CHAPTER - III

MATERIALS AND METHODS
In the present investigation, following material and methods have been employed:

1. COLLECTION OF FISHES: - To evaluate the effect of pesticides (BHC, Endosulfan, Malathion and Carbaryl) on the tissues of fresh water teleost, the fishes selected were Mystus tengara, Family - Bagaridae, Order - Cypriniformes; Heteropneustes fossilis, Family - Heteropneustidae, Order - Cypriniformes and Oreochromis mossambicus, Family - Cichlidae and Order - Perciformes.

Live specimens of Mystus tengara and H. fossilis were collected from the Sagar lake and the fish Oreochromis mossambicus was obtained from the river of Kerala. Fishes of almost the same body weight (M. tengara 12-14 gm., length 10-12 cms., H. fossilis 12-15 gm. and 10-13 cms. length and O. mossambicus 4-5 gm. and 5-7 cms. length) were used in the present investigation. The fishes were kept in a big glass aquaria in the laboratory for a week for proper acclimatization.

2. CHEMICALS USED: - The different chemicals (Pesticides) used in this investigations were of technical grade. BHC (80%) was supplied by M/S. Union Pesticide Ltd., Bhopal, Endosulfan (94.6%) by Excel Industries Ltd., Bombay; Malathion (95%) Cynamide Ltd., Bombay; and Carbaryl by Union Carbide India Ltd., Bhopal.
BHC and Endosulfan belongs to the organochlorine group, 
Malathion to organophosphorus group and Carbaryl to the 
Carbamate Group.

3. CLASSIFICATION AND CHEMISTRY OF INSECTICIDES :

Based on chemical group, the synthetic insecticides accord-
ing to their chemical formulation fall into 3 categories.

A) **Organochlorine Insecticides** :- All compounds belonging to this 
group are called chlorinated hydrocarbons and posses Carbon, 
Chlorine and sometimes Oxygen atoms including a number of 
C-Cl bond. They also have cyclic Carbon chain (including 
Benzene ring). These are non-systematic nerve poisons and 
produce lethal effects in the organisms. In aquatic ecosystem 
these insecticide manifest biological magnification through food 
chain. Examples are Aldrin, BHC, Endosulfan etc.

B) **Organophosphorus Insecticides** :- Most of these are some forms 
of phosphate. These are systematic poisons and produce toxic 
effects by contact. Their toxicity result in the inhibition by 
acetylcholinesterase (ACHE) as a result, acetylcholine accumu-
lates and disrupts the normal functioning of the nervous 
system giving rise to the typical cholinergic symptoms 
associated with poisoning i.e. hyperactivity, convulsions, 
paralysis and death. Examples are Diazinon, Malathion, 
Parathion etc.

C) **Carbamate Insecticides** :- These are reversible cholinesterase 
inhibitory, probably they effect directly acetylcholine recep-
tors. The poisoned animal shows violent convulsions and other 
neuromuscular disturbances. Examples are Sevin, Carbaryl 
etc.
Based on formulations, the insecticides are marketed in different forms which include dust, powder, granule, paste, and water soluble powder etc. These different forms of insecticides depends on their efficiency to kill the insects pest etc.

(a) Dust or powder - Eg. - DDT, BHC, Malathion etc.
(b) Granules - Eg. - Toxaphene, Carbofuran etc.
(c) Wettable powder - Rogar, Enderin.
(d) Aerosols - DDT, BHC, Dieldrin etc.

CHEMISTRY OF INSECTICIDES :

Benzene Hexa-chloride (BHC) :- It is banned in EEC countries and cancelled in the U.S.A. It is categorised by WHO as highly hazardous and is a carcinogen known to 2½ times more toxic than DDT. In India, consumption is about 33000 tonnes (33 million) a year.

Formula and Structure :- The product consists of 8 possible isomers, five of which have been violated in alpha, beta, gama, delta and epsilon forms. A mixture of isomers of 1, 2, 3, 4, 5, 6 hexachlorocyclohexane.

