Observations and results
The range of water temperature varies during different season of a year. The data collected from the Zirpurwadi lake for one year i.e. from January 2001 to December 2001 during January 2001 temperature was 15.1 °C, the temperature started increasing from January upto May, higher during April 34.1 °C and highest during May 35.1 C. From May to September it gradually decreases. In October some increase in temperature noted and then started decreasing upto January.

The seasonal changes in temperature showed that the temperature is maximum in summer and minimum in winter and moderate in monsoon season.

The role of pH is very important for aquatic organisum. During the present study, pH ranges between 7.5 to 8.3 in 2001. During the month January-2001 the values were 7.9 then increases upto May and then decreases in June and July. The pH is highest in May 8.3 from June to January the values are increases slightly.

The seasonal variation in pH shows that it is maximum during summer 8.3 followed by winter 7.9 and monsoon 7.5.

The conductivity of water in the lake is a characteristic which is mainly associated with the dissolved material or solute concentration.
Fig. 4.1 Temperature Range

Fig. 4.2 pH Range

Fig. 4.3 Conductivity Range

Fig. 4.4 TDS Range
present in the water.

Conductivity of a lake during year 2001 ranges between 0.121 to 0.208. The conductivity value in January 0.175, increase upto May 0.208. The value then decrease upto June and July and increases from August to December.

The seasonal trend in the year 2001 was maximum in summer 0.208 followed by winter 0.175 and minimum in mansoon 0.121.

In the present study the monthly variation in the total dissolved solid mg/l ranges between 60.3 to 120.62 mg/l. The values were 61.9 in January which increased upto March 65.3 and decline in April 60.9 then the value increasing rapidly till September 120.62 and show highest peak. After September the value of total dissolve solid decreases upto December 61.9.

The seasonal trend was maximum during rainy season 120.62 followed by post mansoon 72.2, summer 67.9 and winter 61.9.

Chlorides occurs in most of fresh water as the salt of Na, Mg, Ca.

The monthly variataion in total dissolve solid during the present study was between 61.6 to 99.2. In 2001 values were 77.5 mg/l. during January values increases upto May 99.2 then also from June 61.6 values started increasing upto September. 85.2 value increases from
Fig. 4.5 Chloride Range

Fig. 4.6 Sulphate Range

Fig. 4.7 Phosphates Range

Fig. 4.8 Dissolve Oxygen Range
October 66.2 upto December 72.6.

The seasonal changes varied as the maximum chloride was recorded during May (99.2) 2001 and minimum during June 2001.

Natural waters contain higher level of the sulphates contributed from weathering of the rocks. In addition to this, domestic sewage and industrial waste also contribute sulphate to aquatic ecosystem. Sulphate provide hardness to the water.

Values of sulphate ranged between 4.12 to 14.24. During the month January 2001 (4.12) then value increases further upto September 14.24. Then the value decreases upto December 5.11.

Seasonal changes throughout the study period was maximum during post monsoon 14.24 followed by summer 9.73 and minimum in winter 4.12.

It is very essential nutrients of plant and play important role in aquatic ecosystem.

The range of phosphorus in the lake during the present study was between 0.005 to 0.024. The phosphate value increases from January 0.005 upto September 0.024 and then decreases till December 0.008. The seasonal variation throughout the period was maximum in post monsoon 0.024 followed by summer 0.016 and minimum in winter 0.005.
Dissolve oxygen is one of the most important parameter of water quality directly affecting survival and distribution of flora and fauna in an ecosystem.

In the present study dissolve oxygen ranges between 5.4 mg/l to 9.8 mg/l the maximum value recorded in January 9.8 mg/l and minimum value recorded in May 5.4 mg/l. In January 9.8 mg/l dissolve oxygen is highest while it is lowest upto May 5.4 mg/l. and then started increasing till December 2001.

The seasonal variation throughout the year 2001 showed maximum during winter 9.8 mg/l. which is maximum and minimum during summer 5.4 mg/l.

In an aquatic ecosystem free CO₂ is essential for photosynthesis. The range of free CO₂ in the water under study was 0.05 to 4.11 value of free CO₂ during January 1.03 increases upto July 4.11 then decreases till october 0.05. Again increases upto December 0.09.

The seasonal variation throughout the period was maximum during Monsoon followed by summer 3.01 declined in winter.

