SUMMARY
1. The experimental fish, common carp, *Cyprinus carpio*, having a great commercial value were collected from the local fish farm (previously untreated with any pesticide) and adapted to laboratory conditions. The entire data of this investigations on energetics of fish in relation to phosphamidon toxicity was analysed with special emphasis on body-size differences in major carp. The small fish weighs $5 \pm 2$ g and the large fish $15 \pm 2$ g.

2. Studies involving evaluation of toxicity were conducted to determine the LC$_{50}$ values were found to be 30.44 ppm for small fish and 38.73 ppm for large fish. From the LC$_{50}$ values of phosphamidon approximately one thirds value of LC$_{50}$ (i.e. 10 ppm) was chosen as the safe sub-lethal concentration of phosphamidon, for the following physiological and biochemical analysis of this investigation.

3. Time course in the rate of oxygen consumption and opercular activity of the common carp *Cyprinus carpio* during sub-lethal concentration of phosphamidon were studied separately for the small and large fish for a period of 30-days, besides freshwater without phosphamidon as control. O$_2$ consumption and opercular activity in both small and large fish registered an initial increase in 24-hrs exposure period. This initial
increase in $O_2$ consumption and opercular activity of the carp at 24-hrs might be attributed to increased locomotary activity arising out of the animal's tendency to escape from the stress medium. This situation is termed as 'escape reaction' of the animal. Later there is a gradual decline in $O_2$ consumption and opercular activity in 7-day period followed by maximal decrease at the middle period (15-day). But during later half of 30-day exposure period the oxygen consumption and opercular activity raised at the 20-day exposure period from its earlier suppression, reached nearer to the control medium at the 30-day exposure period. Indicating the capacity of both sizes of this fish to recover from the sub-lethal exposure of phosphamidon. Thus the sub-lethal concentration (10 ppm) of the pesticide could cause these physiological systems ($O_2$ consumption and opercular activity) to oscillate outside its normal range of variations, mostly suppressive, yet with time (within 30-days). The $O_2$ consumption and opercular activity could show indications of its return to normal state without suffering lasting effects leading to the maintenance of homeostasis during phosphamidon exposure.

4. The haematological parameters like RBC number and the rate of heart beat were found to be higher in the small fish when compared to their larger counterparts.
Just like the \( \text{O}_2 \) consumption and opercular activity, the RBC number and heart beat in both to exposure. RBC number and the rate of heart beat in both small and large fish registered an initial increase in 24-hrs exposure period. Later there is a gradual decline in RBC number and rate of heart beat in 7-day period followed by a maximal decrease at the middle period (15-day). This showing decrease of the heart beat known as Brody Cardia could be taken as a very good indicator of toxic stress. But in the later half of 30-day exposure period, the RBC number and rate of heart beat raised at the 20-day exposure period from its earlier suppression, reached nearer to the control medium at the 30-day exposure period. Thus there is a linear relationship between the \( \text{O}_2 \) consumption and opercular activity with that of RBC number and heart beat.

5. There is an initial increase in blood glucose with a concomitant decrease in liver-glycogen at the 24-hrs sub-lethal exposure of phosphamidon in both small and large individuals of this common carp \textit{Cyprinus carpio}. Later, from this initial elevation the blood glucose dropped but liver-glycogen raised concomitantly at 7-day period. From 7-day there is a steep and maximum decline in blood glucose at 15-day period. But in the later half of the exposure period the blood glucose
raised at 20-day period and reached nearer to the normal at 30-day exposure period. From 7-day there is a maximal increase in liver-glycogen at 15-day period and decreased at 20-day period and reach nearer to the normal at 30-day exposure period, and bears an inverse relationship with each other. Thus there is a inverse relationship between blood glucose and liver-glycogen. This suggests that the blood glucose is derived from hepatic glycogenolysis in both the sizes of this carp to meet the higher energy demands during phosphamidon exposure.

6. The glycogen utilization in both red and white muscles also increased significantly in this fish suggesting stepping up of glycolysis during phosphamidon exposure, but the glycogen decrease hence its utilisation in white muscle is significantly greater than in the red muscle in both sizes of this carp, suggesting that white muscle is predominantly glycolytic in its metabolism as it has metabolised to a greater extent glycogen in this fish and further the red muscle somewhat like liver, might have supplied glycogen to the white muscle as it is reflected in relatively greater rate of glycolysis in white muscle during the phosphamidon exposure. Thus, the carbohydrate metabolism stepped up in small and large
individuals of this common carp, *Cyprinus carpio* during phosphamidon exposure to meet the higher energy demands and that the hyperglycemia observed in this fish could be considered as a good indicator of the pollutional stress induced by the pesticide, phosphamidon.

