CHAPTER 1

ACUTE TOXICITY
INTRODUCTION

Agriculture plays a prominent role in Indian economy. Pesticides act as a vital component in modern agricultural technology in raising foodgrains output and have made enormous contribution towards green revolution in India. But the indiscriminate use of pesticides in agriculture, public health and improper storage without precautionary measures result in the pollution of environment affecting many non-target species (Muirhead-Thomson, 1971; Matsumara et al., 1972). Pollution is a undesirable change in biological or physico-chemical characteristics of environment that may harmfully affect human life and resources. Consequently today we witness trans-migration of pesticides from site of application to large water bodies and into the systems of animals. Thus the increasing dangers of water pollution by pesticides necessitate establishment of water quality criteria and determination of safety limits for fish (Tarzwell, 1966; Mount and Stephen, 1967).

Besides pesticidal pollution, effluents of chemical industries also posed a serious problem. Effluents containing many toxic chemicals and heavy metals like,
Hg, Pb, Zn, Cd, etc. are drained into water bodies of rivers and sea without pretreatment. Recently it is considered that pesticides and heavy metals head the list of environmental pollutants.

Today about 4500 pesticides are in common use all over the world from which 25% of these have a high toxicity potential to a wide range of flora and fauna of economic importance. These are supposed to be organochlorine compounds. Majority of pesticides and detergents are not readily degradable. They remain in water for considerable period thereby affecting aquatic biota in which fishes are prominent and economically important as proteinous food.

The pesticides are concentrated by bio-accumulation process in aquatic organisms. The pesticides of proven economic potentialities in residual form contaminate aquatic ecosystem and having affinity for living system create series of problems to the aquatic biota especially to the fishes which play a role in bio-magnification. Contamination of aquatic ecosystem initiates a chain of interactions in the organisms. The higher invertebrates and vertebrates feeding upon micro and macroaquatic forms are ultimately vulnerable to the threat of bio-accumulation of pesticides.
Toxicity studies have been carried out on animals like tubificid worms, prawns, shrimps, crustaceans, molluscs, fishes, amphibians, birds and rats (Eisler, 1969; Saunders, 1970; Couch, 1978; Raizada et al., 1979; Rao, 1981; Seuge and Balzat, 1981; Vardia and Durve, 1981; Deshmukh, 1982; Mane et al., 1983; Pawar and Katdare, 1983; Ansari et al., 1984; Dikshith et al., 1984; Bakre, 1985).

Freshwater fishes are the most important not only for their amenity and nutritive value but also due to their high mobility and sensitivity towards the changes occurring in their surroundings. Fishes can potentially avoid pollution-incidents. Hence, fish serve as indices to detect degree of pollution in water bodies concerned. Pesticides used in agriculture persist and accumulate in ecosystem with a consequent biodegradation in the living organisms thereby creating acute as well as chronic hazards.

Considerable literature is available on the mechanism of action of pesticides and other chemicals and their selective toxicities (Henderson et al., 1959;
Lemke and Mount, 1963; Rudd, 1964; Scheier and Cairns, 1966; Ketz, 1969; Muirhead and Thomsen, 1971; Mahajan and Singh, 1973a, b; Russo et al., 1974; Grant, 1976; Maciorowski et al., 1977; Verma et al., 1981, 1982; Subbiah et al., 1985). Similarly much work has been done on acute and chronic toxicity of pesticides and changes in behaviour of fishes due to pesticides (Burdick, 1960; Alderdice, 1967; Anderson, 1971; Sprague, 1973; Basak and Konar, 1976b; Verma et al., 1980; Joshi et al., 1981). Behaviour is usually a very complex phenomenon through which the animal is capable of adjusting its various functions to a constant or changing environment.

Acute toxicity biotesting has been carried out in many species in a variety of ecological systems to detect the sensitive species and for preliminary screening of chemicals for monitoring effluents to determine the extent of risk to aquatic organisms and to determine the extent of component causing death so that it can receive special treatment. Recently the importance of acute toxicity study and its significance have been explained (Gaur et al., 1981). An important consideration
in studies on acute poisoning to fish is the influence of environmental variations on toxicity. Physico-chemical nature of water also influence the toxicity of pesticides.

In India, Mathur (1962) for the first time recorded the toxicological action of pesticide DDT to fishes, *Ophiocephalus punctatus*, *Heteropneustes fossilis* and *Trichogaster fasciatus*. Whereas the polluting effect of DDT in early phase of its wide use was not much understood but the demonstrations about its effects on environment reveals its hazards (Burdick et al., 1964; Reinhert, 1969, and Peakall, 1970).

Studies on toxicity of pesticides and other chemicals demonstrate that the toxicity increases with exposure time (Joshi and Rege, 1980). Similarly fishes exposed to toxic pesticides show behavioural changes (Symons, 1973; Bakthavathsalam and Reddy, 1981; Verma et al., 1975). However, no behavioural changes occur due to sublethal doses of parathion, DDT, BHC and thiordan in *Cyprinus carpio* and *Tilapia mossambica* (Basak and Konar, 1976a, b, 1977). It seems that some fishes are highly sensitive to pesticides showing behavioural changes while others are least affected.
Acute toxicity biotesting of malathion were studied in *Channa punctatus* and *Tilapia mossambica* (Pandey et al., 1976 and Sailatha et al., 1981). Similarly studies were carried out on toxicity of thiodan 35EC, endosulfan and DDT in *Gambusia affinis* and Endosulfan in *Cirrhina mrigala* (Joshi and Rege, 1980; Joshi et al., 1981 and Swarup et al., 1981). Gouda et al. (1981) studied toxicity of dimecron, sevin and lindex to *Anabas scandens* and *Heteropneustes fossilis*. Similarly studies on toxicological effects of DDT were carried out in *Cyprinus carpio*, *H. fossilis* and *Tilapia mossambica* (Basak and Konar, 1976b). Acute toxicity of sumithion, BHC and furadan to *Garra mulya* have also been done (Pawar, 1983). Toxicity of BHC, lindane, endosulfan and malathion to *Rasbora daniconius* and *Puntius ticto* have been tested (Singh and Sahai, 1984). Toxicity of heptachlor and metasystox to *T. mossambica* have been reported by Girija et al. (1985).