Hardness to water is mainly imparted by alkaline earth metallic cations, mainly calcium and magnesium present in it.
Fig. 4.9 Free Carbon dioxide Range

Fig. 4.10 Total Hardness Range

Fig. 4.11 Total Alkalinity Range

Fig. 4.12 Turbidity Range
In the present study the range of total hardness of the lake during investigation was between 145.3 to 212.3. During the month January 2001 the values were 145.3 then increases upto May 212.3 then further decrease in June 159.4 and started increasing upto September 171.3 then decreases till December 149.5.

The seasonal variation in total hardness shows that the total hardness is maximum during summer 212.3 followed by monsoon 166, minimum in winter season 145.3.

During the present study the range of total alkalinity in the lake water under investigation is between 114.82 to 189.35 mg/l. Total alkalinity starts increasing during January 114.82 then increases upto, 189.35, total alkalinity then decrease in June 108.60 then increases upto November 138.31 decreases in December, 2001. The seasonal changes in total alkalinity shows that it is maximum during summer 189.35 followed by winter and decline in monsoon.

The water which is unclear or muddy is called turbid water. It is caused by substances that are having particle size more than 10(9)m.

The value of turbidity ranges between 43 to 82 NTU. The turbidity of lake during January 45 decreases in February 43 then increases in March 55 again decreases in April 53 and gradually increases from May 54 to August 82 then further decreases upto December 64.
Fig. 4.13 Transparency Range

Fig. 4.14 Bicarbonate Range

Fig. 4.15 Calcium Range

Fig. 4.16 Magnesium Range
The seasonal variation in turbidity is maximum during monsoon 82 followed by winter 64 decline in summer 54.

Transparency regulates the productivity of an aquatic ecosystem.

The range of Transparency in the water under study was between 23.1cm to 42.0cm. The value of transparency is maximum during may and lowest in monsoon 23.1cm.

The seasonal variation throughout the period was maximum during summer 42.0 followed by winter 35.0 and decreases in Monsoon.

Bicarbonate content of the lake water is maximum during october 330.01 and less during Jan 269.25.

The value in January 269.25 then increases in may 325.2 then decreases in july 280.03 then increase in Oct 330.03 decrease upto Feb 269.25.

From the graph it is revealed that during January 46.2 the value is to much and decreased immediately in February 3.0 and March 2.6 then the value increases from April 35.0 to May 57.5 and further decline upto December 3.2.

The seasonal changes throughout the study period was maximum during summer season followed by monsoon and decreases in winter.
Fig. 4.17 Sodium Range

Fig. 4.18 Potassium Range
The values of potassium in the investigated water is maximum during June 22.42 and minimum during December. In January value was 13.6 then increases upto June 22.42 and from June value decreases till December.

The seasonal trend changes throughout the period was maximum in rainy season 22.42 followed by summer 20.50 and less in winter (2.13).

The value of calcium in investigated lake water in January 61.13 increases upto to April 80.16. In May 78.24 value decreases upto July 40.23 then further increases upto December 60.32.

The maximum value of calcium is during April 80.16 and minimum during July 40.23.

The seasonal variation throughout the period was maximum during summer followed by winter in decline in rainy season.

Observation on fauna is incorporated in Table.4.3 Zirpurwadi lake shows a faunal diversity. The observation revealed that one species of gastropods were found in the lake. The fishes which live in lake represent two families of which family cyprinidae and family ophiocephalidae. Frog, Rana tigrina and two species of snakes were noticed.
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# PLANKTONS OF ZIRPURWADI LAKE

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**Bacillariophyceae**

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<td>2</td>
<td><em>Cymbella cistula</em></td>
<td>22</td>
</tr>
<tr>
<td>3</td>
<td><em>Diatom valgare</em></td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td><em>Fragelaria capucina</em></td>
<td>09</td>
</tr>
<tr>
<td>5</td>
<td><em>Frustulia rhomboides</em></td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td><em>Navicula radiosa</em></td>
<td>01</td>
</tr>
<tr>
<td>7</td>
<td><em>Pinnularia nobilis</em></td>
<td>03</td>
</tr>
<tr>
<td>8</td>
<td><em>Synedra ulna</em></td>
<td>22</td>
</tr>
<tr>
<td>9</td>
<td><em>Rhopalodia gibba</em></td>
<td>08</td>
</tr>
</tbody>
</table>

**Cyanophyceae**

<table>
<thead>
<tr>
<th></th>
<th>Species</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Anabaena</em> sp</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td><em>Gleocapsa</em> sp</td>
<td>10</td>
</tr>
</tbody>
</table>
Lyngbya sp 08

Microcystis sp 12

Nostoc sp 42

Zooplankton

Rotifera

a) Filinia terminalis 10
b) Brachionus plicatilis 13
c) Cephalodella megalcephala 18
d) Polyarthra vulgaris 08
e) Keratella sp. 09
f) Monostyla closterocera 12
g) Lepadella sp. 03
h) Trichocera sp. 07