These compounds have been widely used outside the United States as a stomach poison insecticide. Their residues were frequently detected in all components of the environment (Edward 1973). The rapid bioaccumulation and biomagnification of persistent BHC residues through food chain posses serious problem of health hazards in human and animal population (Hayes, 1975). The symptoms of acute poisoning from the isomers are hypersensitivity, tremors and convulsion, which may be prevented by pentobarbitol and to a less extent by the isomers. BHC also antagonizes convul-
sions normally produced by metazole, picrotoxin and like theomide. Chronic poisoning with all of the isomers result in an increase in size of the liver with centrolobular hepatic cell enlargement.

**Structure:**

![Structure Diagram]

**Endosulfan:** Endosulfan was developed and introduced by Ferbwerke Hoechst A.G. in 1954 under the registered trade mark "Thiodon (1)". The other alternate names of endosulfan are cyclodon, thimol, thofar, and Malix. It is chemically known as 6, 7, 8, 9, 10, hexachloro, 15, 5, 9, 6, 9, hexahydromethano 2-4, 3-benzodioxathiepine-3 oxideov, -B, 1, 2, 3, 4, 7,7-hexametholene (5-6) Sulfite. The insecticide endosulfan is obtained by the action of thiomylchloride and is butenediol, 1-4 (2).

Endosulfan, an organochlorine pesticide is a stomach poison and has found wide application in ornamental plant, growing agriculture and forestry. Its lack of toxicity towards beneficials is of special significance. It is being widely used and as such it is expected that this insecticide may enter human and animal system either directly or indirectly as environmental contamination.

Endosulfan rapidly breaks down in the organism. There is no danger of accumulation. Longterm feeding trials gave no indications of a chronic toxicity.
**Formula and Structure** :

\[ \text{Formula Image} \]

**Malathion** :- Malathion (O-O dimethyldithiophosphate of dimethylmercepto succinate) is a broad spectrum organophosphorus insecticide. It is a major lethal poison which is widely used because of its efficiency and also its less persistence in the aquatic environment. Though it is less toxic to fish, yet it has been shown to induce histopathological changes in some of vital internal organs (Dubale and Shah 1979). Malathion is used to control a variety of insects on rangeland, forestlands and agricultural lands. The major effects of this insecticide is the reduction of acetylcholinesterase (ACHE) which in turn would bring about the inability of body muscles to perform properly. These are known to attack the animals by inhibiting acetylcholinesterase activity. The tolerance of the toxicity of organophosphate has been studied recently in the mosquito fish (*Gambusia afflians*) by chamber (1976).

**Formula and Structure** :-

\[ \text{Formula Image} \]

**Carbaryl** :- 1-napthalenyl, methyl carbamate carbaryl is a common carbamate insecticide synthesized from 1-napthal and methyl isocyanide widely used for agriculture purpose. It is a lethal poison. Till yesterday, it was widely used and available but after the Bhopal tragedy in which thousand of people were killed, its
production has been stopped. So these days it is not available. It is known to induce histopathological changes in the internal organs of fishes.

**Formula and Structure**:

\[ \text{C}_{10}\text{H}_{7}\\text{OOCHCH}_{3} \]

\[ \text{O} \quad \text{H} \]

\[ \text{C} - \text{O} - \text{N} - \text{CH}_{3} \]

**Bioassay studies** :- The test fishes were observed for any pathological symptoms and placed in a big glass aquaria in the laboratory for a week for the proper acclimatization. Every effort was made to provide optimum condition to the fish. The bioassay methods used were as suggested by American Public Health Association (1985). The water in the aquaria was changed on alternate days and the fishes were fed with fish food every alternate day. If mortality occurred under these conditions dead fish were removed immediately.

To study the effect of pesticides on brain, liver, gills, intestine, kidney and skin, experiments were conducted in two phases. In the first phase of experiment, lethal concentration (LC\textsubscript{100}) and sublethal concentration (LC\textsubscript{50}) of the pesticides was calculated. In the second phase of experiment fishes were kept in sublethal concentration of pesticides over a period of a month.