Acute toxicity testing of different pesticides to different fish species in relation to size have been carried out by many workers. Exposure of fish, *Lepidocephalus thermalis* to LC$_{50}$ concentrations of
dimecron, malathion, metaparathion and aldrin for 48h showed that smaller fishes were more sensitive (Kumari and Nair, 1978). Carbaryl toxicity to *Labeo rohita* of different sizes was done by Tilak *et al.* (1980). Results of toxicity testing of fenitrothion to fish, *Mystus cavasius* and *Labeo rohita* indicated that toxicity decreased as the size increased (Murty *et al.*, 1983). Similarly toxicity of technical grade phosphomidon in two different size groups of freshwater fish, *Sarotherodon mossambicus* have been studied by Rao *et al.* (1984). Results showed that small-sized fishes were more susceptible than large size. Thus, the studies clearly indicate that toxicity of pesticides is size dependent.

Evaluation of toxicity of commercial and technical grade sumicidin, monitor and orthene to fish *Mystus vittatus* showed that commercial grade sumicidin was more toxic, while technical grade was least and others moderately toxic (Verma *et al.*, 1981a). Similar results were obtained by Sailatha *et al.* (1981) while assessing toxicity of malathion (commercial and technical) to fish, *Tilapia*
mossambica. Thus from the available literature on studies of toxicity of pesticides of technical and commercial grade indicate that some technical grade products are more toxic than commercial grade.

Further, the results of toxicity studies of organochlorine, organophosphate and carbamate pesticides in different fishes indicated that toxicity decreased in the order like organochlorine, organophosphate and carbamate, (Verma et al., 1982) studies on toxicity of carbaryl, its 3 formulations and its degradation product 1-naphthol to Channa punctatus showed that metabolite was more toxic than the parent compound (Tilak, 1982). Similar results were obtained in Cyprinus carpio communis exposed to carbaryl and 1-napthol (Panwar et al., 1984).

It is known that the various environmental variables like salinity, pH, water hardness, temperature and chlorine content of water influence the toxicity of pesticides and other toxic chemicals. The toxicity of these substances decreases with increase in water pH (Sprague, 1964; Holcombe, et al., 1980; Kobayashi, 1980; Marking and Bills, 1981; Russo et al., 1981 and Bakre, 1985). Similarly increase in salinity also
causes decrease in toxicity of toxic chemicals (Crandall and Goodnight, 1959; Joshi, 1974).
Increase of water hardness decreases the toxicity of toxic substances (Skidmore and Tovell, 1972; Metz and Brananc in 1975; Calamari et al., 1980; Mason, 1981; Bakre, 1985; Lomte and Alam, 1985).

There is a very little information available on the toxicity of pesticides to the fish, Barilius bendelisis. There is no report on the acute and chronic toxicity of organochlorine, organophosphate and carbamate compound. Similarly there is no report on the effect of various environmental variables on toxicity of pesticides at acute stress. Therefore, in the present investigations, an attempt has been made to study the toxicity of thiodan (35 EC), nuvan and dithane M-45 and effect of salinity, pH and water hardness on pesticide toxicity.

The pesticides used in the present investigation have been elucidated as given below:
Common name

Chemical name (Active ingredient)

Effective concentration

Structural formula

Molecular weight

Melting point

Solubility

Stability

Thiodan

<\beta-1,2,3,4,7,7-hexachlorobicyclo(2,2,1)-heptane-2-bis-(oxymethylene)-5,6-sulphite

35 EC

\[
\begin{array}{c}
\text{H- graph} \\
\end{array}
\]

406.95

70 - 100°C

Insoluble in water, soluble in organic solvents.

Stable towards dilute mineral acids, hydrolyzed rapidly by alkalies
Compatibility

It is compatible with non-alkaline pesticides except bordeaux mixture, calcium arsenate, hydrated lime and other alkaline pesticides. Non-phytotoxic.

Antidote

If swallowed, a gastric lavage with warm water may be given. It is followed by administration of a mixture containing 2 parts activated charcoal, 1 part magnesium oxide and 1 part tannic acid in a half a pint of warm water. To induce sedation and control convulsions, phenobarbital (0.7 g/day) may be given.

Formulations

17.5% and 35% E.C. - liquid
17.5% 35%, 50% wettable powder,
5% granules, 10%, 3%, 4%,
5% dusts.
**Uses**

It is a broad spectrum acaricide, non-systemic contact and stomach poison. Highly effective against sucking and chewing insect pests like aphids, jassids, thrips, white flies, shoot flies, gall flies, beetles, weevil's, bugs, caterpillars, borers, grubs, termites etc.

<table>
<thead>
<tr>
<th>(2) Common name</th>
<th>Chemical name</th>
<th>Structural formula</th>
<th>Boiling point</th>
<th>Purity</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nuvan</td>
<td>$0,0$-dimethyl $0-2,2$ dichlorovinyl phosphate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$(\text{CH}_3\text{O}) \underset{0}{\overset{O}{\text{P}}}-\text{CH} \equiv \text{CCl}_2$</td>
<td>74°C</td>
<td>76%</td>
<td>It is amber coloured liquid soluble in water and also miscible in organic solvents</td>
</tr>
</tbody>
</table>
Stability
In presence of trace moisture, on standing it breaks down with the formation of acidic products which further catalyse the decomposition. 4% epichlorohydrin is added to tie up the acidic substances and improve the conditions for storage.

Uses
A short lived, wide spectrum, contact and stomach poison with fumigant and penetrant action and non-phytotoxic. It is used as household and public health fumigant especially against mosquitoes and other diptera in addition to crop protection uses.

Formulations
50 to 100% EC
0.4 to 1% aerosols; 0.5% granules.

Antidote
Atropine supported by 2 PAM
(2 pyridine-aldoxime-methyliodide)
(3) Common name  Dithane M-45
       Chemical name  Manganese ethylene bisdithiocarbamate

Structural formula

\[
\begin{array}{c}
\text{Mn} \\
\text{CH}_2\text{NHCS} \\
\text{Zn} \\
\text{CH}_2\text{NHCS}
\end{array}
\]

Molecular weight  265.3

Composition  (10% Zineb + 70% Maneb)

75% of co-ordination product of zinc ion and manganese ethylene bisdithiocarbamate.

Solubility  Yellow crystalline substance. Slightly soluble in water and insoluble in organic solvents.

Compatibility  It is compatible with most insecticides and fungicides except lime, lime sulphur and bordeaux mixture.
Uses:

It is widely used in grape gardens as fungicide for controlling most plant diseases like leaf blights, fruit, rots, leaf spots, downy mildew, anthracnose and tikka disease of groundnut. It is also used for seed treatment.