Cladocera

a) Daphnia sp. 20
b) Daphnia similis 06
c) Bosmina sp. 04
d) *Sida* sp. 02

e) *Moina brachiata* 06

f) *Alona* sp. 05

g) *Leptodora* sp. 08

3) Copepoda

a) *Diaptomus* sp. 15

b) *Osphranticum* sp. 11

c) *Senecell* sp. 09

d) *Cyclop* sp. 12

e) *Eucyclops* sp. 07
Fig No. 4.19 - *Brachionus plicatilis*

Fig No. 4.20 - *Cephalodella megalcephala*

*SOME ROTIFERS OF ZIRPURWADI LAKE*
Fig No. 4.21 - *Flinia terminalis*

Fig No. 4.22 - *Polyarthra vulgaris*

**SOME ROTIFERS OF ZIRPURWADI LAKE**
Fig No. 4.23 - *Monostyla closterocera*

**SOME ROTIFERS OF ZIRPURWADI LAKE**

Fig No. 4.24 - *Eucyclops prionophorus* (Copepods)
Table - 4.3
Fauna of Zirpurwadi Lake

1) Mollusca
   Fam : Lymnaeidae
       Lymnaea

2) Crustaceans
   *Palaemon malcolmsonii*
   *Paratelphusa jaquemonti*

3) Pisces
   Fam : Cyprinidae
       *Catla catla* (Ham.)
       *Labeo rohita*
       *Cirrhihinus mrigala*
       *Cyprinus carpio*
   Fam : Ophiocephalidae
       *Channa Punctatus*

4) Reptiles
   Fam : Boridae
       *Eryx conicus*
   Fam : Colubridae
       *Ptyas mucosus*
From the Table 3.2 it is clear that the water used for holding the fish *Labeo rohita* did not contain any substance toxic to fish. Mortality was not observed in control experiment.

The result for toxicity evaluated for pesticide endosulfan are indicated graphically in fig. 4.37 to 4.40 and their relative toxicities are depicted in Table 4.4 to 4.7.

Exposure to different concentrations of endosulfan showed the following trend.

As the concentration increased the rate of mortality also increased.

The LC_{16}, LC_{50} and LC_{84} values at 24, 48, 72 and 96 hr are given in the Table 4.4 to 4.7 the LC_{16}, LC_{50} and LC_{84} values at different time interval were calculated by the Litchfield and Wilcoxon (1948) method.

The LC_{16} values were 1.97, 1.92, 1.77, and 1.71 mg/l at 24, 48, 72 and 96 hr of exposure period respectively.

The LC_{50} values at 24, 48, 72 and 96 hr were 2.13, 2.09, 1.99, and 1.86 mg/l. respectively. The LC_{50} values decreased with increase in exposure period. The values were higher at 24 hr and lowest at 96 hr of treatment.
The LC₄₄ values were 2.30, 2.26, 2.22 and 2.05 mg/l. at 24, 48, 72 and 96 hr respectively.

The factor of LC₅₀ values at 24 hr was found to be 2.13 and upper and lower confidence limit of LC₅₀ at 24 hr were 2.178 and 2.082 mg/l. Slope factor, upper and lower limit of slope functions values at 24 hr are given in Table 4.4.

The LC₅₀ factor value at 48 hr was 2.09 upper and lower confidence limit of LC₅₀ at 48 hr were 2.144 and 2.037 mg/l. respectively. Slope factor, upper and lower values of slope function at 48 hr are given in Table 3.2.

The factor of LC₅₀ at 72 hr was 1.99 and upper and lower confidence limit of LC₅₀ at 72 hr were 2.065 and 1.917 mg/l. respectively. This slope factor and upper and lower limit values for slope factor are given in Table 4.6.

The factor of LC₅₀ at 96 hr was found to be 1.86 and upper and lower confidence limit of LC₅₀ at 96 hr were 1.900 and 1.819 mg/l. respectively. The slope factor and upper and lower limit values for slope factor are given in Table 4.7.

