposure (Table xvi to x.x).

Under the toxicant stress the enhanced adrenaline of the kidney throughout the experiment except on the 45th day (Table x.x) might be responsible for increasing the force, amplitude and frequency of heart beat and decreasing the
Fig. 23: Effect of 15 PPm BHC on Noradrenaline content of testis.
Fig. 24: Effect of 15 PPM BHC on Noradrenaline content of Ovary.
Fig. 25: Effect of 15 PPM BHC on Noradrenaline content of Kidney.
peripheral circulating resistance with a dilation of the skeletal blood vessels. Enhanced noradrenaline of all the tissues throughout the experiment under toxic stress probably brought constriction of the blood vessels of the testis, skin, muscles and increased the total peripheral resistance as well as increasing blood pressure mediated by β-adrenoergic receptors (Holmgren 1977) in H. fossilis.

Generally the catecholamine disappears very rapidly from the circulation in normal animals. However, the enhanced value of the epinephrine and norepinephrine in the pesticide exposed fish may be due to the inhibition of the enzyme catechol-o-methyl transferase (De Schaepdryver and Krishna 1961). Since their metabolic degradation involves methylation catalysed by the said enzyme. This enhanced epinephrine mediate its effect on glycogen metabolism by a scheme of enzyme activation operating through the "second messenger" cyclic AMP, and activate the gamma amylase to release of glucose. The epinephrine depress the rate of glucose utilisation which may be due to the inhibition of adrenocortical steroids or act at the level of the cell membrane and reduce the rate of entry of glucose into the cells. The enhanced epinephrine in the pesticide treated testis and ovary supported by Bullough (1955) in vitro and have important effects on water and electrolyte metabolism.

It is known that adrenaline may operate under certain
condition to promote the release of ACTH from the anterior hypophysis with the consequent release of certain adrenal cortical steroids although pituitary activation may be only a minor function of adrenaline under stress condition.

Fig. 26: Schematic diagram during Toxic stress
(Modified after Ghosh and Bannerjee 1983)

However, the mechanism involved in the neuroendocrine adjustment to stress or stimuli are extremely complicated and await full classification. Both adrenaline and noradrenaline are involved in the stress adjustment. It is assumed that the
Noradrenaline is released in enhanced quantum under BHC stress which alter the blood pressure level of the fish. Thus noradrenaline is released in response to need for circulatory adjustments while adrenaline is of primary importance in metabolic adjustments, confirm more nearly to cannon's concept of an "emergency hormone".

From the endocrinological stand point there may be two possible explanations of the mechanism of gonadal inhibition during toxic stress (1) may be due to an increased level of ACTH release during BHC toxicant stress which in turn supressed GTH and caused the gonadal atrophy ; or (II) it may be that increased level of circulating corticoids has some direct inhibiting effect on the gonads (Christian 1967). The ready response of epinephrine on all occasions suggests that it also act as a line of defence in condition of stress. (Ghosh and Banerjee 1983).
Summary:

1) The adrenaline and noradrenaline were estimated by the method of U.S.V. Euler (1950).

2) The adrenaline and the noradrenaline of the BHC exposed gonads were increasing up to 90th day except on the 45th and the 75th day. The highest level of adrenaline was recorded on the 30th day. Similarly, kidney adrenaline and noradrenaline also exhibited enhanced trend over the control counterpart throughout the period of experimentation.

3) In kidney, adrenaline might be responsible for increasing the force and amplitude. But enhanced noradrenaline under toxic stress might be responsible for the constriction of the blood vessels of the testis and ovary.

Present investigation suggests that these two hormones might be responsible to meet emergency situation.
Histopathology of BHC exposed Testis, Ovary and Kidney of *H. fossilis*:
Scanning Electron microscopic study
Histopathology of BHC exposed Testis, Ovary and Kidney of H. fossilis; Scanning Electron microscopic study

Introduction:

The visual estimation of tissue entities under the microscope is the most direct and still most unequivocal method of determining the cellularity of a given tissue. The technique is free from subjective error and with a sufficient number of readings is capable of attaining a high degree of accuracy in the most heterogenous types of tissue.

