MATERIAL & GENERAL EXPERIMENTAL PROCEDURES
"Labeo Rohita" is abundantly available in the fresh water tanks in and around the Anantapur. It have a great commercial value and a good source of food. It is shown to be a suitable test animal for toxicity studies. It known to have adaptability to laboratory conditions. Hence this fish Labeo Rohita is selected for the present investigation as the experimental material.

Biology of Labeo Rohita:

The Indian major carp Labeo Rohita belongs to the family cyprinidae, found all over Northern and Central India up to river Godavari. This is the most famous major carp. This carp is commonly called as rohu, having elongated body and abdomen was moderately rounded. Head was prominent with pointed snout, terminal mouth with thicker frienged lips. The colour of the body was brownish grey to blackish. The scales are with orange to reddish in centre. This species is fast growing and feeding from non-vegetable to vegetable matter.

In India, this carp is found to be the most valuable food, growing very quickly up to 45 cm. It can maximally grow upto a length of 91 cms in three years. (The wealth of India, fish and fisheries, 1962). Within two years it can sexually matured. Recently in many countries this carp culture has become very important. For human consumption this carp is very rich in protein. Hence, this carp is economically very important and has a great commercial value. Because of,
easy and abundant availability and adaptability to varied environmental conditions, *Labeo rohita* is selected as the ideal experimental animal in the present investigation.

**Pesticide selected:**

In 1955 R.Beriger first synthesized the phosphomidon pesticide which is an organophosphate in nature. Great deal of work has been done in the Research laboratories of CIBA Limited in Basle, regarding phosphomidon in general. It has low phytotoxicity, and a systemic insecticidal activity. It is easily soluble in water. The name Dimecron is the familiar name of phosphomidon. Since it possesses all these properties phosphomidon was recognised suitable for the use of plant protection agent.

**Physico-Chemical Properties:**

Pure phosphamidon is a mixture of L and R - isomers. It is in the group of enol phosphates and known as vinyl phosphate. This organophosphate pesticide phosphamidon is a systemic insecticide.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Phosphamidon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familiar name</td>
<td>Dimecron</td>
</tr>
<tr>
<td>Chemical name</td>
<td>O(2-chloro - 2-(diethyl carbonyl)-1-methyl-vinyl)-O, O-dimethyl phosphate</td>
</tr>
<tr>
<td>Empirical formula</td>
<td>C₁₀H₁₉O₅ NCIP</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>299.7</td>
</tr>
</tbody>
</table>
Solubility:
- Mixible with water and all organic solvents except saturated hydrocarbons, in which it is soluble only to a limited extent.

Boiling point:
- 0.04 mm of Hg, 94°C

Specific gravity:
- d₂₅ = 1.2132

Colour:
- Pine

Odour:
- Faint, pleasant

Structural formula:

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The Vol. 48 of the Review of Applied Entomology (75) first published the common name of phosphamidon for the insecticide. This review is recognised by the British Standards Institution.

Chemical degradation of Phosphamidon:

Phosphamidon in the first stage in an alkaline medium gives hydrochloric acid, considerably more slowly, acetic acid, the final product being glycolic and diethylamide. Due to these successive stages of hydrolysis phosphamidon should be protected from moisture during storage.

The phosphamidon breaks down in biological substrates in three ways.

1. Phosphamidon is hydrolysed to give dimethyl phosphoric acid and N, N-diethylchloroacetoacetamide.
2. The phosphate group is demethylated to give desmethyl phosphamidon.

3. By the removal of one ethyl group from the acid amide by a reaction whose mechanism has not yet been elucidated, desethylphosphamidon results.

Water quality:

Since it is known that water chemistry influence bioassessment to toxicity of chemical, it is essential to maintain uniform water quality. The composition of the water used for the maintenance of fish is given below.