The observed LC₅₀ values showed constant decrease from 24 hr to 96 hr of exposure. It clearly indicated that very low concentration was also toxic to the animal.
Table 4.4

Litchfield and Wilcoxon (1948) toxicity evaluating method steps for endosulfan exposed Labeo rohita fish at 24 hours treatment.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Conc. used</th>
<th>Percent Mortality</th>
<th>Difference a-b</th>
<th>Contribution to (CHI)^2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Observed (a)</td>
<td>Expected (b)</td>
<td>C1</td>
</tr>
<tr>
<td>1</td>
<td>1.97</td>
<td>10</td>
<td>16</td>
<td>06</td>
</tr>
<tr>
<td>2</td>
<td>2.01</td>
<td>30</td>
<td>25</td>
<td>05</td>
</tr>
<tr>
<td>3</td>
<td>2.08</td>
<td>40</td>
<td>38</td>
<td>02</td>
</tr>
<tr>
<td>4</td>
<td>2.11</td>
<td>40</td>
<td>46</td>
<td>06</td>
</tr>
<tr>
<td>5</td>
<td>2.14</td>
<td>50</td>
<td>51</td>
<td>01</td>
</tr>
<tr>
<td>6</td>
<td>2.21</td>
<td>60</td>
<td>66</td>
<td>06</td>
</tr>
<tr>
<td>7</td>
<td>2.23</td>
<td>70</td>
<td>70</td>
<td>00</td>
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<tr>
<td>8</td>
<td>2.27</td>
<td>80</td>
<td>75</td>
<td>05</td>
</tr>
<tr>
<td>9</td>
<td>2.30</td>
<td>90</td>
<td>84</td>
<td>06</td>
</tr>
</tbody>
</table>

Total number of animals used = 90, \( \text{Total C}_2 = 0.0995 \)

1. \( K = \) Total Number of concentration = 9
2. \( N = \) Degree of freedom = \( K - 2 \) = 7
3. Total number of animal used
   \[ \text{Total number of concentration used (K)} = 10 \]
4. \( (\text{CHI})^2 = \) \[ \frac{\text{Total contribution to (CHI)^2}}{\text{Total no of animal used}} \]
   \[ = 0.995 \]
5. \( (\text{CHI})^2 \) value read from table (2) for degree of freedom n i.e. 7 = 11.1

As 0.995 is less than 11.1 therefore the data are not significantly heterogenous and the curve is regarded to be good fit.
1. Slope function (S)

\[ S_1 = \text{Slope function for endosultan at 24 hours of exposure.} \]

\[ \frac{L_{C_{84}}}{L_{C_{50}}} = \frac{L_{C_{50}}}{L_{C_{16}}} \]

\[ S_1 = \frac{2.30}{2} = \frac{2.13 + 1.97}{2} = 1.080 \]

2. Slope of LC50 24 hours (fLC50)

\[ f_{L_{C_{50}}} = S^{\frac{2.77}{\sqrt{R}}} = 1.080^{\frac{2.77}{\sqrt{80}}} = 1.080^{0.30} \]

\[ = 1.023 \]

Where \( N' \) = Total No of animals treated at those concentrations whose expected effects were between 16 and 84 percent.

3. Upper confidence limit of LC50 at 24 hours for endosulfan.

\[ = L_{C_{50}} \times f_{L_{C_{50}}} = 2.13 \times 1.023 = 2.178 \]

Lower confidence limit of LC50 at 24 hours for endosulfan.

\[ = L_{C_{50}} \div f_{L_{C_{50}}} = 2.13 \div 1.023 = 2.082 \]

4. Factor of slope (FS)

\[ FS = \frac{A^{10}(K-1)}{K \sqrt{N'}} = \frac{1.45^{10(9-1)}}{9 \sqrt{80}} = 1.45^{0.99} \]

\[ = 1.444 \]

Where \( R \) = Concentration range ratio

= largest ÷ Smallest concentrations tested

By using values \( R \) and \( S \) read value designed as "A" from Nomograph number 3.

5. Upper limit of slope function

\[ = \text{slope function} \times \text{factor of slope} \]

\[ = 1.080 \times 1.444 \]

\[ = 1.559 \]

Lower confidence limit of slope function

\[ = \text{slope function} \div \text{factor of slope} \]

\[ = 1.080 \div 1.444 \]

\[ = 0.747 \]
Fig. No.: 4.37
Dose mortality relationship in endosulfan exposed *Labeo rohita* fish at 24 hours exposure period.
Table 4.5:
Litchfield and Wilcoxon (1948) toxicity evaluating method steps for endosulfan exposed Labeo rohita fish at 48 hours treatment.

