7. SUMMARY

Pesticides are recognised as serious pollutants especially in the aquatic environment with deleterious effects on the associated non-target organisms like fishes and amphibian larvae. Carbaryl and methyl parathion are two important and commonly used pesticides in India.

Frogs are useful to man in many ways; as experimental animal, food source for fish in nature, biological agent to control mosquitoes and delicious food item (frog leg). With an objective on the conservation of *Rana tigrina* population in nature, a bioassay study and a subsequent stagewise study on the effect of sublethal concentrations of carbaryl and methyl parathion on the survival and various food utilization parameters like feeding, absorption, excretion, conversion and metabolism (bimodal respiration) were studied in the feeding tadpole phase (I, II and III stages). The duration and energy cost of metamorphosis were investigated in the non-feeding metamorphic phase (IV stage). Rearing, different stages of tadpoles in the 10 and 30% sublethal concentrations of carbaryl and methyl parathion, histopathological study was carried out in tissues like liver, intestine and muscles of the emerged froglets.

*R. tigrina* tadpoles were produced in the laboratory by hypophysation. Bioassay experiments were carried out when the tadpoles attained 15 mg wet weight. Adopting the standard bioassay techniques, 96 hr LC$_{50}$ was calculated for carbaryl (5.7 ppm) and methyl parathion (4.4 ppm). The 10, 20 and 30% of 96 hr LC$_{50}$ of the two pesticides were chosen as sublethal concentrations and the tadpoles were reared in that medium upto the froglet stage. Carbaryl affects the
survival at the fourth stage but methyl parathion causes mortality in the third stage itself. The individuals exposed to 30% methyl parathion also suffered vertebral damages (scoliosis) during long term study. Accounting the mortality in IV stage, $LC_{50}$ was calculated and the sublethal levels were chosen (10, 20 and 30% $LC_{50}$); these concentrations were maintained for further studies.

The present observation suggests the need for a survival study throughout the larval period of the amphibians to fix the sublethal levels of the toxicants. In both the long term and short term studies, methyl parathion affects the survival of the tadpoles more than that of carbaryl.

During the ontogeny of amphibians, their alimentary canal undergoes morphological modifications. Besides growth, tadpoles undertake energetically expensive metamorphosis. These peculiar features of amphibians are capable of significantly influencing the pattern of ingestion and allocation of energy. Hence, bioenergetics of $R. \ tigrina$ tadpoles exposed to chosen sublethal concentrations of carbaryl and methyl parathion could provide scope for the construction of energy budget under pesticide stress.

The International Biological Programme (IBP) formula was adopted to express the scheme of energy balance i.e.,

$$C = F + U + R + P$$

where $C$ is the food energy consumed, $F$ the energy of feces egested, $U$ the nitrogenous excretory wastes, $R$ the energy spent on metabolism and $P$ the growth.
Food utilization parameters are significantly influenced by the developmental stages and pesticide concentrations. When the tadpole grows, the rates of feeding, conversion and metabolism are significantly decreased. Tadpoles during their first stage show 4.4, 4.1, 0.54 and 3.5 KJ/g/day as feeding, absorption, conversion and metabolic rates, respectively and these rates are reduced to 1.40, 1.0, 0.21 and 0.8 KJ/g/day in the third stage. Both the chosen pesticides enhanced the feeding rate significantly. The average feeding rate of all stages shows an increase of 29.2% from the control in tadpoles exposed to 30% LC$_{50}$ of both carbaryl and methyl parathion.

Quantitative study of nitrogenous excretion with reference to the development stages of the tadpoles and concentrations of the pesticides provides an opportunity to find the fraction of ingested energy spent on excretion and fluctuation in the pattern of nitrogen excretion. Following phenol hypochlorite method, NH$_3$-N concentration in the water sample was determined. The same method was followed to estimate urea-N concentration after incubating the sample with urease solution for 20 min at 30°C. The rate of urea-N excretion increases whereas NH$_3$-N excretion decreases in relation to stage; this indicates a shift in the pattern of excretion from ammonotelism to ureotelism in the developing tadpoles. In every stage, both the pesticides enhance the excretion rates of NH$_3$-N and urea-N ultimately increasing the total excretion of the tadpoles in the pesticide medium. Both the pesticides enhance the urea excretion dramatically when the stages are in progress indicating more energy utilization for excretion.

Both carbaryl and methyl parathion reduce the conversion rate and efficiency significantly. The conversion rate of control tadpole averages to 0.33
KJ/g live tadpole/day, and it is only 0.20 and 0.14 KJ/g live tadpole/day in those exposed to maximum concentrations (30% of LC$_{50}$) of carbaryl and methyl parathion, respectively. Similarly, the conversion efficiency declines from 15.5% (Control) to 7.2 and 5.5% in tadpoles exposed to 30% LC$_{50}$ of carbaryl and methyl parathion, respectively.

Both aquatic and aerial respirations were determined simultaneously in a specially designed and constructed respirometer. The sublethal concentrations of carbaryl and methyl parathion influence the bimodal respiratory pattern of the tadpoles. Carbaryl causes a decline in aquatic respiration in IV stage; methyl parathion, however, decreases the aquatic respiration from II stage onwards. Aerial respiration (an average of all stages) increases significantly from 71.9 J/g live tadpole/day in control group to 95.3 and 113.5 J/g live tadpole/day in the maximum sublethal (30% of LC$_{50}$) concentrations of carbaryl and methyl parathion, respectively.

Energy budget studies reveal that the control tadpoles invest 14.0% on production spending 6.6% on defecation and 0.08% on excretion. However, test animals exposed to the highest concentrations (30%) of carbaryl and methyl parathion allocate only 6.4 and 4.2% respectively on production spending more energy on defecation and excretion.

Carbaryl does not alter the larval duration compared to the control; methyl parathion, however, extends the larval and metamorphic durations considerably. The extension of larval duration may be to accumulate adequate energy for metamorphosis. Both carbaryl and methyl parathion reduce the metamorphic
efficiency from 84.1% in control to 65.0 and 48.2% in 30% LC₅₀, respectively. The metamorphic metabolic rate (MMr) significantly increases in the tadpoles exposed to carbaryl. However, methyl parathion (30% of LC₅₀) reduces the MMr considerably (24%) compared to the control.

To assess the effects of pesticides, tissue pathology will be an effective tool. Histolopathological study furnishes evidences for physiological changes in the tadpoles exposed to pesticides. This study has been carried out in liver, intestine and muscle tissues of the newly emerged froglets, that were developed from the larvae exposed to 10 and 30% of LC₅₀. Pathological symptoms occur in the liver, intestine and muscle tissues of the emerged froglets. An intense granulomatous reaction in the liver tissue, massive accumulation of macrophages in the intestinal villi and the neoplastic growth in the submucosa of intestine are observed in tadpoles exposed to methyl parathion (30%).

The safe level concentrations were calculated from the LC₅₀ values according to the lowest application factors of carbaryl (0.026) and methyl parathion (0.024) suggested for the carbamate and organophosphate pesticides (Verma et al., 1979). The safe concentrations calculated with reference to the short term (96 hr)/long term (throughout larval period) study conducted on R. tigrina tadpoles are 0.2/0.06 and 0.1/0.03 ppm for carbaryl and methyl parathion, respectively.

The present study emphasizes the need for long term study for determining the safe concentrations particularly for the developing amphibians. Among the two chosen pesticides, methyl parathion is more toxic than carbaryl
with reference to its safe level and various physiological studies. These low safe concentration values for the *R. tigrina* tadpoles, will be absolutely safe levels for most of the fish species which co-inhabit with the amphibians in nature.