Scanning electron microscopy (SEM) provides a means of visualising surface structure of cells and tissues and because of its great depth of focus, it affords easy appreciation of the 3 dimensional (3D) relationship of the surface ultrastructure, and because of these features the SEM has been used extensively to study a variety of biological material. Hitherto SEM studies of the gonads and kidney of the Indian cat fish Heteropneustes fossilis are not attempted. In aquatic environment the pesticide is diluted to sublethal levels producing chronic histopathological effect on some organs. The kidneys of fish are hyperosmotic regulators. The kidneys and the gonads have no direct contact with the pollutants dissorved in water but get affected through the blood vascular system. The pesticide present in water
causes various structural alteration in the gonads and kidneys as shown in few studies (Grant and Mehrle, 1970; Konar, 1970, 1980; Shastry and Sharma 1979; Mandal and Kulashrestha, 1980; Kulashrestha et al., 1984). Nijaguna et al., (1990) reported the SEM studies of the alimentary tract of Channa gachua. Baruffaldi et al., (1989) observed a ultrastructural changes after exposing the hepatocytes for a period of 15 days and observed increased number of ergastroplasm, cytoplasmic lipid inclusion which was found more roundish than the control. Braunbeck et al., (1989) studied ultrastructural change of liver in Zebra fish exposed to 4-nitrophenol and revealed sex distinction effect. The male fish primarily reacted with a proliferation of smooth endoplasmic reticulum, whereas female fish displayed a high degree of fenestration within the rough endoplasmic reticulum.

Lee et al., (1989) studied the reversibility of testicular atrophy under electron microscope when induced by ethylene glycol monomethyl ether in rat. Here spermatocytes have changed their structure and specific stage of reproduction is damaged. Hence this work elucidates the light and SEM studies on the microanatomy of the gonads and the kidney of H. fossilis under 15 ppm BHC stress for a period of 90 days.

Materials and Methods:

Two sets of fishes were considered in this
investigation. The control group was exposed to 1 ml. of acetone in 15 litre of water while the test group were exposed to 15 ppm. BHC (in 1 ml. acetone) as described under materials and methodology (chapter-II).

Results:

The results observed under light and scanning Electron Microscope are presented below:

TESTIS:

Light microscopic studies of the control group of testis of *Heteropneustes fossilis* is composed of seminiferous tubules bordered by basal lamina. Germ cells and sertoli cells were noted inside the tubules. Interstitial cells and germ cells are gathered in seminiferous cyst in which they differentiate synchronously. The spermatogonia individually surrounded by sertoli cells are not gathered in cyst. They occur along with the entire length of the tubule and in the apical part of the tubule immediately beneath the epithelium which borders the gonad. The testis of *H. fossilis* is "unrestricted spermatogonial testis type". Spermatogonia are located along with the entire length of the tubule.

The testes of the BHC treated fish revealed damages
in the spermatogenic stages. The outer covering acquired thick deposition of fibrous tissue and other cells of this wall were atrophied. The seminiferous tubules were shrunken considerably. The different cell types of spermatogenesis were intermingled and bulk of atrophied masses were formed within the seminiferous tubule. The diameter of the seminiferous tubule are greater than that of the control. The sperm mother cell, the primary and secondary spermatocytes had only traces of the cytoplasm and nuclei were in degenerative condition. However, few of these cells were greatly hypertrophied. The outer wall of the primary spermatocyte appeared thicker than the control group. The interstitial cells present loose connective tissue. The lumen of sperm duct were completely devoid of sperm and eosinophilic sections. The squamous layer while lined the lumen of the duct exhibited hypertrophy and many cytoplasmic proliferation appeared due to fibrous accumulation.

Similarly seminal vesicles lost their characteristic feature. The muscularis gathered significant thickness than these noted in control. The innermost epithelial fold contained hypertrophied and elongated cells. The muscular layer detached from the epithelial cells and the nuclear size significantly increased.

The control testis under SEM exhibited follicles,
podocytes and few number of blebs. Intact testicular follicles with microprojections, cytopodia and columnar epithelial cells have been observed (Plate 1...). The BHC exposed group of testis revealed rough surfaces on its follicle. The 30 days BHC treated testis showed the appearance of increased number of blebs, disintegration between follicles along with the ruffles (plate 2...). However, 60th day treated testis exhibited disruption and disintegration of epithelial cells of the follicles. But reduction of number of blebs with distinct microprojection were noted on 60th day. (Plate 3...). The 90th day treated testis showed a revival effect with the appearance of distinct follicular structures, cytopodia and podocytes. (Plate 4...).