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration used (C)</th>
<th>Percent Mortality</th>
<th>Difference a-b</th>
<th>Contribution to (CHI)$^2$</th>
<th>Nomograph No. 1 C2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed (a)</td>
<td>Expected (b)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.96</td>
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<td>24</td>
<td>12</td>
<td>0.0000</td>
</tr>
<tr>
<td>2</td>
<td>2.01</td>
<td>30</td>
<td>35</td>
<td>5</td>
<td>0.0150</td>
</tr>
<tr>
<td>3</td>
<td>2.05</td>
<td>40</td>
<td>42</td>
<td>2</td>
<td>0.0020</td>
</tr>
<tr>
<td>4</td>
<td>2.07</td>
<td>50</td>
<td>46</td>
<td>4</td>
<td>0.0080</td>
</tr>
<tr>
<td>5</td>
<td>2.11</td>
<td>50</td>
<td>55</td>
<td>5</td>
<td>0.0125</td>
</tr>
<tr>
<td>6</td>
<td>2.13</td>
<td>60</td>
<td>56</td>
<td>4</td>
<td>0.0075</td>
</tr>
<tr>
<td>7</td>
<td>2.17</td>
<td>70</td>
<td>66</td>
<td>4</td>
<td>0.0080</td>
</tr>
<tr>
<td>8</td>
<td>2.21</td>
<td>80</td>
<td>75</td>
<td>5</td>
<td>0.0112</td>
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<td>9</td>
<td>2.24</td>
<td>90</td>
<td>82</td>
<td>8</td>
<td>0.0270</td>
</tr>
</tbody>
</table>

Total number of animals used = 90, Total C = 0.0912

1. K = Total Number of concentration = 9
2. N = Degree of freedom = K - 2 = 7
3. Total number of animal used = 90
4. Total number of concentration used (K) = 10
5. \((CHI)^2 = \frac{\text{Total contribution to (CHI)}^2 \times \text{Total no of animal used}}{k}\)

\(= 0.912\)

6. \((CHI)^2\) value read from table (2) for degree of freedom n i.e. 7 = 11.1

As 0.912 is less than 11.1 therefore the data are not significantly heterogeneous and the curve is regarded to be good fit.
1. Slope function (S)

\[ S_1 = \text{slope function for endosulfan at 48 hours of exposure.} \]

\[ \frac{L_{C_{50}}}{L_{C_{16}}} = \frac{2.16}{2.09} = \frac{2.09}{1.92} = 1.084 \]

2. Slope of LC50 48 hour (fLC_{50})

\[ f_{LC_{50}} = S^{2.77\sqrt{R}} = 1.084^{2.77\sqrt{70}} = 1.084^{0.33} \]

\[ = 1.026 \]

Where \( N' \) = Total No of animals treated at those concentrations whose expected effects were between 16 and 84 percent.

3. Upper confidence limit of LC50 at 48 hours for endosulfan.

\[ L_{C_{50}} \times f_{LC_{50}} = 2.09 \times 1.026 = 2.144 \]

Lower confidence limit of LC50 at 24 hours for endosulfan.

\[ L_{C_{50}} \div f_{LC_{50}} = 2.09 \div 1.026 = 2.037 \]

4. Factor of slope (FS)

\[ FS = A^{10(K-1)/K} \sqrt{N'} = 1.70^{10(0.9-1)/9} \sqrt{70} = 1.70^{1.0524} \]

\[ = 1.757 \]

Where \( R \) = Concentration range ratio

= largest \( \) Smallest concentrations tested

By using values \( R \) and \( S \) read value designed as “A” from Nomograph number 3.

5. Upper limit of slope function

\[ = \text{slope function } \times \text{factor of slope} \]

\[ = 1.084 \times 1.757 \]

\[ = 1.3045 \]

Lower confidence limit of slope function

\[ = \text{slope function } \div \text{factor of slope} \]

\[ = 1.084 \div 1.757 \]

\[ = 0.616 \]
Fig. No.: 3.38
Dose mortality relationship in endosulfan exposed *Labeo rohita* fish at 48 hours exposure period.
Table 4.6:
Litchfield and Wilcoxon (1948) toxicity evaluating method steps for endosulfan exposed Labeo rohita fish at 72 hours treatment.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Conc. used</th>
<th>Percent Mortality</th>
<th>Difference a-b</th>
<th>Contribution to (CHI)^2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Observed (a)</td>
<td>Expected (b)</td>
<td>CI</td>
</tr>
<tr>
<td>1</td>
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<td>12</td>
<td>02</td>
</tr>
<tr>
<td>2</td>
<td>1.76</td>
<td>20</td>
<td>15</td>
<td>05</td>
</tr>
<tr>
<td>3</td>
<td>1.88</td>
<td>30</td>
<td>32</td>
<td>02</td>
</tr>
<tr>
<td>4</td>
<td>1.92</td>
<td>40</td>
<td>38</td>
<td>02</td>
</tr>
<tr>
<td>5</td>
<td>1.96</td>
<td>50</td>
<td>42</td>
<td>08</td>
</tr>
<tr>
<td>6</td>
<td>2.00</td>
<td>50</td>
<td>51</td>
<td>01</td>
</tr>
<tr>
<td>7</td>
<td>2.08</td>
<td>60</td>
<td>61</td>
<td>01</td>
</tr>
<tr>
<td>8</td>
<td>2.14</td>
<td>80</td>
<td>72</td>
<td>08</td>
</tr>
<tr>
<td>9</td>
<td>2.19</td>
<td>90</td>
<td>80</td>
<td>10</td>
</tr>
</tbody>
</table>