Antidote

If taken internally, induce vomiting by inserting finger into throat. Drink copious quantity of water and obtain medical attention for gastric lavage.
MATERIAL AND METHODS

The fish *Barilius bendelisis* were collected from river Girna near Lohaner (Nashik district) and brought to the laboratory. The fishes of similar weight (5-7 g) and size (7-8 cm long) were selected and acclimatized to laboratory conditions in aquaria for a period of 30 days before being used for experiment. During the period of acclimatization the fishes were fed on algae and pieces of live earthworms. The water was replaced daily after feeding the fishes. Clear, aged and dechlorinated tap water was used to maintain the fish as well as for the toxicity tests.

Three pesticides of technical grade provided by Hoechst Industries (thiodan), Ciba-Geigy (Nuvan) and Indofil chemicals Ltd. Bombay (Dithane M-45) were used for toxicity biotesting. The stock solutions (100 mg/ml) of these pesticides were prepared by dissolving in acetone and further dilutions were prepared using tap water.
Pilot experiments were conducted to choose test range concentrations for 24h for each pesticide which have a resultant mortality range from 0 - 100% and then 6 to 8 concentrations were used in the final experiment. The test concentrations were 0.009 to 0.042, 0.02 to 1.4 and 0.25 to 0.85 ppm for thiodan, nuvan and dithane M-45, respectively.

The effect of environmental variables like pH, salinity and water hardness on toxicity of these 3 pesticides were also carried out. For testing the pH tolerance, pilot experiments were performed to determine limits of pH tolerance. The fish was unable to tolerate pH below 6 and above 10, and hence the pH ranges selected were 6.5 and 9. The lower or high pH of test water was adjusted by addition of acetic acid/H₂SO₄ or sodium hydroxide as the case may be. The pH of water was maintained twice a day (morning and evening).

The water hardness (100 and 200 ppm) was adjusted by using naturally occurring hard water from wells and hardness was tested following the EDTA method using Erichrome black T as an indicator.
Similarly the effects of low (0.5%) and high (1%) salinity on acute toxicity of pesticides were carried out. Sodium chloride was used for adjusting the desired salinity of test water.

The physico-chemical parameters of the tap water used in the experiments were analysed as per standard methods (APHA, 1975).

Static bioassays were carried out as per standard method of APHA (1975). Ten fishes approximately of similar size (7-8 cm long) and weight (5-7 g) were kept in aquaria containing solution of the pesticide along with controls. The volume of the solutions were kept 20 l in all the cases. Before exposing the fishes to pesticide solutions, they were starved for 24h. During bio-testing feeding was discontinued. The treatment was carried out for a period of 96h and after every 24h the solutions of pesticides were replaced by freshly prepared solution of corresponding concentrations. Observations were made on mortality, and survival of the fishes after 24, 48, 72 and 96h and physical behaviour of the fish was also recorded. The fishes that did not respond to tactile stimulus were considered dead and removed immediately.
Mortality was recorded to calculate 24, 48, 72 and 96 h LC$_{50}$ values (lethal concentration showing 50 % death of test animal) for different pesticides by probit analysis of log dose against probits mortality using the methods of Robert and Boyce (1972). The regression equations were calculated by approximate weighted regression method and the values of LC$_{50}$ were calculated from the equations. Dose mortality curves (Probit regression lines) were also drawn (Figs. A-4, A-5, and A-6). The results are presented in tables A-1 to A-3. The LC$_{50}$ values have been expressed in terms of concentration in ppm.

RESULTS

Tables (A1, A-2, A-3) show the comparative LC$_{50}$ values, regression equations and fiducial limits of the pesticides thiodan, nuvan and dithane M-45 for different time periods i.e. 24, 48, 72 and 96h both in normal laboratory conditions and with various water variables (like pH, water hardness and salinity) to which B. bendelisis were exposed. Fig. A-4, A-5 and A-6 represent dose mortality curves for the toxicities of three pesticides after 96 h of exposure.
**Fish behaviour**

No change in the behaviour were observed in the fishes exposed to 0.009 ppm thiodan. There was no mortality (LC₀) observed at this concentration. While with higher doses the fish show high excitation with irregular and increased opercular movements; fishes leap out of water and loss equilibrium before death. The fish secretes excessive mucous particularly around gills. Mortality increases as the dose increases. The swimming and opercular movements were similar to that of the control fishes at low dose (0.2 ppm LC₀) of nuvan. At high dose the fish showed more excitation, increased swimming rate and opercular movements during initial two hours which decreased later an. Hyperexcitation with increasing opercular movements, surfacing activity, shivering movements, loss of balance before death was observed. A thick mucous covering was observed around gills. Mortality increased with higher dose. The fish showed no excitation at lowest concentration (0.25 ppm LC₀) of dithane M-45 and physical behaviour was similar to that of the control. While exposure to higher concentrations of pesticide, the fish showed decreased opercular movements and setting down at the bottom of aquarium. Initially no remarkable changes in swimming movements was observed but rapid opercular movements, jerky movements at water surface and loss of balance was obvious before death. The gills had thick mucoid coating whereas the body was enveloped by a thin mucoid covering.
Table A-1

Regression equation, 95% fiducial limits with observed and calculated LC\textsubscript{50} values for *Barilius bendelisis* exposed to thiodan at various water variables.