**Test Solutions** :- Test solution of different concentrations were prepared from common stock solution. For BHC and Endosulfan 500 mg. was dissolved in 1% acetone solution and was further diluted with distilled water. For carbaryl 1 gm. pesticide was dissolved in 1% dimethyl formamide solution. For malathion the stock solution
was prepared by dissolving 1 ml. of pesticide in 100 ml of water.

$\text{LC}_0$, $\text{LC}_{50}$ and $\text{LC}_{100}$ values: $\text{LC}_0$, $\text{LC}_{50}$ and $\text{LC}_{100}$ values for each pesticide for 4, 10, 15 and 30 days intervals were calculated and are shown in the Table No. 1 - 9. During the second phase of experiment about 200 fishes were acclimatized in the laboratory aquaria for a week at a temperature of 21 ± 5°C. They were exposed to the above mentioned pesticides at a sublethal concentration. Simultaneous controls were maintained. The water and the pesticide were renewed on alternate days during the experimental period.

HISTOPATHOLOGICAL AND ANALYTICAL STUDIES:— For histopathological and analytical studies 15 fishes were taken out at 96 hrs, 10 days, 15 days and 30 days. Each fish was removed from the water and immediately stunned with a blow on the head. The tissues were taken out and rinsed in physiological saline and debris removed and then used for both the studies.

PROCESS FOR HISTOPATHOLOGICAL STUDY:— For this study, the tissues (Liver, intestine, gills, kidney and skin) were fixed in aqueous Bouin’s solution and brain were fixed in both Hieldhain’s SUSA and aqueous Bouin’s. All the possible precautions were taken to ensure a proper fixation of tissues. The tissues were kept in the respective fixatives for 24 hrs.

After proper fixation the material was thoroughly washed in running tap water. The material was dehydrated in different ascending grades of alcohol, kept in methyl benzoate for overnight and then in Benzene for about 1 hr. After this, material was transferred to paraffin wax in the oven with temperature maintained at
at 62°C and after giving 3 changes in wax of 30 minutes each, blocks were prepared. Sections cut at 7 μ thickness and stained with Heidenhain's Azan.

PROCESS FOR ANALYTICAL STUDIES :- Qualitative identification of pesticide residues and pesticide metabolites in the brain of *H. fossilis* was done by thin layer chromatography (TLC). It is inexpensive and compact apparatus, simple in technique and gives quick results. This qualitative method is being used widely. For the detection and confirmation of the presence of pesticides in the tissues, this method has been particularly suitable. It is a specific, rapid and sensitive method widely used for qualitative determinations and the sensitivity of the detection is also increased. Quantity as small as 0.1 mg can be detected on thin layer plates.

1. Isolation from Fish Tissues :- Various methods are available for the isolation of pesticide from the fish tissues. In the present work the method of Walker and Beroza (1963) has been used.

2. Tissue Sampling :- After 10, 15 and 30 days of exposure, brain (0.5 gm.) was removed and crushed in acetone with anhydrous sodium sulphate and was fixed for 24 hr in a mixture of hexane : ether (90 : 10) for the extraction of organochlorine pesticide (BHC and Endosulfan). Hexane : Acetone (80 : 20) mixture for organophosphorus pesticide and acetone for carbamate pesticide.

   Silica gel 'G' plates of 250 μ thickness were prepared and employed in the chromatographic separation of components of pesticides.
3. Preparation of Plates:

A) Washing of Plates: - First of all glass plates were thoroughly washed with chromic acid and then rinsed with the detergent solution. The plates were rinsed with distilled water and dried.

B) Absorbants: - In the present work, Silica gel 'G' was used as absorbant, 30 gm silica gel 'G' was dissolved in 60 ml of distilled water. Slurry was placed in the chamber of applicator. Desired plates of uniform thickness viz. 250 
 were obtained. Plates were dried at room temperature and finally activated for 1 hr. at 60°C in an oven.