7. In the present investigation phosphamidon induced marked changes in AchE activity (acetylcholinesterase activity) in both small and large fish. AchE activity is the target enzyme of the organophosphorus pesticide phosphamidon, the inhibition or suppression in AchE activity, resulted in the concomitant accumulation of Ach content in different tissues of this carp. As the AchE enzyme was phosphorylated because of phosphamidon, as result of inactivation in AchE leading to inhibition in hydrolysis of Ach into choline takes place. The differential inhibition of AchE activity in different tissues of carp during phosphamidon exposure is in the order of brain white muscle gill red muscle intestine kidney liver etc., thus brain tissue exhibited maximal inhibition in AchE activity suggesting that it is more sensitive to organophosphate pesticide like phosphamidon. Hence present investigation on brain AchE activity appeared to be a better indicator of phosphamidon toxicity to fish. Thus inhibition in AchE activity reflected the environmental
poisoning by phosphamidon. But recovery in AChE activity at 30-day sub-lethal exposure period is indicative of restoration towards normalcy.

8. Amongst the adenylate nucleotides, the ATP concentration was much higher than the AMP concentration with ADP having intermediate level between ATP and AMP, in all the tissues like gill, kidney, intestine, brain, liver, muscle (red muscle and white muscle) in small and large individuals of the fish, _Cyprinus carpio_ in control medium (freshwater without phosphamidon). The high concentration of ATP and low concentration of AMP resulted in the fully charged adenylate system, indicates prevalence of high energy charge. The energy charge \( \frac{(ATP+\frac{1}{2}ADP)}{(ATP+ADP+AMP)} \) was found to be greater in the order kidney > brain > gill > intestine > liver. Red muscle > white muscle in both small and large fish.

9. From their levels in control medium, the energy rich nucleotide concentrations like ATP and ADP and energy charge level increased in all the tissues in both the size groups of the fish during 24-hrs exposure period, indicating availability of high energy charge in tissues to meet the increased locomotary activity arising out of the fish escape reaction during initial exposure of phosphamidon.
During subsequent sub-lethal exposure periods of phosphamidon like 7-day, 15-day, the energy rich nucleotides and the energy charge registered a significant decrease in all the tissues, whereas the energy poor nucleotide AMP registered a corresponding elevation exhibiting an inverse relationship between ATP and AMP levels.

The % decline in these energy rich nucleotides and energy charge but the % elevation in AMP concentration are maximal at the 15-day sub-lethal exposure period of phosphamidon, is suggestive of the decreased rate of TCA cycle with impairment of oxidative metabolism at mitochondrial level ultimately leading to depression in cellular energy in the form of low ATP production and availability of low energy charge. This inhibition in the cellular energy process might be due to the accumulation of des-methyl phosphamidon, the toxic metabolite of phosphamidon in the liver of this carp.

Interestingly in all the tissues in both small and large fish when the ATP level is higher the ATPase activity is decreased significantly this indicates the hydrolysis of ATP by the activity of ATPase releasing ultimately the greater amount of AMP which might be the cause for the least energy charge in the tissues. Initially during 24-hrs exposure the ATPase
activity was found to be decreased, then increased at 7-day, and maximally elevated at 15-day, later in the second half of exposure, the ATPase activity was decreased through 20-day period and reached nearer to the normal level of ATPase activity at 30-day exposure period. But the variations in ATP and energy charge are diametrically opposed to the variations observed in ATPase activity and AMP concentration in all the exposure periods of phosphamidon. Thus there is an inverse relationship between AMP and energy charge and ATP and ATPase activity.

12. During the 30-day exposure period all these tissues exhibited a fairly good level of recovery in the concentration of ATP, ADP, AMP, ATPase activity and energy charge, ultimately reaching nearer to the levels of adenylate nucleotides and energy charge of the control medium. This might be due to disappearance of des-methyl phosphamidon through the activation and enhancement of detoxifying enzymes in the liver of this carp. Thus the sub-lethal concentration (10 ppm) of the pesticide phosphamidon could cause these energy parameters like ATP, ADP, AMP, ATPase activity and energy charge to oscillate outside their normal range of variations mostly suppressive, yet with time (in 30-days) these energy nucleotides and the energy
charge could return to almost normal level with fairly very good amount of recovery without suffering lasting effects as exhibited by this common carp *Cyprinus carpio*. Hence it could be safely inferred that phosphamidon at sub-lethal concentration does not seem to affect the regulatory mechanisms involved in energy relations of this carp, *Cyprinus carpio*.