<table>
<thead>
<tr>
<th>Water Variable</th>
<th>Exposure period (hours)</th>
<th>Regression equation</th>
<th>Fiducial limits</th>
<th>Calculated LC\textsubscript{50} (ppm)</th>
<th>Observed LC\textsubscript{50} (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory conditions</td>
<td>24</td>
<td>$Y = -24.0604 + 11.4304 \times X$</td>
<td>0.04890 0.02996</td>
<td>0.03485</td>
<td>0.03400</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>$Y = 4.1257 + 0.3653 \times X$</td>
<td>0.04235 0.02576</td>
<td>0.02631</td>
<td>0.02550</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>$Y = -9.5109 + 6.3298 \times X$</td>
<td>0.02665 0.01985</td>
<td>0.02087</td>
<td>0.02095</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>$Y = -10.0217 + 4.7060 \times X$</td>
<td>0.02651 0.01485</td>
<td>0.01560</td>
<td>0.01514</td>
</tr>
<tr>
<td>pH 6.5</td>
<td>24</td>
<td>$Y = -40.4034 + 18.3171 \times X$</td>
<td>0.04020 0.02990</td>
<td>0.03010</td>
<td>0.03000</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>$Y = -10.7049 + 6.5748 \times X$</td>
<td>0.05101 0.02350</td>
<td>0.02452</td>
<td>0.02540</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>$Y = -7.0971 + 5.3664 \times X$</td>
<td>0.02234 0.01650</td>
<td>0.01796</td>
<td>0.01800</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>$Y = -7.6700 + 4.0512 \times X$</td>
<td>0.02467 0.01315</td>
<td>0.01349</td>
<td>0.01413</td>
</tr>
<tr>
<td>pH 9</td>
<td>24</td>
<td>$Y = -35.0098 + 15.6590 \times X$</td>
<td>0.04220 0.03486</td>
<td>0.03589</td>
<td>0.03600</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>$Y = 208.3198 - 83.1854 \times X$</td>
<td>0.03870 0.02674</td>
<td>0.02770</td>
<td>0.02700</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>$Y = -7.8387 + 5.5317 \times X$</td>
<td>0.02446 0.01996</td>
<td>0.02094</td>
<td>0.02100</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>$Y = -10.4607 + 3.5040 \times X$</td>
<td>0.02278 0.01585</td>
<td>0.01616</td>
<td>0.01622</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.04260</td>
<td>0.03085</td>
<td>0.03390</td>
</tr>
<tr>
<td>Water hardness 100 ppm</td>
<td>24</td>
<td>$Y = -26.0327 + 12.2640 \times X$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>$Y = 7.404 - 0.9062 \times X$</td>
<td>0.03240</td>
<td>0.02625</td>
<td>0.02652</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>$Y = -10.3445 + 6.6696 \times X$</td>
<td>0.02570</td>
<td>0.01890</td>
<td>0.01972</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>$Y = -6.6633 + 5.1424 \times X$</td>
<td>0.02175</td>
<td>0.01410</td>
<td>0.01417</td>
</tr>
<tr>
<td>Water hardness 200 ppm</td>
<td>24</td>
<td>$Y = -27.7201 - 9.1859 \times X$</td>
<td>0.04227</td>
<td>0.03350</td>
<td>0.03420</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>$Y = -12.9125 + 7.3443 \times X$</td>
<td>0.03172</td>
<td>0.02650</td>
<td>0.02748</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>$Y = -0.7948 + 2.5058 \times X$</td>
<td>0.02642</td>
<td>0.01974</td>
<td>0.02054</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>$Y = -7.6997 + 5.7153 \times X$</td>
<td>0.02174</td>
<td>0.01595</td>
<td>0.01667</td>
</tr>
<tr>
<td>Salinity 0.5%</td>
<td>24</td>
<td>$Y = -17.1161 + 8.7265 \times X$</td>
<td>0.04275</td>
<td>0.03389</td>
<td>0.03422</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>$Y = -11.4426 + 6.8637 \times X$</td>
<td>0.03197</td>
<td>0.02412</td>
<td>0.02486</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>$Y = -8.6136 + 5.9617 \times X$</td>
<td>0.01971</td>
<td>0.01885</td>
<td>0.01922</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>$Y = -10.5069 + 6.9560 \times X$</td>
<td>0.01777</td>
<td>0.01618</td>
<td>0.01695</td>
</tr>
<tr>
<td>Salinity 1%</td>
<td>24</td>
<td>$Y = -22.2038 + 10.7201 \times X$</td>
<td>0.05037</td>
<td>0.03324</td>
<td>0.03451</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>$Y = -10.477 + 6.4681 \times X$</td>
<td>0.03214</td>
<td>0.02378</td>
<td>0.02471</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>$Y = 1.5682 + 1.5185 \times X$</td>
<td>0.02417</td>
<td>0.01750</td>
<td>0.01320</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>$Y = -3.6236 + 3.9785 \times X$</td>
<td>0.02174</td>
<td>0.01425</td>
<td>0.01471</td>
</tr>
<tr>
<td>Water variable</td>
<td>Exposure period (hours)</td>
<td>Regression equation</td>
<td>Fiducial limits</td>
<td>Calculated LC&lt;sub&gt;50&lt;/sub&gt; (ppm)</td>
<td>Observed LC&lt;sub&gt;50&lt;/sub&gt; (ppm)</td>
</tr>
<tr>
<td>----------------</td>
<td>------------------------</td>
<td>---------------------</td>
<td>----------------</td>
<td>------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Laboratory conditions</td>
<td>24</td>
<td>Y = -166.2551 + 85.4714 X</td>
<td>1.1288</td>
<td>1.04546</td>
<td>1.0890</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>Y = -5.4902 + 5.3967 X</td>
<td>0.8926</td>
<td>0.8724</td>
<td>0.8786</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>Y = -6.2102 + 6.0902 X</td>
<td>0.7867</td>
<td>0.6898</td>
<td>0.6929</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>Y = -1.17513 + 3.9940 X</td>
<td>0.5543</td>
<td>0.4874</td>
<td>0.4903</td>
</tr>
<tr>
<td>pH 6.5</td>
<td>24</td>
<td>Y = -7.0082 + 6.0295 X</td>
<td>1.0220</td>
<td>-0.9775</td>
<td>0.9806</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>Y = -6.9691 + 6.2358 X</td>
<td>0.8742</td>
<td>0.8290</td>
<td>0.8307</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>Y = -9.9385 + 8.2721 X</td>
<td>0.7285</td>
<td>0.6324</td>
<td>0.6397</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>Y = -5.6869 + 6.3078 X</td>
<td>0.5274</td>
<td>0.4710</td>
<td>0.4768</td>
</tr>
<tr>
<td>pH 9</td>
<td>24</td>
<td>Y = -22.6629 + 13.4444 X</td>
<td>1.2015</td>
<td>1.1085</td>
<td>1.1410</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>Y = -11.1772 + 8.2695 X</td>
<td>0.9425</td>
<td>0.8925</td>
<td>0.9040</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>Y = -6.2429 + 6.0413 X</td>
<td>0.8024</td>
<td>0.7125</td>
<td>0.7262</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>Y = -7.1772 + 7.0371 X</td>
<td>0.5864</td>
<td>0.5275</td>
<td>0.5389</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>--------</td>
<td>----</td>
<td>----------------------------------------</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Hardness 100 ppm</td>
<td>48</td>
<td>Y = -7.5163 + 6.5158 X</td>
<td>0.9015</td>
<td>0.8174</td>
<td>0.8354</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>Y = -7.1635 + 6.6886 X</td>
<td>0.7282</td>
<td>0.6475</td>
<td>0.6586</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>Y = -2.5914 + 4.5309 X</td>
<td>0.5204</td>
<td>0.4705</td>
<td>0.4736</td>
</tr>
<tr>
<td>Hardness 200 ppm</td>
<td>48</td>
<td>Y = -24.6792 + 15.1947 X</td>
<td>0.9402</td>
<td>0.8924</td>
<td>0.8980</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>Y = -5.5368 + 5.6738 X</td>
<td>0.8172</td>
<td>0.7025</td>
<td>0.7196</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>Y = -3.4412 + 4.7524 X</td>
<td>0.6017</td>
<td>0.5914</td>
<td>0.5948</td>
</tr>
<tr>
<td>Salinity 0.5%</td>
<td>24</td>
<td>Y = 5.1442 - 0.07249 X</td>
<td>1.1069</td>
<td>0.9724</td>
<td>0.9780</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>Y = -6.4463 + 6.1495 X</td>
<td>0.8413</td>
<td>0.7325</td>
<td>0.7413</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>Y = -9.9698 + 8.2892 X</td>
<td>0.6874</td>
<td>0.6317</td>
<td>0.6400</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>Y = 26.0759 - 12.2328 X</td>
<td>0.4950</td>
<td>0.4702</td>
<td>0.4843</td>
</tr>
<tr>
<td>Salinity 1%</td>
<td>24</td>
<td>Y = -13.2728 + 8.9125 X</td>
<td>1.1742</td>
<td>1.1195</td>
<td>1.1220</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>Y = -7.1067 + 6.0296 X</td>
<td>1.0233</td>
<td>0.9395</td>
<td>0.9450</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>Y = -6.2351 + 6.0371 X</td>
<td>0.7603</td>
<td>0.7214</td>
<td>0.7263</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>Y = -4.9782 + 5.7182 X</td>
<td>0.5872</td>
<td>0.5510</td>
<td>0.5560</td>
</tr>
</tbody>
</table>
Table A-3