C) Apparatus: - The apparatus used in this experiment consists of:

1. Rectangular glass jar with a ground rim on which a glass lid was placed. Grease was applied to the rim to make the glass tank airtight.
2. Glass plates - size 22.5 x 10 Cm.
4. Sprayer or glass automizer.
5. Suitable solvent of analytical grade.

4. Solvent Systems and Spray Reagents:

A) Solvent System: - For the development of chromatogram following solvent systems were used:

(1) For organophosphorus pesticides.

   i. nHexane : Acetone (80 : 20)
   ii. Hexane
(2) For organochlorine pesticides.
   i. nHexane : Ether (90 : 10)
   ii. nHexane : Benzene (50 : 50)
   iii. Chloroform : Methanol (90 : 10)

(3) For carbamate pesticide
   i. Cyclohexane : Acetone (80 : 20)
   ii. Benzene : Acetone (95 : 5)
   iii. Ether : Hexane (80 : 20)

5. Spray Reagents :
   A) For Organophosphorus Pesticides : - 0.5 gm. palladium chloride
      was dissolved in 100 ml of water containing a few drops of
      15% HCl. This solution was sprayed over the developed
      chromatogram. Yellow coloured spots were obtained.

   B) For Organochlorine Pesticide : - 2 gm of o'dianicidine was
      dissolved in 100 ml of absolute alcohol and sprayed over the
      developed chromatogram and brown spots were obtained.

   C) For Carbamate Pesticide : - Tollen's reagent was used as
      spray reagent for the detection of carbamate residue and was
      prepared by mixing equal amount of 2% AgNO₃ and 10% NaOH in
      distilled water. To this solution liquor amonia was added till
      the solution become colourless. After spraying, the plates
      were kept in an oven at 60°C for 1 hr. Golden yellow and
      brown spots were obtained.

6. Spotting of the Plates : - With the help of capillary tubes,
   solution of samples were applied on the plates. The area of
   application should be kept as small as possible, because the
   smaller the area of application the sharper will be resolutions.
   The spots were observed about 15 cm above from the bottom of the
   plates.
METHODS

Approximately 100 ml of each suitable solvent was poured into the developing glass jar. These jars were covered with the coverlids and made airtight. They were placed in the same condition for an hour, so as to saturate the chamber with the vapour of the solvent system. The spotted plates were placed in the chamber making approximately an angle of 40° to the base of chamber for ascending development and consequently the solvent ascends, when solvent reached about 10 cm above the distance from the point of application, the plates were removed and air dried. The plates were sprayed with specific reagent concerned. The Rf values of spots were calculated with the help of the following formula.

\[ Rf = \frac{\text{Distance of spot centre from the start point}}{\text{Distance of solvent from start point}} \]

Rf values varies with layer thickness. Therefore in this study, layer thickness was always kept constant at 250 \( \mu \).
From the exploratory tests conducted $LC_{100}$, $LC_{50}$ and $LC_0$ values were recorded. The experimental concentration i.e. the concentration for intermediate test, was calculated for each pesticide using the formula.

$$SEC = \frac{(96 \text{ h } LC_{50})^2 \times A}{24 \text{ h } LC_{50}}$$

where, SEC = Safe Experimental Concentration.

$A = $ Application factor i.e. 0.3.

Experiments were setup for 30 days to study the toxic effect. Fishes were separated into groups of 10 and exposed to sublethal concentration of pesticide, calculated by the above mentioned formula in different glass aquaria. Simultaneous control were also maintained.

Safe experimental concentration (SEC) for the intermediate term toxicity test last from 8 to 90 days, (APHA, et al. 1985) was calculated for all the pesticides to each fish on the basis of 24 and 96 h. $LC_{50}$ values using a slight modification of the formula suggested by Hart et al. (1945). The formula of Hart et al. (1945) is -

$$C = 48 \text{ h TLM} \times A/s^2$$

where, $C = $ Harmless concentration;

$A = $ Application factor its value was suggested by Hart et al. (1945) to be 0.3

$s^2 = 24 \text{ h TLM}/48 \text{ h TLM}$

The harmless concentration calculated by the formula
was not applicable as the safe experimental concentration because mortality of the fishes occurred within the test period. So modification had to be made by replacing 48 h TLM with 96 h TLM i.e. 96 h LC$_{50}$.