13. There is a differential toxicity of phosphamidon amongst the tissues of this carp, *Cyprinus carpio*. Hence the phosphamidon toxicity is found to be tissue-specific. The % depletion in energy rich nucleotides like ATP, ADP, AMP, energy charge and ATPase activity is significantly greater in the order of kidney > brain > gill > liver > intestine > red muscle > white muscle.

Further, the % recovery in these adenylate nucleotides ATP, ADP, AMP and ATPase activity and energy charge at 30-day exposure period of phosphamidon followed the same trend as given above amongst different tissues of the fish. This suggests that mostly osmoregulatory tissues like kidney, gill and intestine became more sensitive, hence affected but highly recoverable than the non-osmoregulatory tissues like brain, liver, red muscle and white muscle, to phosphamidon toxicity.

Next to brain, gill is involved and affected to the highest extent. This is because gills are the
most efficient and remarkable respiratory organs of
the fish and insecticides gain entry largely through the
gills of fishes hence the first organs to be affected
by the pesticide like phosphamidon of the present study.
Following the gill, liver tissue is found to be affected
to a greater extent. It might be possible because
liver is an important metabolic centre for a variety of
interconversions and storage of food stuffs. More
specifically during phosphamidon exposure, the accumulation
of des-methyl phosphamidon hence detoxification
mechanisms might be operating to reduce the phosphamidon
toxicity in the liver of this fish. Next to liver,
intestine is involved and the role of intestine is less
in the freshwater medium. Hence intestine is least
affected during phosphamidon toxicity when compared
to the other osmoregulatory tissues like kidney and
gill. Therefore, the variations of the parameters during
phosphamidon exposure as far as osmoregulatory tissues
are concerned, are maximal in the kidney and minimal
in the intestine, the gill occupying the intermediate
position. Following the intestine, muscle (Red followed
by white) tissue is affected to a greater extent.

These above variations could be corroborated
with the physical observation of this carp in sub-lethal
exposure of phosphamidon producing loss of muscular
activity and equilibrium, resulting out of the
enhancement of anaerobic metabolism, to compensate the concomitant reduction in oxidative metabolism. As red muscle is relatively more oxidative than the white muscle, the red muscle is affected to a greater extent than the white muscle in this carp. Thus aerobic tissues like kidney, brain, gill, liver, are very much affected but at the same time very much recovered than the anaerobic tissues like red muscle and white muscle during phosphamidon toxicity in *Cyprinus carpio*.

15. The findings of this investigation also indicate that the phosphamidon toxicity to this commercial fish, *Cyprinus carpio* is not only tissue-specific but also found to be profoundly influenced by the size of the fish. Hence, there appears to be a differential toxicity of phosphamidon based on size of this carp. Thus during sub-lethal exposure of phosphamidon the above mentioned physiological and biochemical systems are affected significantly to a greater extent and the extent of utilization of metabolic energy through energy reserves it meet the high energy demands during phosphamidon stress is found to be greater in the case of small fish than the larger. But the capacity of this carp to recover in these physiological and biochemical parameters towards the end of phosphamidon exposure i.e. at the 30-day exposure period is
interestingly found to be much more in larger fish than the smaller. Thus, relative to large, small individuals of *Cyprinus carpio* exhibit significantly lower LC$_{50}$ values, greater % initial elevation in O$_2$ consumption greater initial % decline in liver glycogen and greater initial % increase in muscle glycogen, and greater initial % elevation in energy rich nucleotides ATP, ADP and energy charge and greater initial % decline in energy poor nucleotide like AMP, ATPase activity in all the tissues like gill, kidney, intestine, brain, liver, red muscle and white muscle. Further, the small fish relative to large has registered a greater maximum suppression in O$_2$ consumption, maximum % suppression in blood glucose, maximum % elevation in liver glycogen, maximum % suppression in energy rich nucleotides like ATP, ADP, and energy charge and maximum % elevation in energy poor nucleotide like AMP, ATPase activity during 30-day exposure of phosphamidon. Thus the larger the fish *Cyprinus carpio* is, the more of a pesticide phosphamidon is required to have an influence on it. And thus, through this investigation, larger individuals of *Cyprinus carpio* are found to be less sensitive and susceptible but more resistant, and recoverable to phosphamidon toxicity than the smaller ones, indicating ultimately that the large individuals of *Cyprinus carpio* are more efficient pollutionally with reference to phosphamidon than their smaller counterparts.