### Physico-chemical factors of the water used for experiment:

<table>
<thead>
<tr>
<th>Factor</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salinity</td>
<td>0.191 ml/litre</td>
</tr>
<tr>
<td>pH</td>
<td>7.4 to 7.6</td>
</tr>
<tr>
<td>Chlorinity</td>
<td>0.111 mg/litre</td>
</tr>
<tr>
<td>Sodium</td>
<td>1.22 m.moles/litre</td>
</tr>
<tr>
<td>Potassium</td>
<td>30.5 m.moles/litre</td>
</tr>
<tr>
<td>Calcium</td>
<td>4.31 m.moles/litre</td>
</tr>
<tr>
<td>Carbon dioxide</td>
<td>2.09 mg/litre</td>
</tr>
<tr>
<td>Oxygen percent Saturation</td>
<td>8</td>
</tr>
<tr>
<td>Dissolved Oxygen</td>
<td>6 to 7 ml/litre</td>
</tr>
</tbody>
</table>

General experimental procedures:

Variations in the toxicity are approximately nullified to a satisfactory level by the following factors.
Since significant difference in the toxicity of pesticides between static and flowing water in fishes was reported by Burke and Ferguson (1969), the experiments of the present investigation were conducted in static water conditions as suggested by Dondoroff et al., 1951.

**Temperature:**

The toxicity of number of pesticides was reported in increase with rise in temperature of the medium (Wacek et al., 1969; Parvathi, 1982; Shahnawaz and Bashamohiddeen, 1985; Shahnawaz 1985). Therefore throughout the present investigation the temperature of the water is maintained at 28°C, 35°C and 15°C.

**Hardness of water:**

Several investigations have demonstrated that toxicity is often dependent on hardness of water and rapid breakdown of toxic products occur at higher pH values in hard or soft water (Pickering and Henderson, 1966; Henderson et al., 1960 and Alagaster 1969). Hence throughout the present study the water used had a hardness of 150 ± 10 ppm (pH 7.6 ± 0.4).

**Density of fish:**

Since an increase in fish density was known to increase the toxicity (LC₅₀) of the pesticide (Muirhead Thomson, 1971; Holden, 1970) a constant ratio of fish biomass to water volume was maintained by taking approximately 1 of fish per 1 litre of water.
Time of exposure:

The period for which the median tolerance limit (LC₅₀) values is determined was fixed for 40-hrs, because of its relatively shorter period than 72 or 96-hrs where variations in O₂ and phosphamidon concentration may be more (Pickering et al., 1962; Kabeer Ahmed, 1979).

Holding captivity:

The detoxification and metabolism of xenobiotics in fishes, is influenced by holding in captivity (Adamson, 1967; Dawaide, 1971). Therefore great care was taken in maintenance and the fishes were handled very gently.

Maintenance of the fish:

The fish Labeo rohita weighing 20 + 2 of were collected from the Government. Fisheries Department in Anantapur and brought to the laboratory within two hrs. This fish were stored in the large aquaria and the aquaria with the water having fishes was aerated twice for a day. The fish were fed daily with ground nut cake and with frogs muscle twice for a week. Fishes were adapted for a minimum period of fifteen days to the laboratory conditions. The temperature in aquaria was 27°C ± 1°C and the same is maintained as normal temperature throughout the course of this investigation and the fish were exposed to the natural photoperiod. In order to study the effect of temperature on phosphamidon toxicity in this fish (Labeo rohita) at normal temperature (28°C) were adapted to lower temperature (15°C) and to a
higher temperature (35°C) separately for a period of ten
days. Because the phosphamidon exposure is fixed for 30 days
in further studies of this investigation the fish after
attaining the stabilised level in \( \text{O}_2 \) consumption was allowed
to continue in the same adaptation medium lower and higher
over to 30-days extension period. As the present investigation
deals with the effect of temperature on phosphamidon toxicity,
three different set of fish namely lower (15°C) temperature
adapted, normal (20°C) exposed to higher (35°C) temperature
adapted fishes were used in the further studies of this
investigation.