Regression equations, 95% fiducial limits with observed and calculated LC$_{50}$ values for _B. bendelisis_ exposed to dithane M-45 at various water variables.

<table>
<thead>
<tr>
<th>Water variable</th>
<th>Exposure period (hours)</th>
<th>Regression equation</th>
<th>Fiducial limits</th>
<th>Calculated LC$_{50}$ (ppm)</th>
<th>Observed LC$_{50}$ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory conditions</td>
<td>24</td>
<td>$Y = -6.0796 + 6.2249X$</td>
<td>0.6885</td>
<td>0.6140</td>
<td>0.6485</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>$Y = -7.4110 + 7.1708X$</td>
<td>0.6402</td>
<td>0.5290</td>
<td>0.5380</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>$Y = -0.7038 + 3.4957X$</td>
<td>0.4862</td>
<td>0.4210</td>
<td>0.4867</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>$Y = -4.2602 + 5.8600X$</td>
<td>0.4424</td>
<td>0.3980</td>
<td>0.3981</td>
</tr>
<tr>
<td>pH 6.5</td>
<td>24</td>
<td>$Y = -16.4856 + 12.0650X$</td>
<td>0.6285</td>
<td>0.6012</td>
<td>0.6037</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>$Y = -14.6965 + 11.2184X$</td>
<td>0.6702</td>
<td>0.5515</td>
<td>0.5699</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>$Y = -16.4815 + 12.6171X$</td>
<td>0.6112</td>
<td>0.5148</td>
<td>0.5202</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>$Y = -0.0931 + 3.1951X$</td>
<td>0.4102</td>
<td>0.3815</td>
<td>0.3890</td>
</tr>
<tr>
<td>pH 9</td>
<td>24</td>
<td>$Y = -19.6188 + 13.5807X$</td>
<td>0.6815</td>
<td>0.6495</td>
<td>0.6537</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>$Y = -3.4571 + 1.6891X$</td>
<td>0.5802</td>
<td>0.5495</td>
<td>0.5508</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>$Y = -9.1128 + 8.1487X$</td>
<td>0.5704</td>
<td>0.5305</td>
<td>0.5394</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>$Y = -6.6023 + 7.1578X$</td>
<td>0.4665</td>
<td>0.4285</td>
<td>0.4205</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>----------</td>
<td>----------</td>
<td>--------------------</td>
<td>------------------</td>
<td>-----</td>
<td>--------</td>
</tr>
<tr>
<td><strong>Water hardness 100 ppm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>$Y = -16.4856 + 12.0650 X$</td>
<td>0.6840</td>
<td>0.5985</td>
<td>0.6038</td>
<td>0.6200</td>
</tr>
<tr>
<td>48</td>
<td>$Y = -9.1469 + 8.2659 X$</td>
<td>0.5742</td>
<td>0.5095</td>
<td>0.5145</td>
<td>0.5150</td>
</tr>
<tr>
<td>72</td>
<td>$Y = -9.7135 + 8.7798 X$</td>
<td>0.5174</td>
<td>0.4705</td>
<td>0.4720</td>
<td>0.4800</td>
</tr>
<tr>
<td>96</td>
<td>$Y = -5.4581 + 6.6646 X$</td>
<td>0.4438</td>
<td>0.3955</td>
<td>0.4010</td>
<td>0.3890</td>
</tr>
<tr>
<td><strong>Water hardness 200 ppm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>$Y = -15.7081 + 11.6535 X$</td>
<td>0.6545</td>
<td>0.5940</td>
<td>0.5984</td>
<td>0.6150</td>
</tr>
<tr>
<td>48</td>
<td>$Y = -8.0125 + 7.6322 X$</td>
<td>0.5322</td>
<td>0.4985</td>
<td>0.5008</td>
<td>0.5000</td>
</tr>
<tr>
<td>72</td>
<td>$Y = -9.8767 + 8.9721 X$</td>
<td>0.5389</td>
<td>0.4505</td>
<td>0.4557</td>
<td>0.4600</td>
</tr>
<tr>
<td>96</td>
<td>$Y = -8.7304 + 8.7178 X$</td>
<td>0.4282</td>
<td>0.3696</td>
<td>0.3758</td>
<td>0.3715</td>
</tr>
<tr>
<td><strong>Salinity 0.5%</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>$Y = 4.5891 + 5.3217 X$</td>
<td>0.6874</td>
<td>0.6264</td>
<td>0.6336</td>
<td>0.6500</td>
</tr>
<tr>
<td>48</td>
<td>$Y = 5.7757 + 6.8849 X$</td>
<td>0.5942</td>
<td>0.5135</td>
<td>0.5892</td>
<td>0.5400</td>
</tr>
<tr>
<td>72</td>
<td>$Y = 6.0717 + 6.5608 X$</td>
<td>0.5012</td>
<td>0.4805</td>
<td>0.4871</td>
<td>0.4950</td>
</tr>
<tr>
<td>96</td>
<td>$Y = 3.1474 + 5.9080 X$</td>
<td>0.4224</td>
<td>0.4000</td>
<td>0.4020</td>
<td>0.3981</td>
</tr>
<tr>
<td><strong>Salinity 1%</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>$Y = -10.7160 + 8.7148 X$</td>
<td>0.6839</td>
<td>0.6310</td>
<td>0.6356</td>
<td>0.6500</td>
</tr>
<tr>
<td>48</td>
<td>$Y = -7.7680 + 7.3872 X$</td>
<td>0.5877</td>
<td>0.5405</td>
<td>0.5477</td>
<td>0.5500</td>
</tr>
<tr>
<td>72</td>
<td>$Y = -12.5618 + 10.3270 X$</td>
<td>0.5107</td>
<td>0.4695</td>
<td>0.4721</td>
<td>0.4900</td>
</tr>
<tr>
<td>96</td>
<td>$Y = -11.1764 + 9.9701 X$</td>
<td>0.4704</td>
<td>0.4085</td>
<td>0.4192</td>
<td>0.4169</td>
</tr>
</tbody>
</table>
Fig. A-4  Dose mortality curves of *B. bendelisis* in relation to pH and toxicities of three pesticides.
Fig. A-4  LOG CONCENTRATION
Fig. A-5: Dose mortality curves of *B. bendelisis* in relation to water hardness and toxicities of three pesticides.
Fig. A-5

