MATERIAL AND METHODS GENERAL
BIOLOGY OF TEST SPECIES

The fresh water snail, "Pila globosa" (Swainson) was used as the experimental animal in the present investigation. It is one of the largest fresh water gastropod abundantly found in ponds, tanks, rice-fields and in water having succulent vegetation. It is found in oriental and Ethiopian regions. It is common species of Northern India. It was formerly called "Ampullaria globosa". Its popular name is the "Apple snail". It is herbivorous. It creeps very slowly with its massive foot. Its slow movements are proverbial, "Snail's speed". It merely covers about 50 mm in a minute at full speed. It is an amphibious animal. When disturbed, it withdraws into the shell and closes its mouth with a lid. It can withstand long periods of drought by remaining dormant within its tightly closed shell buried in mud. This period of inactivity is called "summer sleep or aestivation". Sexes are separate. Breeding takes place in rainy season. Fertilization is internal. Eggs are laid at some sheltered spot on land. There is no parental care. Development is direct.

As an experimental animal, the snail was selected because it is common inhabitant of fresh waters and rice fields, where the
The applicability of phenthoate for the eradication of pests is quite frequent. Further it has some economic value, because it is consumed by some poor folk in certain areas of South India. It also contributes to the natural manurisation of the soil (Kotpal, 1975).

MAINTENANCE OF SNAILS

Gastropod snails of the species *Pila globosa* (Swainson) were collected from fresh water ponds around Tirupati. They were brought to the laboratory and kept in glass troughs (20x10cm) with 2" columns of depth orinated tap water. Care was taken to minimise the rate of mortality by uniformly distributing them without crowding. They were fed with leaves like *Amaranthus viridis*. The water in the troughs was changed regularly once in every day. They were acclimated to laboratory conditions for 7 to 10 days. After acclimation they were used for experimentation. In view of the abundant availability and adaptability to laboratory conditions, this species was selected as an experimental animal.

PESTICIDE EMPLOYED

The insecticide, “Phenthoate” was used for the present investigation. It is an organophosphate pesticide obtained from Motilal
pesticides (India) Pvt. Ltd., New Delhi is a technical grade sample of 70% purity. The physical and chemical properties of phenthoate are as follows:

**SPECIFICATIONS**

Common name : Phenthoate  
Trade name : Elsan  
Chemical name : O,O-dimethyl, S-(ethoxy-carbonyl benzyl) phosphorothiolothionate.

![Structural formula](image)

Molecular weight : 320.36

**Physical properties :**

Colour : Reddish Yellow (Liquid)  
Odor : Aromatic odor  
Solubility : Soluble in all organic solvents  
Bioling point : 186-187°C/5, Hg.  
Melting point : 16°C to 17°C (pr grade)
Stability : Stable in neutral except in acidic and basic media where it undergoes hydrolysis. Pesticide Manual (BCPC): 'In water: stable under neutral and acidic conditions: degrades under alkaline conditions'

Concentration : 70% purity (Technical grade). The remaining 30% be the dissolving media.

TOXICITY DETERMINATION

For toxicity determination technical grade sample of phenthoate is used. Since the pesticide is not completely soluble in water, little of acetone was used as a solvent to obtain uniform distribution of the test solution. A stock solution of 1000 ppm of phenthoate was prepared in acetone. For working concentration, required dilutions were made with tap water. Fresh stock solutions and required dilutions were prepared for each exposure. Since a small quantity of acetone was used for stock preparation, it is reported to be no toxic to snail (Pickering et al., 1962). However, acetone controls were also maintained to nullify possible effects if any.
EXPOSURE STUDIES

Snails (n=7) were exposed to lethal 14.2 ppm and sublethal 4.8 ppm for 48h. Equal number of snails exposed to the same quantity of acetone for the same period served as control.

FACTORS LIKELY TO INFLUENCE TOXICITY

Size of snail

Since the size of the animal has an effect on pesticide toxicity (Mount, 1962; Pickering et al., 1962) almost equal size of snails weighing about 110-120gms were used throughout the investigations.

Temperature

Since a rise in temperature, increase the toxicity of pesticides (Macek et al., 1969) in the present study, the temperature of the water was maintained at 27 ±1°C throughout the investigation.