Total number of animals used = 90, Total C2 = 0.1308

1. K = Total Number of concentration = 9
2. N = Degree of freedom = K - 2 = 7
3. Total number of animal used
   Total number of concentration used (K) = 10
4. (CHI)^2 =
   Total contribution to (CHI)^2 x Total no of animal used
   \[ \frac{k}{k} \]
   = \[1.308\]
5. (CHI)^2 value read from table (2) for degree of freedom n i.e. \[7 = 11.1\]

As 1.308 is less than 11.1 therefore the data are not significantly heterogenous and the curve is regarded to be good fit.
1. Slope function (S)

\[ S_1 = \text{Slope function for endosultan at 72 hours of exposure.} \]

\[ \frac{L_{C_{84}}}{L_{C_{50}}} = \frac{L_{C_{50}}}{L_{C_{16}}} = \frac{2.22}{1.99} = \frac{1.99}{1.77} = 1.1199 \]

2. Slope of LC 50 72 hour (fLC 50)

\[ fLC_{50} = S^{2.77/\sqrt{R}} = 1.1199^{2.77/\sqrt{90}} = 1.1199^{0.33} \]

\[ = 1.038 \]

Where \( N' \) = Total No of animals treated at those concentrations whose expected effects were between 16 and 84 percent.

3. Upper confidence limit of LC 50 at 72 hours for endosulfan.

\[ = Lc_{50} \times fLC_{50} = 1.99 \times 1.038 = 2.065 \]

Lower confidence limit of LC50 at 24 hours for endosulfan.

\[ = Lc_{50} \div fLC_{50} = 1.99 \div 1.038 = 1.917 \]

4. Factor of slope (FS)

\[ FS = A^{10} (K - 1) / K \sqrt{N'} = 1.16^{10(9-1)} / 9 \sqrt{70} = 1.16^{1.062} \]

\[ = 1.170 \]

Where \( R \) = Concentration range ratio

\[ = \frac{\text{largest}}{\text{smallest concentrations tested}} \]

By using values \( R \) and \( S \) read value designed as “A” from Nomograph number 3.

5. Upper limit of slope function

\[ = \text{slope function} \times \text{factor of slope} \]

\[ = 1.119 \times 1.170 \]

\[ = 1.310 \]

Lower confidence limit of slope function

\[ = \text{slope function} \div \text{factor of slope} \]

\[ = 1.119 \div 1.170 \]

\[ = 0.956 \]
Fig. No. : 4.39
Dose mortality relationship in endosulfan exposed *Labeo rohita* fish at 72 hours exposure period.
Table 4.7:
Litchfield and Wilcoxon (1948) toxicity evaluating method steps for endosulfan exposed Labeo rohita fish at 96 hours treatment.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Conc. used</th>
<th>Percent Mortality</th>
<th>Difference a-b</th>
<th>Contribution to $(\text{CHI})^2$</th>
<th>Nomograph No. 1</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Observed (a)</td>
<td>Expected (b) C1</td>
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<td></td>
</tr>
<tr>
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<td>0.0025</td>
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<td>28</td>
<td>02</td>
<td>0.0020</td>
</tr>
<tr>
<td>3</td>
<td>1.83</td>
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<td>34</td>
<td>06</td>
<td>0.0200</td>
</tr>
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<td>4</td>
<td>1.85</td>
<td>50</td>
<td>48</td>
<td>02</td>
<td>0.0020</td>
</tr>
<tr>
<td>5</td>
<td>1.87</td>
<td>50</td>
<td>54</td>
<td>06</td>
<td>0.0180</td>
</tr>
<tr>
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<td>1.91</td>
<td>60</td>
<td>64</td>
<td>04</td>
<td>0.0079</td>
</tr>
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<td>7</td>
<td>1.93</td>
<td>70</td>
<td>74</td>
<td>04</td>
<td>0.0075</td>
</tr>
<tr>
<td>8</td>
<td>1.96</td>
<td>80</td>
<td>84</td>
<td>04</td>
<td>0.0070</td>
</tr>
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<td>9</td>
<td>2.00</td>
<td>90</td>
<td>88</td>
<td>02</td>
<td>0.0020</td>
</tr>
</tbody>
</table>