PROBIT MORTALITY vs. LOG CONCENTRATION

- O THIODON ——— $\log_{10}$ cond.
- ● NUVAH ——— WH 100 ppm.
- △ DITHANE ——— WH 200 ppm.
Fig. A-6: Dose mortality curves of *B. bendelisis* in relation to salinity and toxicities of three pesticides.
Fig. A-6  LOG CONCENTRATION

- THIODON — Lab. cond.
- NUVAN —— S. 0.5%
- DITHANE —— S. 1%
DISCUSSION

Acute toxicity tests are generally carried out to determine the level of toxic agents that produce adverse effects on test organisms in a short period of time. A dose of pesticide which causes a biological system to deviate from its normal range of variation is called as acute dose. Available literature shows a huge amount of work on toxicity of pesticides and heavy metals referring to different aspects like mortality, biochemical and physiological changes, behavioural and histopathological changes of invertebrates and vertebrates.

The toxicological effects at acute stress generally occur due to the direct action of the pesticide on target organs. In majority of the toxicity tests, toxic effects are dose dependent. However, certain factors like temperature, oxygen, type of toxicant, pH of medium, water hardness, salinity and sulphate levels of water influence the toxicity of pesticides. It is also known that living organisms have ability to adapt themselves when exposed to new environment. But severe changes in the environment cause damage to fish life by affecting its resistance mechanism (Mason, 1981).
In the present study, results of bioassay tests have shown that the toxicity of thiodan, nuvan and dithane M-45 to Barilus bendelisis was a function of concentration and duration of exposure (Table A-1 to A-3). Behavioural changes in B. bendelisis due to exposure to different concentrations of pesticides were noted on the basis of definite symptoms of uncoordinated movements, hyperexcitability, restlessness reflected by fast swimming and increased opercular movements. These symptoms are clearly noticeable in higher concentrations of thiodan. Excitability of fish was observed earlier in thiodan than in nuvan and dithane M-45. It was observed that fishes rarely dashed against the wall of aquarium. Fish activities became lethargic indicating a slightest balance in movements, possibly due to disorder in central nervous system discernible from the jerky movements (especially in dithane M-45) before death.

The colour change from normal to pale yellow in the higher concentrations of thiodan and dithane M-45 was more pronounced than nuvan. These observations are in accordance to the findings of Verma et al., (1980).
The LC50 values for different exposure periods for different pesticides have been reported by several workers. Grant and Schoettger (1972) and Symons (1973) reported the impact of organochlorine pesticides on physiological functions of the fish. Knauf and Schulze (1973) reported 48 hr LC50 values of endosulfan (35 EC) for the mosquito fish Gambusia affinis and the gold fish, Carassius auratus as 1 and 10 μg/l, respectively. Verma et al. (1979) reported 0.0108 ppm as 96 h LC50 for thiodan to Saccobranchus fossilis. The toxicity of endosulfan to Labeo rohita, Catla catla, Heteropneustes fossilis, Mystus cavasius, Mystus vittatus and Anabas testudineus found to be 0.011, 0.018, 0.011, 0.019, 0.022, and 0.022 ppm as 96 h LC50 values, respectively (Rao, 1979). Similarly Krishna Gopal et al. (1981) evaluated acute toxicity of endosulfan to the fish, Clarias batrachus and showed the median lethal concentration (LC50) values 0.0225, 0.0175 and 0.0140 ppm for 24, 48, and 96 h, respectively. Chronic toxicity of thiodan (35 EC) for 3 weeks to fish Gambusia affinis and Gymnocorymbus ternetzi have been studied by Joshi et al. (1981) and showed that maximum acceptable toxicant concentration (MATIC) lied in the range of 0.0001 and 0.0094 ppm.
Similarly studies on toxicity, biotransformation and elimination of endosulfan in *Anabas testudineus* recorded LC$_{50}$ values as 3.0, 2.4, 1.5 and 1.2 ppb for 24, 48, 72 and 96 h respectively. Toxicity is associated with accumulation of endosulfan in excess amounts which may be metabolised and stored in various tissues; the accumulated pesticides are eliminated through urine or feces or both because liver, kidney and intestine are the main organs where biodegradation, metabolism and excretion of the toxic materials take place (Rao and Murthy, 1980).