Time of exposure

All experiments were conducted after 48 hrs of exposure. 48h period was selected, because it is relatively longer period than 24h. Wherever its effects are fluctuating. Besides, this exposure period is relatively lower compared to 72 or 96h, where variations in oxygen
content and phenthoate concentration in the medium (water) are likely to influence the physiological and metabolic state of the snail. Any other factor which is likely to influence the toxicity of phenthoate was nullified by maintaining normal snails under similar experimental conditions as that of the phenthoate exposed snails (but without phenthoate in the medium).

Experimental design

Species : *Pila globosa*
Pesticide : Phenthoate
Exposure concentrations : Sublethal 4.8 ppm
                          : Lethal 14.2 ppm
Exposure time : 48 h
Tissues selected : Foot, mantle and Hepatopancreas

SEPARATION OF TISSUES

The animal selected for experimentation are first kept on filter paper and the water is blotted from the animals. Later, the shell of the animal is broken with a forceps and removed. Later tissues like foot, mantle and hepatopancreas are separated and kept on a porceline tile in ice bath in a cold room.
HOMOGENIZATION

The tissues thus isolated are weighed accurately on electric balance. For homogenization, Yorco tissue homogenizer having electrically controlled teflon pestle in a glass homogenizer was used. When ever required for experimentation the tissue homegenates are centrifuged at 2,000 g for 10-15 min.

The details of individual procedures adopted for assay of enzyme activity or estimation of any metabolite are given in the concerned chapters.

VALIDITY OF ANALYTIC PROCEDURES

Aliquots for assay

Aliquots selected for the assay initial rates were approximated as nearly as possible, yet providing sufficient product to fall in convenient range for spectrophotometric measurement range.

Substrate requirements

All assays were made under conditions following zero order reaction.
Lambert-Beer Law

The products of the reactions were measured spectrophotometrically, wherein the optical density (absorbance) of the resulting coloured complexes were proportional to the concentration of the reaction products.

Units

The units were expressed for all lipid profiles as mg/g wet weight for foot, mantle, hepatopancreas respectively in order to maintain uniformity. The enzyme activity was expressed in standard units i.e., \( \mu \text{Mol of product formed or cleaved/ mg protein/h} \).

STATISTICAL ANALYSIS OF THE EXPERIMENTAL DATA

For each parameter, the mean of individual observations (for both control and experimental groups) were taken into consideration. Statistical significance of the data was analysed through two way ANOVA (analysis of variance); SNK (Student-Newman-Keuls) test and regression analysis (Zar, 1984). A p-value <0.001 was considered as significant.
AIM AND OBJECTIVES

Present study is designed to study the *in vivo* effect of sub-lethal and lethal concentrations of phenthoate on selected aspects of lipid metabolism in the tissues of the snail, *Pila globosa*.

PLAN OF WORK

1. Toxicity evaluation of phenthoate to the snail *P. globosa* (Swaison) in static bioassay system for determining the LC_{50} concentration.

2. Exposure of *P. globosa* to lethal and 1/3 lethal concentrations of phenthoate and in the tissues like foot mantle and hepatopancreas the following aspects of lipid metabolism be studied.
   a. Total lipids, phospholipids, neutral lipids, tri-di and monoglycerides, cholesterol, glycerol and fatty acids.
   b. To measure the enzymatic activity like triglyceride acyl hydrolase, diglyceride acyl hydrolase, phospholipase and palmitoyl carnitine transferase in the control and phenthoate exposed snail tissues,
   c. To measure the oxygen consumption and CO_{2} liberation and there by to calculate the respiratory quotient (R.Q.) values of the
control and phenthoate exposed foot, mantle and hepatopancreas tissues of the snail *P. globosa*.

d. To measure the oxidation of selected fatty acids by the mitochondria from the control and phenthoate exposed foot, mantle and hepatopancreas tissues of the snail *P. globosa*.

e. To study the impact of different doses of phenthoate in the control and phenthoate exposed snail tissues lipase and phospholipase activities to identify whether these two key enzymes act as biomarkers to assess the toxicity of phenthoate in experimental models.