MATERIAL AND GENERAL EXPERIMENTAL PROCEDURES
1. MATERIALS:

The experimental animal selected for the present investigation is *Labeo rohita*.

The Indian Major carp *Labeo rohita*, an economically important food fish having great commercial value. It is easily available in and around Anantapur. Because of easy availability and adaptability to varied laboratory conditions, this has been selected as the ideal animal for the present investigation of toxicity studies.

**SYSTEMATIC POSITION OF *L. rohita***:

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Chordata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-phylum</td>
<td>Vertebrata</td>
</tr>
<tr>
<td>Division</td>
<td>Gnathostomata</td>
</tr>
<tr>
<td>Super-class</td>
<td>Pisces</td>
</tr>
<tr>
<td>Class</td>
<td>Osteichthyes</td>
</tr>
<tr>
<td>Sub-class</td>
<td>Actinopterygii</td>
</tr>
<tr>
<td>Super-order</td>
<td>Teleostei</td>
</tr>
<tr>
<td>Order</td>
<td>Cypriniformes</td>
</tr>
<tr>
<td>Family</td>
<td>Cyprinidae</td>
</tr>
<tr>
<td>Genus</td>
<td><em>Labeo</em></td>
</tr>
<tr>
<td>Species</td>
<td>rohita</td>
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</tbody>
</table>

**BIOLOGY OF THE CARP**:

*Labeo rohita* is commonly called as rohu. It is the most famous Indian major carp, easily found all over north and central India. *Labeo rohita* is the fresh water fish, growing to a length of 91 cms. in three years (wealth of India, fish and fisheries 1962). *L. rohita* has an elongated body with moderately rounded abdomen. The colour of the body is brownish grey to back. It has a prominent head with a blunt snout at the tip of which is the transverse and semi-oval mouth with thick fringed lips. A soft and movable horny covering with a sharp margin on the inner side of the one or both the lips. Barbles are absent. Air bladder is usually large and divided into an anterior and posterior part. The body is covered with orange to red coloured large scales. It feeds on both vegetable and non-
vegetable matter. Within two years it becomes sexually mature. *Labeo* is the main source of food. It is rich in proteins and very suitable for human consumption. Hence it has a great commercial value and it is economically very important. It is found all over Northern & Central India. It is also now found in Godavari & Krishna rivers in the South and easily available in and around Anantapur. Because of its easy availability and adaptability to varied laboratory conditions *Labeo rohita* has been selected for the present investigation.

II. PESTICIDES SELECTED:

The present investigation deals with the comparative evaluation of three different commercial organochlorines, Organophosphorous and carbamate compounds namely Endosulfan 35% E.C., Malathion 50% EC and Sevin 50% E.C. The commercial grade Endosulfan, Malathion and Sevin were purchased from the local pesticide stores.

(A) COMMERCIAL GRADE ENDOSULFAN (CgE):

Commercial grade Endosulfan 35% E.C. of liquid formulation was obtained from local agro-chemical stores. The physical properties and other details of endosulfan are as follows:

**DEFINITION:**

6, 7, 8, 9, 10, 10 - Hexachloro - 1, 5, 5a, 6, 9, 9a - hexahydro - 6, 9 - methano - 2, 4, 3 - benzo (e) dioxathiapin - 3 - oxide.

**EMPIRICAL FORMULA :** C₉H₆Cl₆O₃S

**STRUCTURAL FORMULA :**

![Structural formula of Endosulfan](attachment:image.png)
MOLECULAR WEIGHT : 406.95

MELTING POINT : $\infty$ - Isomer = 109.2°C, $\beta$ - Isomer = 213.3°C

V.P. : $9 \times 10^{-3}$ mm Hg. at 80°C

DENSITY : 1.745

SOLUBILITY : Practically insoluble in water but isomeric mixture soluble in most organic solvents.

DESCRIPTION : It is commercially known as Thiodan. Technically, product ($\infty$ & $\beta$ stereo - isomers) is a brownish crystalline solid.

(B) COMMERCIAL GRADE MALATHION (CgM):

The commercial grade malathion (CgM) is a clear brown to colourless liquid with mercaptan Odour. It is obtained from the local agro-chemical shop. The physical properties and other details of Malathion are as follows:

DEFINITION : $S[1,2$ - di (ethoxy carbaryl) - ethyl] dimethyl phosphorothiolothionate

EMPIRICAL FORMULA : $C_{10}H_{19}O_6PS_2$

STRUCTURAL FORMULA:

\[
\begin{array}{c}
\text{CH}_3\text{O} \\
\text{P.S.C.H.CO}_2\text{C}_2\text{H}_5
\end{array}
\]

MOLECULAR WEIGHT : 330.4

V.P. : $4 \times 10^{-5}$ mm Hg. at 30°C

B.Point: 156 - 157°C at 0.7 mm Hg.

M.Point: 2.85°C

DENSITY : 1.23

SOLUBILITY : 145 ppm in water at 15°C, miscible with many organic solvents.
DESCRIPTION: Pure malathion is a colourless liquid. Technical malathion is a colourless to light amber liquid.