Raizada *et al.* (1981) noted (5.2 mg/L) 24 h LC$_{50}$ of endosulfan to *Gambusia affinis*. Singh and Srivastava (1982) reported 96 h LC$_{50}$ values (0.0033 - 0.0002 mg/L) for *Heteropneustes fossilis* on exposure to endosulfan. Verma *et al.* (1982) conducted a bioassay tests for seven organochlorine (thio tox, endosulfan, heptachlor, chlordane, aldrin, lindane and BHC) pesticides on *Saccobranchus fossilis* and determined the LC$_{50}$ values for a period of 24, 48, 72 and 96 hours by graphical interpolation and probit analysis, but only 96h LC$_{50}$ values were calculated. The evaluation of toxicity of endosulfan to *Barbus stigma* showed 0.0043 ppm as 96 h LC$_{50}$ (Manoharan and Subbiah, 1982). Ruparelia *et al.* (1984) in their status
report on acute toxicity of thiodan to freshwater fishes reported LC$_{50}$ values for *Cirrhina mrigala* as 0.007 and 0.00092 ppm for 24 and 96 h, *Mystus vittatus* 0.00024 ppm for 96 h, *Macrolebias aculeatus* 3.5 ppm for 96 h and for *Gymnocorymbus ternetzi* 0.023 and 0.006 ppm for 24 and 96 h.

In the present bioassay of *Barilus bendelisis* to thiodan (35 EC), the LC$_{50}$ values obtained for 24, 48, 72 and 96 h are 0.03485, 0.02613, 0.02087 and 0.01560 ppm, respectively. The results of the present investigations show that the LC$_{50}$ values for various exposure periods are in concurrence with the findings of Krishna Gopal et al. (1981) and Rao (1979). It indicates that *B. bendelisis* is more sensitive to thiodan as appears in *Clarias batrachus*, *Catla catla*, *Mystus vittatus* and *Heteropneustes fossilis*.

Further, it has been observed that the toxicological effects of thiodan increased with exposure period which is evident from the LC$_{50}$ values. It is possible that the increase in toxicity may be associated with accumulation of excess amount of endosulfan that may be metabolised or stored in various tissues (Rao and Murthy, 1980). Gupta et al. (1981) while studying the
toxicity of endosulfan and manganese chloride demonstrated cumulative toxicity rating of the endosulfan. Studies on biological magnification of endosulfan in *Tilapia mossambica*, show that there is gradual accumulation of endosulfan in kidney and liver (Subbiah et al., 1985). But when accumulation of residues exceed the tolerable limit, mortality sets in. Mortality rate increases with increase in exposure period. Observations on toxicity tests also indicate the thiodan is highly toxic to the fish. Observations on behaviour of *Barilius bendelisis* exposed to thiodan show quick response, hyperactivity, loss of balance and increase mucus secretion in gills with consequent decreased oxygen consumption affecting respiratory rate and ultimate death of the fish due to asphyxiation.

Acute toxicity of organophosphorus pesticides to different fishes have been reported by number of workers. Verma et al. (1982) reported acute toxicity of dichlorovos to the fish *Saccobranchus fossilis* and 96 h LC$_{50}$ value ranged between 6 to 6.61 ppm. Similarly Ruparelia et al. (1984) in their status report quoted the 96 h LC$_{50}$ values of dichlorovos to
Channa (Ophioccephalus) punctatus, Mystus vittatus and Cirrhina mrigala as 2.3, 0.45 and 0.29 ppm respectively.

In the present investigations, the LC₅₀ values at acute stress of nuvan are 1.0890, 0.8786, 0.6929 and 0.4903 ppm for 24, 48, 72 and 96 h, respectively. The 96 h LC₅₀ of dichlorovos to Mystus vittatus is 0.45 ppm (Ruparelia et al., 1984) which appears more or less similar to the value obtained for B. bendelisis. This indicates that both fish species are almost equally sensitive to nuvan. It is very clear from the LC₅₀ values, that the toxicity of nuvan increases with increase in exposure period. The fish is more sensitive to dimecron (Kamble 1984) than nuvan. Observations on behaviour of B. bendelisis exposed to nuvan show toxic symptoms like impairment in respiration specially suffocation may be due to extended opercular flaps before death (Gouda et al., 1981).

Konar and Ghosh (1983) evaluated 96h LC₅₀ (0.18 ppm) of dithane M-45 to fish Tilapia mossambica. Toxicity of carbamate pesticide, sevin to B. bendelisis showed 2.67 ppm as 96 h LC₅₀ (Kamble, 1984). Ruparelia et al. (1984) in their status report gave 96 h LC₅₀ values of carbamate pesticide, carbofuran to Ophioccephalus punctatus (0.18 ppm), Mystus vittatus (0.31 ppm), Saccobranchus
fossilis (0.547 ppm) and Cirrhina mrigala (0.260 ppm). The LC$_{50}$ values obtained for acute toxicity of dithane M-45 to B. bendelisis in the present study are 0.6485, 0.5380, 0.4867 and 0.3981 ppm for 24, 48, 72 and 96 h, respectively. Comparison of these LC$_{50}$ values of the present fish with others indicate that Tilapia mossambica is more sensitive to dithane M-45 than B. bendelisis. Sensitivity of Mystus vittatus to carbofuran and B. bendelisis to dithane M-45 appear to be similar.

From the above discussion, it is clear that the variation in sensitivity of different species to different pesticides is dependent on factors like age, sex, weight, physiological state of the animal and presence or absence of enzyme system that can degrade the pesticide (Nagratnamma and Rammurthi, 1981). It is also revealed from the results that thiordan is more toxic to B. bendelisis than dithane M-45 and nuan. From the available literature and findings of present investigations, it seems that organochlorine pesticides are highly toxic to various fish species than organophosphate and carbamates (Ruparelia et al., 1984; Verma et al., 1982). The pesticides can be arranged in order of their toxicities as thiordan > dithane M-45 > nuan.
Crandall and Goodnight (1959) working on toxicity of pentachlorophenate to fathead minnow showed that toxicity decreased with increasing pH. Davies (1973) reported optimum pH (7.5) for fish survival. Holcombe et al. (1980) showed that toxicological effects of 2,4-dinitrophenol at acute stress decreased when the pH of water increased. Kobayashi (1980) also reported that the toxicity of pentachlorophenol decreased as pH increased. With the increase in pH, toxicity in terms of $\text{HNO}_2\text{N}$ is increased (Russo et al., 1981). Hydrogen cyanide is toxic especially in the molecular form; any change in pH which reduces the degree of dissociation will increase the toxicity of the solution without any change in the total concentration of cyanide. Mason (1981) reported that the toxic effects of pollutants vary with the quality of water, pH and water hardness.