OVARY:

Sections of the ovary under light microscope shows ovigenous lamellae covered by germinal epithelium extend to the lumen. Each lamellae contains developing oogonia and oocytes of different sizes. Germinal epithelium gives rise to oogonia which in turn developed into primary oocytes. Mature ovary consisted of maturing and ripe oocytes. The lumen of the ovary got obliterated due to enlarged oocytes in the lamallae. A few atretic oocytes are also observed in the mature ovary. Smaller oocytes are characterized by a large
PLATE NO. 1 A, B, C, D & E

Shows the control Testis of H. fossilis with epithelial cells and few blebs in different magnification.

Blebs (Bl), Microprojection (MP)
PLATE NO. 2

30 days BHC treated testis of *H. fossilis* shows increase number of blebs and disintegration between follicles.
60 days BHC treated testis of *H. fossilis* shows disruption and disintegration of epithelial cells along with the reduction number of blebs and microprojections.
90 days BHC treated testis of *H. fossilis* shows a revival effect with the appearance of distinct follicular structure, cytopodia and podocytes.
nucleus which occupies more space than the basophilic cytoplasm around it. Nuclei shows deeply staining nucleoli along with the chromatic network. Atretic oocytes are found in the ovaries even at the early stages. During degenerative changes of the oocyte the follicular cells undergo hyperthropy.

Severe histopathological changes were observed with the fibrinogenesis in the tunica albuginea under toxicant stress. Damage was observed in the germinal epithelium and degeneration of ovigenous lamellae. Many immature oocytes were seen scattered in the ovocoel. At some places the immature oocytes showed reduction in their size and their nuclei were finally stained. Atresia of mature oocytes showed cytoplasmic changing and their nuclei showed karyohypertrophy. Some oocytes were highly wrinkled.

The control ovary under SEM exhibited a distinct follicular structure with the microprojection on its surrounding. (Plate.§...). The 15th day and 30th Day treated ovary exhibited rupture of membranes along with the bend microprojection with the increasing polygonal epithelial cells, there are increased number of blebs and fillopodia (Plate..§...). More number of blebs are also observed over the surface. Small microvilli, approximately 0.1 µ in diameter of variable length are distributed along with the
PLATE NO. 5 A, B, C & D

Shows the control ovary of H. fossilis with distinct follicular structure and microprojection.
30 days BHC treated ovary of *H. fossilis* shows rupture of membranes along with bend microprojection and increase number of blebs and fillopodia.

Bleb (Bl), Microprojection (MP) and Fillopodia (Fl)
follcular margin and sparsely over the follicular centre. Infrequently there are also small ruffles at the margin. The 60th day treated ovary exhibited disintegration of surface structure except for a few fillopodia at the follicular margins (Plate.7..). The surfaces are free of microvilli, while at 90th day ovarian follicle has exhibited distinct structure with a very few numbers of blebs and microprojections. Minor differences in the number of small microvilli, small blebs and slender fillopodia are found over its respective control. (Plate..8...).

KIDNEY:

Light microscopic studies exhibited that 30th day exposure to 15 ppm. BHC the renal peritonium was completely fragmented. However, after 45 days recovery is noticed which is substantiated and after 60 days the renal peritoneum was completed. The flattening of the cells of renal epithelium is noticed after 15 days. The widening of the lumen of the tubule are also noticed. After 45 days the reorganization of cellular structure, narrowing of tubule lumen and recovery of damaged tubules are noticed. After 60 days the renal tubules have exhibited near control/normal structure. The glomeruli exhibited shrinkage and breakdown of outer wall after 15 days and continued up to 30 days. But after 45 days regeneration of the outer wall of the
PLATE NO. 7

60 days BHC treated ovary of H. fossilis shows disintegration of surface structure having few fillopodia. Fillopodia (Fl).
PLATE NO. 8 A, B, C & D

90 days BHC treated ovary of *H. fossilis* shows distinct structure with a very few number of blebs, microprojection and slender fillopodia.
8C × 2000

8D × 1500
glomerulus/slarted and near control structure are noticed after 60 days.