(C) COMMERCIAL GRADE SEVIN (CgS):

The commercial grade sevin is procured from a local Agro-chemical shop. It is a contact and stomach poison with slight systemic property. The physical properties and other details of sevin are as follows:

TECHNICAL NAME: Carbaryl
COMMERCIAL NAME: Sevin
DEFINITION: Carbaryl is the ISO name for 1 - Naphthyl methyl carbamate.

EMPIRICAL FORMULA: $C_{12}H_{11}NO_2$

STRUCTURAL FORMULA:

\[
\text{\begin{tikzpicture}
    \draw (0,0) node[above] {O.CO.NHCH$_3$} -- (1,0) -- (1,-1) -- (0,-2) -- cycle;
    \draw (0,-1) -- (1,-1);
    \draw (0,-2) -- (1,-2);
    \end{tikzpicture}}
\]

MOLECULAR WEIGHT: 201.2
M. POINT: 145°C
V.P.: Less than 0.005 mm Hg. at 26°C
DENSITY: 1.232

SOLUBILITY: In water, less than 0.1%; soluble in most polar Organic solvents eg: Dimethyl formamide, Dimethyl sulphoxide.

DESCRIPTION: White crystalline solid

STABILITY: Stable to light, heat and hydrolysis under normal storage conditions.

TOXICITY: Acute oral LD$_{50}$ for rats 850 mg/kg.
Acute dermal LD$_{50}$ for rats more than 4000 mg/kg.

APPLICATION: Used on a wide range of crops like cotton, fruit, crops and vegetable for the control of chewing and sucking pests.

Nandakumar (1983) reported the presence of residues of malathion and methyl parathion in the inland waters of Rayalaseema. The pesticides entry into the aquatic environment causes great havoc to the aquatic organisms like crabs and fishes (Darsie and Corriden 1956, Mulla 1961, Basha mohideen and Onurrappa, 1986).
Because of easy availability of these pesticides and their deleterious effects on fishes, endosulfan, malathion and sevin are selected as ideal pollutants to evaluate their comparative toxicity on the individuals of Indian major carp, *Labeo rohita*.

III. GENERAL EXPERIMENTAL PROCEDURES:

(A) FACTORS INFLUENCING PESTICIDE TOXICITY:

i) WATER QUALITY: Because water chemistry influence bioassessment to toxicity of the chemical, it is essential to maintain uniform water quality.

The composition of the water used for the maintenance of fish is given below.

<table>
<thead>
<tr>
<th>PHYSICO-CHEMICAL FACTORS OF THE WATER USED FOR EXPERIMENT:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity: 0.191 ml/ltr.</td>
</tr>
<tr>
<td>PH: 7.4 - 7.6</td>
</tr>
<tr>
<td>Chlorinity: 0.111 gm/litre</td>
</tr>
<tr>
<td>Sodium: 1.22 m.moles/litre</td>
</tr>
<tr>
<td>Potassium: 30.5 m.moles/litre</td>
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<tr>
<td>Calcium: 4.31 m.moles/litre</td>
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<tr>
<td>Carbondioxide: 6 - 7 ml/litre</td>
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<tr>
<td>Oxygen % saturation: 8</td>
</tr>
<tr>
<td>Dissolved oxygen: 160 ppm (as CaCO₃)</td>
</tr>
<tr>
<td>Hardness of water: 87 ppm (as CaCO₃)</td>
</tr>
</tbody>
</table>

ii) TEMPERATURE: Temperature is known to influence the toxicity of a large number of pesticides (Macek et al, 1969). Therefore, throughout the present investigation, temperature of the water is maintained at 29 ± 1°C.

iii) DENSITY OF WATER: The toxicity of pesticide is influenced by the density of the fish (Muirhead Thomson, 1971, Holden, 1970). So, a constant ratio of fish biomass to water volume was maintained by taking approximately 1 gram of fish per litre of water.

iv) FLOW OF WATER: Since significant difference in the toxicity of pesticides between static and flowing water in fishes was reported by Burke and Fergusson (1969), the
experiments of the present investigation are conducted in static media as suggested by Doudroff et al (1951).

v) HOLDING IN CAPTIVITY: Fishes were handled very gently and carefully. Greater care was taken in maintenance, as detoxification and metabolism of Xenobiotics in fishes is influenced by holding in capacity (Adamson 1967).

(B) MAINTENANCE OF EXPERIMENTAL ANIMALS:

The Indian major carp *Labeo rohita* having the size of 5 + 2 gms. were collected from local fish farm in Anantapur. The fish were transferred to large glass aquaria in the laboratory. The water in the aquaria having fishes was aerated twice a day for a duration of one hour each time so as to provide fresh water rich in oxygen. The water was changed every alternate day to remove the excreta accumulated. The fish were fed daily with groundnut cake and twice a week with frog muscle and acclimated to the laboratory conditions for a minimum period of ten days. The temperature in the aquaria was 29 ± 1°C and the same is maintained throughout the course of this investigation and the fish were exposed to the natural photo period.