Total number of animals used = 90, $\text{Total C}_2 = 0.0689$

1. $K = \text{Total Number of concentration} = 9$
2. $N = \text{Degree of freedom} = K - 2 = 7$
3. Total number of animal used
   
   \[
   \text{Total number of concentration used (K)} = 10
   \]
4. $(\text{CHI})^2 = \frac{\text{Total contribution to (CHI)$^2 \times \text{Total no of animal used}}}{{k}}$
   
   \[
   = 0.689
   \]
5. $(\text{CHI})^2 \text{value read from table (2) for degree of freedom n i.e. } 7 = 11.1$

As 0.689 is less than 11.1 therefore the data are not significantly heterogenous and the curve is regarded to be good fit.
1. Slope function (S)

\[ S_1 = \text{Slope function for endosultan at 96 hours of exposure.} \]
\[ LC_{96} = 1.71 \quad LC_{50} = 1.86 \quad LC_{84} = 2.05 \]

\[ \frac{LC_{84}}{LC_{50}} = \frac{LC_{50}}{LC_{16}} = \frac{2.05}{1.86} + \frac{1.86}{1.71} = 1.094 \]

2. Slope of LC 50 96 hour (fLC 50)

\[ fLC_{50} = S^{2.77/\sqrt{R}} = 1.094^{2.77/\sqrt{90}} = 1.094^{0.25} = 1.022 \]

Where \( N^* \) = Total No of animals treated at those concentrations whose expected effects were between 16 and 84 percent.

3. Upper confidence limit of LC 50 at 96 hours for endosulfan.

\[ = LC_{50} \times fLC_{50} = 1.86 \times 1.022 = 1.90 \]

Lower confidence limit of LC50 at 24 hours for endosultan.

\[ = LC_{50} \div fLC_{50} = 1.86 \div 1.022 = 1.819 \]

4. Factor of slope (FS)

\[ FS = A^{10,(K-1)} / \sqrt{N^*} = 1.70^{10(9-1)} / 9 \times \sqrt{90} = 1.70^{0.937} = 1.644 \]

Where \( R \) = Concentration range ratio

\( = \frac{\text{largest}}{\text{Smallest concentrations tested}} \)

By using values \( R \) and \( S \) read value designed as “A” from Nomograph number.

5. Upper limit of slope function

\[ = \text{slope function} \times \text{factor of slope} \]
\[ = 1.094 \times 1.644 \]
\[ = 1.798 \]

Lower confidence limit of slope function

\[ = \text{slope function} \div \text{factor of slope} \]
\[ = 1.094 \div 1.644 \]
\[ = 0.66 \]
Fig. No. 4.40
Dose mortality relationship in endosulfan exposed *Labeo rohita* fish at 96 hours exposure period.
Fig. - 4.25 T.S. of intestine of the control fish *Labeo rohita*.

Fig. - 4.26 T.S. of intestine of the fish *Labeo rohita* exposed to endosulfan for 48 hr.

Fig. - 4.27 T.S. of intestine of the fish *Labeo rohita* exposed to endosulfan for 72 hr.

Fig. - 4.28 T.S. of intestine of the fish *Labeo rohita* exposed to endosulfan for 96 hr.

---

Ac = Absorptive cells  
Sh = Shrinkage of intestinal epithelium  
Vc = Vacuolization of mucosal cell  
Sw = Swelling in mucosal cell  
Rv = Ruptured villi
The intestine of control fish is lined by a simple columnar epithelium consisting of absorptive cells with centrally placed nuclei and mucus secreting cells having basal nuclei. (Fig. 4.25) A few granular cells, basophilic or acidophilic in nature, are also present in the mucosa. The muscularis of the intestine is thin and consists of an outer layer of longitudinal and inner layer of circular muscle fibres.

During early hours of exposure to the endosulfan, the changes were not marked. But at 48 hr of exposure shrinkage in intestinal epithelium observed. After 96 hr of exposure, vacuolization of mucosal cells was observed. The absorptive cells got damaged and villi were ruptured. Swelling in mucosal cells and enlargement of their nuclei was prominent. The villi became very close to each other without any intervillar sapce. Mucus layer was observed covering the mucosal cells.