Thus,

$$SEC = \frac{(96 \text{ h } LC_{50})^2 \times A}{24 \text{ h } LC_{50}}$$

where, SEC = Safe Experimental Concentration

A = Application factor (0.3 as suggested by Hart et al. (1945)).

This formula was found to be correct with all the pesticide used.
### TABLE NO. 1 - TOXICITY OF BHC TO HETEROPNEUSTES FOSSILIS

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Temperature</th>
<th>Dosage in mg/l.</th>
<th>Duration</th>
<th>Mortality</th>
<th>Lc Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHC</td>
<td>21-23°C</td>
<td>3.75</td>
<td>96 hrs.</td>
<td>All died</td>
<td>Lc&lt;sub&gt;100&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.15</td>
<td>96 hrs</td>
<td>Half died</td>
<td>Lc&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.4</td>
<td>96 hrs</td>
<td>All alive</td>
<td>Lc&lt;sub&gt;0&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.8</td>
<td>4,10,15 &amp; 30 days</td>
<td>All alive</td>
<td>Sublethal dose</td>
</tr>
</tbody>
</table>

Preparation of stock solution: - 0.5 gm BHC dissolved in 0.1% Acetone.

### TABLE NO. 2 - TOXICITY OF ENDOSULFAN TO HETEROPNEUSTES FOSSILIS

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Temperature</th>
<th>Dosage in mg/l.</th>
<th>Duration</th>
<th>Mortality</th>
<th>Lc Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endosulfan</td>
<td>22-24°C</td>
<td>0.0037</td>
<td>96 hrs.</td>
<td>All died</td>
<td>Lc&lt;sub&gt;100&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0035</td>
<td>96 hrs</td>
<td>Half died</td>
<td>Lc&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.003</td>
<td>96 hrs</td>
<td>All alive</td>
<td>Lc&lt;sub&gt;0&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.001</td>
<td>4,10,15 &amp; 30 days</td>
<td>All alive</td>
<td>Sublethal dose</td>
</tr>
</tbody>
</table>

Preparation of stock solution: - 0.5 gm BHC dissolved in 0.1% Acetone.
### TABLE NO. 3 - TOXICITY OF MALATHION TO *HETEROPNEUSTES FOSSILIS*

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Temperature</th>
<th>Dosage in mg/l.</th>
<th>Duration</th>
<th>Mortality</th>
<th>Lc Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malathion</td>
<td>23-25°C</td>
<td>38</td>
<td>96 hrs.</td>
<td>All died</td>
<td>$Lc_{100}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>96 hrs</td>
<td>Half died</td>
<td>$Lc_{50}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24</td>
<td>96 hrs</td>
<td>All alive</td>
<td>$Lc_0$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>4, 10, 15, 30 days</td>
<td>All alive</td>
<td>Sublethal dose</td>
</tr>
</tbody>
</table>

Preparation of stock solution: - 1 ml malathion dissolved in 100 ml of water

### TABLE NO. 4 - TOXICITY OF CARBARYL TO *HETEROPNEUSTES FOSSILIS*

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Temperature</th>
<th>Dosage in mg/l.</th>
<th>Duration</th>
<th>Mortality</th>
<th>Lc Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbaryl</td>
<td>21-23°C</td>
<td>65</td>
<td>96 hrs.</td>
<td>All died</td>
<td>$Lc_{100}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>58</td>
<td>96 hrs</td>
<td>Half died</td>
<td>$Lc_{50}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>51</td>
<td>96 hrs</td>
<td>All alive</td>
<td>$Lc_0$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17</td>
<td>4, 70, 15, 30 days</td>
<td>All alive</td>
<td>Sublethal dose</td>
</tr>
</tbody>
</table>

Preparation of stock solution: - 1 gm carbaryl dissolved in 1% Dimethyl formamide.
### TABLE NO. 5 - TOXICITY OF BHC TO MYSTUS TENGRA