Aly and Eldib (1971) and Hoeler (1975) reported that the rate of hydrolysis was accelerated with increasing pH and the products were more toxic than the parents. Tilak et al. (1981) while assessing carbaryl and 1-hapthol (metabolite of carbaryl) toxicity to Catla catla, Anabas testudineus, Mystus cavasius and Mystus vittatus showed that degradation products were more toxic than parent compound. Marking
and Bills (1981) studied *Cyprinus carpio*, *Ctenopharyngodon idela*, *Aristichthyes nobolis* and *Hypothalamichthyes molitrix* and correlated the toxicity of GD-174 with pH which increased at high pH. The physical factors like temperature and pH have modifying effect on the toxicity of pesticides and other pollutants (Vardia and Durve, 1981).

In the present study, the effects of pH on toxicity of thiodan, nuvan and dithane M-45 indicate that the toxicity of all the pesticides increases at pH 6.5 and decreases at pH 9.0. The increase in toxicity at 6.5 (low) pH might be attributed to saponification or hydrolysis (degradation) of toxicants which may have been accelerated and the resulting degraded products (isomers) are more toxic than the original compounds (Hosler, 1975).

The effect of water hardness on toxicity of different chemicals have been studied in detail by many workers (Sprague, 1964; Pickering and Henderson, 1966, Skidmore and Tovell, 1972; Masen, 1981). Studies show that pollutants tend to be more toxic in soft waters while hardness of water decreases the toxicity at any pH. Chloride cells present in gills increase in hard water than in soft water (Skidmore
and Tovell, 1972). Detoxification mechanism mainly depends on the number and activity of chloride cells and amount of water hardness (Metz and Branancian, 1975). Toxicity of cadmium to *Salmo gairdneri* increases with the decrease of water hardness (Calamari, et al., 1980). While assessing the effect of water hardness on toxicity of endosulfan, malathion and sevin to *Rasbora daniconius*, Khalid (1985) showed that endosulfan and sevin toxicity increased at 100 ppm and decreased at 200 ppm water hardness. No significant change in toxicity of malathion was observed.

Findings of the present investigations indicate that 100 ppm water hardness has little effect on toxicity of thiodan and nuvan; but toxicity decreases at 200ppm water hardness. The toxicity of carbamate pesticide, dithane M-45 decreases at 100 and increased at 200 ppm water hardness. The decrease or increase of toxicity in relation to water hardness cannot be attributed to a specific reason.

Changes in salinity affect toxicity of pollutants. Jones (1940) reported that toxicity of HgCl₂ to *Bufo bufo* decreased with increase in sodium chloride (upto 0.09%).
A little increase in salinity reduces the toxicity of copper (Sprague, 1964). Joshi (1974) have similar findings in the mosquito fish, Gambusia affinis. Zhumbora and Somkina (1976) in Heteropneustes fossilis and Lucioperca lucioperca and Al-Dahm and Bhatti (1977) in G. affinis observed that 2-4 dinitrophenol was ineffective at low salinity but had deleterious effects at high salinity. Holcombe et al. (1980) reported the decrease in toxicity of 2-4 dinitrophenol due to addition of salts in test water. High quantity of free chlorine in water causes death of fish under normal conditions (Mason, 1981).

Bakre (1985) reported that the toxicological effects of mercury in combination with salinity has severe effect on Gambusia affinis than mercury alone. Deshmukh et al. (1985) while studying the naphthalene toxicity to the speckled prawn, Metapenaeus monoceros demonstrated the toxicity is salinity dependent i.e., less toxicity at salinity 17.5%, and 26.25%, and more at 3.5%, 8.75%, and 35%. Khalid (1985) found that toxicity of endosulfan to Rasbora daniconius decreased at 0.9% salinity and did not show any remarkable change at 0.45%. Whereas toxicity of malathion and carbaryl decreased at 0.45% and 0.9% salinity.
Data obtained in the present studies show that at acute stress, toxicity of dithane M-45 decreases at 1% salinity and remains unaffected at 0.5%. Toxicity of thiodan decreases at 0.5%, and increases in 1% salinity. The toxicity of nuvan increases at 0.5% and decreases at 1% salinity. The findings of the present investigations show that change in salinity affects nuvan toxicity remarkably than thiodan and dithane M-45.

**SUMMARY**

1. Acute toxicity studies of the pesticide thiodan, nuvan and dithane M-45 were conducted on B. bendelisis.
2. The toxicity of thiodan, nuvan and dithane M-45 is to the fish a function of concentration and duration of time.
3. Different environmental variables like change in pH, hardness and salinity of water modify the toxicity of pesticides.
4. Thiodan is found to be highly toxic to this fish than nuvan and dithane M-45.
5. B. bendelisis appears more resistant to nuvan than thiodan and dithane M-45.
6. Symptoms of poisoning are:

a) Uncoordinated movements
b) Change in the rate of opercular movements
c) Hyperexcitation, increased swimming and surfacing activity
d) Loss of balance.
REFERENCES


Al-Dahm, N.K. and Bhatti, M.N. (1977). Salinity tolerance of Gambusia affinis (Baird and Girard) and Heteropneustes fossilis (Bloch).


APHA, AWWA and WPCF (1975). Standard methods for the examination of water and waste water, 14th Ed.


Progressive Fish Culturist, 35: 157.


Holcombe, G.W., James, T. and Fipp, G. (1980). Effect of pH increase and sodium chloride additions on the acute toxicity of 2-4 dinitrophenol to the fathead minnow.


Jones, J.R.E. (1940). Antagonism between salts of the heavy metal and alkaline earth metals in their toxic action on the tadpole of the toad *Bufo bufo* L.


Ph.D. thesis, Bombay University, Bombay, India.


*J. Environ. Biol.*, **2**: 43-57.


Ph.D. thesis, Marathwada University, Aurangabad, India.


Ph.D. thesis, Marathwada University, Aurangabad, India.


Geobios, 10: 222-225.


Mason, C.F. (1981) "Biology of fresh water pollution"


Experientia, 18: 506.


Environmental toxicity of pesticides.

In: Excretion (Wessing, A. ed).


Faber and Faber - London, pp. 320.

Acute toxicity of nitrate to rainbow trout
(*Salmo gairdneri*).


Indian J. exp. Biol., 18: 75-76.

Toxicity of carbaryl and 1-napthol to four species of freshwater fish.

Hydrobiologia, 77: 155-159.


J. Ichthyol., 16: 511-514.

*NOT SEEN IN ORIGINAL