SEM studies exhibited that parital epithelium forms a capsule that is remarkably thin and nearly spherical in shape in control kidney (Plate.9.). It often inhibits intendment in its surface. The outer partial surface is comparatively smooth, presence of basal lamina over the epithelial cells are noticed. Very fine collagenous fibrils of the interstitium insert into this lamina. The inner surfaces of the parital cells occasionally exhibited cillia arise from near the centre of each cell. This cillia might be long or short. A sphere population of fingerlike or bleblike microprojection also characterise the free surfaces of the parital cells. (The microprojection are particularly numerous along with the cell borders and allow to distinguish the irregular polygonal surface outlines of this cell). The centrally nucleated region of parital cells often protude into the capsular lumen. A prominent space, bowman's capsular space separate the partial epithelia from the glomerulus.

The visceral epithelium is composed of podocytes (however unlike that of a normal) and each podocytes consists of an enlarged centrally nucleated cell body from
PLATE NO. 9

Shows the control kidney of H. fossilis

9 (a) Plate shows visceral epithelium with glomerulus (G), Capsular space of Bowman (S) and Parietal epithelium (P)

9 (b) Glomerular arteride (A), Parietal epithelium (P), Capsular space of Bowman (S).

9 (c) Cut portion of control kidney with smaller fingerlike pedicles (P)
which variously sized armlike processes wrapped around the glomerular lobe. Bleblike or fingerlike microprojection also arises over the visceral epithelium. Tall columnar epithelial cells are also noticed. Undifferentiated podocytes are tightly packed around primitive glomerular capillaries and give the appearance of "medullary globules". The globular endothelium are also observed. There are some endothelial pores the proximal tubule is characterized by a dense microvilli brush border distinguished on the luminal surface of the parital epithelium. Although most of the microvilli making up the brush border were tall and fingerlike, some irregularly shaped and bleblike microprojection were also present. Each proximal tubule cell possesses one and occasionally two central primary cilia are similar to those found in the renal capsule.

SEM images of the treated kidney exhibited that the cell bodies became very swollen and lumpy in appearance in 15 and 30 days (Plate 10, 4, 11). The major processes arising out less discrete and exhibited bulbous protrusion of their surface. Occasionally pore like openings of variable size (0.1-1.5 μm diameter) were visible on the cell bodies and major processes. There was loss of podocytes pedicles. However, sharp process along with in the podocytes were recorded in 60 days (Plate 13), and 90 days of kidney
PLATE NO. 10

15 days BHC treated kidney of *H. fossilis* shows presence of Podocytes (PC) with swollen and lumpy appearance with bulbus portutions (B)
PLATE NO. 11

30 days BHC treated kidney of *H. fossilis* shows pedicles of Podocytes (PC) can no longer be distinguished. Microbridge (mb) also formed due to BHC treatment by fusion of two nublike protrusions arising from adjacent pedicles (P).
PLATE NO. 12

45 days BHC treated kidney of *H. fossilis* shows loss of podocytes pedicles.
PLATE NO. 13 A, B, C & D

60 days BHC treated kidney of *H. fossilis* shows the traces of appearance of Podocytes pedicles.
An assessment of the effect of BHC on the gonads and kidney of H. fossilis in the histological level had been undertaken for an effective period of 90 days. The investigation revealed the histopathological changes of the gonads and kidney which could be understood to be degenerative up to 45th day. However, the tissues attempted to restore their normal structure was observed after 60th day.

The degenerative changes in the gonads and kidney as observed in this investigation were disintegration of follicles, increased number of blebs in testis, rupture of membranes along with the bend microprojection in the ovary and formation of capsule in parital epithelium, occasional appearance of silica in kidney upto 45th day suggestive of the detrimental effect of BHC. It had been found that the rupture of peritoneal lining, flattening of renal epithelial cells, thereby widening of the tubules, migration of epithelial nuclei, necrosis of haemopoietic tissue, shrinkage...
PLATE NO. 14

90 days BHC treated kidney of H. fossilis

14 (a) Shows the reappearance of Podocytes (P).
14 (b) Tubular cells (A) having free surface microprojections, whereas (B) with very few microprojection and (C) shows cilia on its surface.
and disintegration of glomeruli were the major alterations.
The recovery of the gonadal and kidney tissue after 45
days of exposure has not been reported so far. The
prolonged exposure probably produced immunity substances
in the body which helped the fish to develop resistance
against pesticide. The occurrence of immunity substances in
fish blood and mucus has been reviewed by Ingram (1980).