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Temperature</th>
<th>Dosage in mg/l.</th>
<th>Duration</th>
<th>Mortality</th>
<th>Lc Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHC</td>
<td>23-25°C</td>
<td>1.1</td>
<td>96 hrs.</td>
<td>All died</td>
<td>Lc&lt;sub&gt;100&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.95</td>
<td>96 hrs</td>
<td>Half died</td>
<td>Lc&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.75</td>
<td>96 hrs</td>
<td>All live</td>
<td>Lc&lt;sub&gt;0&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.25</td>
<td>30 days</td>
<td>All alive</td>
<td>Sublethal dose</td>
</tr>
</tbody>
</table>

Preparation of stock solution: - 0.5 gm BHC dissolved in 0.1% Acetone.

### TABLE NO. 6 - TOXICITY OF MALATHION TO MYSTUS TENGRA

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Temperature</th>
<th>Dosage in mg/l.</th>
<th>Duration</th>
<th>Mortality</th>
<th>Lc Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malathion</td>
<td>23-25°C</td>
<td>12</td>
<td>96 hrs.</td>
<td>All died</td>
<td>Lc&lt;sub&gt;100&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>96 hrs</td>
<td>Half died</td>
<td>Lc&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>96 hrs</td>
<td>All alive</td>
<td>Lc&lt;sub&gt;0&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>30 days</td>
<td>All Alive</td>
<td>Sublethal dose</td>
</tr>
</tbody>
</table>

Preparation of stock solution: - 1 gm malathion dissolved in 100 ml of water.
### Table No. 7 - Toxicity of Carbaryl to Mystus Tengra

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Temperature</th>
<th>Dosage in mg/l.</th>
<th>Duration</th>
<th>Mortality</th>
<th>Lc Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbaryl</td>
<td>21-23°C</td>
<td>24</td>
<td>96 hrs</td>
<td>All died</td>
<td>Lc&lt;sub&gt;100&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18</td>
<td>96 hrs</td>
<td>Half died</td>
<td>Lc&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>96 hrs</td>
<td>All alive</td>
<td>Lc&lt;sub&gt;0&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>30 days</td>
<td>all alive</td>
<td>Sublethal dose</td>
</tr>
</tbody>
</table>

Preparation of stock solution: - 1 gm carbaryl dissolved in 1% dimethyl formamide.

### Table No. 8 - Toxicity of BHC to Oreocharmis Mossambicus

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Temperature</th>
<th>Dosage in mg/l.</th>
<th>Duration</th>
<th>Mortality</th>
<th>Lc Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHC</td>
<td>25-26°C</td>
<td>0.75</td>
<td>96 hrs</td>
<td>All died</td>
<td>Lc&lt;sub&gt;100&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.65</td>
<td>96 hrs</td>
<td>Half died</td>
<td>Lc&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>96 hrs</td>
<td>All alive</td>
<td>Lc&lt;sub&gt;0&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.17</td>
<td>30 days</td>
<td>all alive</td>
<td>Sublethal dose</td>
</tr>
</tbody>
</table>

Preparation of stock solution: - 0.5 gm BHC dissolved in 0.1% Acetone.
TABLE NO. 9 - TOXICITY OF MALATHION TO OREOCHROMIS MOSSAMBICUS

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Temperature</th>
<th>Dosage in mg/l.</th>
<th>Duration</th>
<th>Mortality</th>
<th>Lc Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malathion</td>
<td>26-27°C</td>
<td>6.0</td>
<td>96 hrs</td>
<td>All died</td>
<td>Lc&lt;sub&gt;100&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.0</td>
<td>96 hrs</td>
<td>Half died</td>
<td>Lc&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.7</td>
<td>96 hrs</td>
<td>All alive</td>
<td>Lc&lt;sub&gt;0&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.9</td>
<td>30 days</td>
<td>All alive</td>
<td>Sublethal dose</td>
</tr>
</tbody>
</table>

Preparation of stock solution:— 1 ml malathion dissolved in 100 ml of water.