Histologically, the trunk kidney is made up of a large number of nephrons, each consisting of a renal corpuscle and the tubule. The intertubular space is full of lymphoidal tissue which is unevenly distributed (Fig. 4.30). The section of control kidney shows the transverse sections of renal corpuscle containing a well vascularised glomerulus, the proximal segment lined by tall columnar cells, the distal segment of tubule lined by relatively shorter clear cells and the collecting ducts lined by narrow intermediate cells with centrally placed nuclei. The haemopoietic tissue occupying intertubular space has parenchymatous cells which are round to polygonal in shape with distinct nuclei in the centre and shows numerous erythrocytes at different stages of developments.
Fig. - 4.29  T.S. of kidney of the control fish *Labeo rohita*.

Fig. - 4.30  T.S. of kidney of the fish *Labeo rohita* exposed to endosulfan for 48 hr.

Fig. - 4.31  T.S. of kidney of the fish *Labeo rohita* exposed to endosulfan for 72 hr.

Fig. - 4.32  T.S. of kidney of the fish *Labeo rohita* exposed to endosulfan for 96 hr.

\[ \begin{align*}
G &= \text{Glomerulus} \\
GD &= \text{Glomerulus damage} \\
VC &= \text{Proximal tubule cell vacuolization}
\end{align*} \]
After 24 hr of exposure to endosulfan degeneration of parenchymatous cells of haemopoietic tissue was observed. The blood cells flowing through glomeruli started showing signs of disintegration. After 96 hr of exposure, there was marked reduction in protoplasmic material of parenchymatous cells of haemopoietic tissue. All the tissues of kidney were severely affected. Cells of proximal tubule showed vacuolization. Glomerulus was more or less damaged and tissue organization was lost (Fig 4.31 & 4.32).

The liver of *Labeo rohita* consists of polygonal hepatic cells (Fig. 4.33). It also shows the sinusoids and sections of bile duct and blood capillaries. The pancreas extends into the liver forming hepatopancreas. The exocrine cells of pancreas are of large size and are columnar in shape with large nucleus. They are arranged around the blood capillaries. Each exocrine cell has a basal portion containing homogenous cytoplasm and an apical part containing a large number of zymogen granules.

Though it has no direct contact with the pesticide, it is indirectly affected through its contact with the blood. Histologically, the hepatic changes were characterised by disorganization of the lobular architecture accompanied by various grades of degenerative changes. The hepatocellular changes after 96 hr exposure to endosulfan include perilobular and centrilobular cirrhosis, cellular, necrosis, proliferation of bile duct epithelial cells leading to the formation of new bile canaliculi. Fibrinous thrombi with a large number of leucocytic infiltrations were noted within the central vein leading to obliteration of the vessels lumen.
Fig. - 4.33  T.S. of liver of the control fish *Labeo rohita*.

Fig. - 4.34  T.S. of liver of the fish *Labeo rohita* exposed to endosulfan for 48 hr.

Fig. - 4.35  T.S. of liver of the fish *Labeo rohita* exposed to endosulfan for 72 hr.

Fig. - 4.36  T.S. of liver of the fish *Labeo rohita* exposed to endosulfan for 96 hr.

=Cm = Clumping
=Cr = Cirrosis
=Do = Disorganization
=Hc = Hepatic cell pro-nuclei
=Pr = Prolifération of bile duct
=SE = Shrinkage of blood vessel
=VC = Vacuolization
After 24 hr of exposure, the nuclei of hepatic cells become prominent and large. Dark granular patches were observed in between the hepatocytes. Shrinkage of blood vessels clumped erythocytes and widely separated bile canaliculi were noticed. After 96 hr of exposure some hepatic cells showed vacuolization and others showed clumping (Fig. 2.5 & 2.6). The pancreatic cells around the blood capillaries showed enlargement after 24 hr but they appeared degenerated, and empty after 96 hr of exposure. Thus the findings of the present investigation clearly revealed the cytotoxic nature of endosulfan.

Behaviour is highly adoptive response to environmental variables of physical, chemical or biological nature. The behavioural response of the fish varied in accordance to the test concentrations. In behavioural changes the onset of symptoms were noted in 30 minutes after exposure to lethal concentration of endosulfan.

In early hours of treatment there was no jumping over the water surface. Mucus secretion was observed on the body surface. Initially they swim flat laterally on one side of the body. As the concentration increase further the fishes lose their balance and erratic movements were seen. Some fishes frequently dashed against the wall of aquarium. At higher concentration and at 72 to 96 hr of exposure period the fish showed jerky movements were seen for some time.

The fishes started jumping over the surface of water and swim flat laterally on one side of the body and died with in 24 hr.