Harilal and Sahai (1990) reported severe damage in
testis due to administration of sublethal dose of BHC. They
noted deformities in seminal vesicles, fibrosis of connective
tissue and broken disorganised columnar epithelial cells
after 4 days of exposure. Sanglang and O'Halloran (1974)
suggested that there may be inhibition of spermatogenesis due
to inhibition of steroid synthesis in trout after Cdcl₂
treatment. Pandey and Sehagal (1985) noted the deformed
and atrophied primary and secondary spermatocytes in
Tilapia mossambica at 2 ppm and 4 ppm concentration of BHC.
Further, Pandey and Shukla (1985) found pathological
changes in the testicular history of Sarotherodon
mossambicus. Bhattacharya et al. (1989) suggested that due
to the sequential inhibition of the releasing hormone
gonadotrophin steroid hormones, which consequently arrest
the spermatogenesis in the fish, is arrested.
The seminal vesicles are androgen dependent and they also undergo atrophy which proves the nonavailability of androgen in all experimental insecticides exposed fishes.

In another report Pandey and Shukla (1985) showed the adverse effect on the hypothalamic nuclei i.e. nuclei preopticus (NPO) and nucleus laterelis Tuberis (NLT) which regulates the reproduction through release of releasing hormones for pituitary activation.

In some oocyte, the 'nuclear bleb' appear extruding from nucleus in the ooplasm, which gradually shift towards periphery of the oocyte (Mishra and Munnet 1979). Flat follicular cells surrounds the oocyte.

The nuclear membrane disappears and the follicular layer becomes thick. In immature oocytes theca consists of fibroblasts and granulosed cuboidal cells. The latter becomes columnar in mature oocytes (Belsare 1974). The follicular cells are primarily concerned with supplying nourishment to the developing oocyte and help in yolk deposition (Ginburg, 1968, Hoar, 1969).

Saxena and Garg (1978) found higher oocytes percentage on treatment of C. punctatus with carboxyl and
fenitrothion. Srivastava (1989) observed changes in the ovarian histology of *H. fossilis* after 2 - 5 ppm. BHC treatment for a period of 30 days due to the inhibition of hormones responsible for the oogenesis. The delayed oogenesis and failure of oogenesis at certain stages result in decrease in the fecundity and gonosomatic index.

The ovary of BHC treated fish was somewhat loose in texture. The control ovary showed normal features and the ovary were intact. Different stages of the oogenesis were clearly demarcated and the germinal layers were intact. The gonosomatic index of the control showed gradual increase with the increase in duration of the experiment. The ruffles, appearing on the surface of the toxicant stressed ovary might cause contact inhibition among the toxicity infested cells. However, later stage of exposure showed significant arrangement and movement of ruffles and blebs over the cells.

The inter-renal tissue of *H. fossilis* is embedded in the anterior end of the kidney and associated with post cardinal vice. The inter-renal cells are polygonal columnar and spindle shaped as appeared under SEM. The cells contain lipid droplets and ascorbic acid, cholesterol; glucose 6phosphatase (G6PG) dehydrogenase and $\Delta 5 3\beta$
dehydrogenase.

Patches of chromaffin cells (produces catecholamines, adrenaline and noradrenaline) are found in the region of the post cardinal venous drainage of the kidney. Chromaffin cells are generally more uniform and rounded in appearance than are the internal cells and their cytoplasm is pale and slightly basophilic. Both the anterior kidney region and the plasma of teleost have been shown chemically to contain adrenaline and noradrenaline.

The swollen and lumpy appearance in the 30 days BHC treated kidney was due to an accumulation of variously sized intracellular cell bodies sometime seen in SEM images probably due to the rupturing of large intracellular vacuoles. The interpedial microbridge probably represent the sites of contact in the formation of extensive occluding junctions between adjacent pedicles. The SEM images have been used to demonstrate a loss of podocytes pedicels in alloxan dibetic rats (Hagg and Winblad 1975), in response to the nephrotic syndrome induced by injection of N, N-diacyetylbenzidine (Carroll et al., 1974) and in a renal biopsy taken from a patient suffering from unilateral occlusalve hydronephros (Lehtonen et al., 1973).
The vacuoles observed in the pesticide exposed kidney and gonads may be originated from liposomes (Durfort 1982) which are cisterns of endoplasmic reticulum with scattered ribosomes or also dialations of distal dictiosome cisternae (Poquet 1986). The abundance of small vacuoles indicates an enhancement of excretory activity of the kidney. Within the same type of response, distal dialation of dictiosomes may also indicate relevant synthesis of glycoproteins (Tartakoff 1980).

There have been several investigations of acute renal failure reported in the literature. Cox et al., (1974) observed that podocytes major processes and pedicles appeared "crowded" and indistinguishable in response to acute renal failure. This failure might be brought from the vaso-constriction produced by infusion of enhanced norepinephrine under toxic stress. These observation led us to suggest that a decrease in glomerular capillary permeability may be present in the failure of renal activity. Stein et al., (1975) reported similar observation in response to acute renal failure resulting from the administration of uranyl nitrate. However, Dach and Kurtzman (1976) noted that the visceral epithelium appeared unaltered and that the glomerular endothelium is filled with debris, erythrocytes and sometime fibrinlike materials.
The observations in the present investigation of gonads and kidney of *H. fossilis* exposed to 15 ppm. concentration of BHC are studied under SEM and these three organs exhibit that at an early stage the 3D structures have been changed. In all these cases the 45th day appeared to be a critical period. However, thereafter the organs attempted to restore the normal level activity thereby suggesting that at longer periods i.e. on the 90th day the gonads and kidney of *H. fossilis* have become resistant over the toxic effect. It has been mentioned that the fish develops the immunity against xenobiotic material. This immunity/resistance is facilitated by the rupture of the ruffle which appeared to be useful in isolating toxicant infested cells or otherwise triggering the antibodies against this after 45 days. Thus detoxicating mechanism will include both physiological response i.e. (a) sequestration in concertions and binding to the cytoplasmic proteins, and (b) subsequent enhancement of apocrine excretion (Marigomez et al., 1990). In fact Fowler (1972) reports the extrusion of cytoplasmic masses from methyl mercuries exposed rat kidney tubule in association with proteinuria and Dillaman (1980) reports the occurrence of reticular bodies and kidney functions appeared to be normal (Marigomez 1989), and at present it is difficult to find support for direct effect of toxicant on glomerular function even in the well studied chronically exposed mammals.(Cheville 1980).
Summary:

1) The tissues of H. fossilis i.e. gonad and kidney were observed under SEM starting with fixative, dehydration etc. The dried specimens were fixed with metal coating and observed under SEM.

2) The control testis under SEM exhibited intact follicles with microprojection, podocytes and a few number of blebs. The early part of treated testis exhibited disintegration of follicles, increased number of blebs, but the reappearance of follicles with cytopodia and podocytes were observed later.

3) The control ovary exhibited a distinct follicular structure with microprojection. On the 15th and 30th day treated ovary, the rupture of membranes along with bend microprojection as well as increased number of blebs were observed.

4) In kidney the chromaffin cells are uniform and rounded in appearance than the internal cells. The parital epithelium is spherical in shape and outer surface is smooth with basal lamina whereas inner surface exhibits cilia. Finger or bleb like microprojections are observed on the free surface of parital cells. In the visceral epithelium finger or bleb like microprojections are observed. The 15th and
30th day treated kidney exhibits a swollen and lumpy appearance. However, on the 60th and 90th day the loss of podocytes was observed. Though the proximal tubules altered during experiment yet the visceral epithelium remained unaltered.

5) Increased excretion and protein synthesis leads to the alteration in cellular transport which results in a saturation of the homeostasis regulatory capacity. Subsequently, cellular autolysis takes place resulting in nuclear pycnosis and cytoplasmic hydropic degeneration.