STUDIES ON HAEMATOLOGICAL, BIOCHEMICAL AND HISTOPATHOLOGICAL CHANGES IN MALATHION POISONING IN POULTRY

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No part of the thesis has been submitted for any other degree or diploma or has been published. Published part has been fully acknowledged. All the assistance and help received during the course of the investigations have been duly acknowledged by him.

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LIST OF CONTENTS

Number	Content	Page No.
1	INTRODUCTION	1 - 4
2	REVIEW OF LITERATURE	5 - 20
2.1	Body weights	5
2.2	Haematological studies	7
2.3	Biochemical Studies	12
2.3.1	Serum Glutomic Pyruvic transaminase activity	12
2.3.2	Serum cholinesterase activity	14
2.4	Gross and Histopathological changes	17
3	MATERIALS AND METHODS	21 - 36
3.1	Haematological Investigations	23
3.1.1	Packed cell volume	23
3.1.2	Haemoglobin estimation	24
3.1.3	Total erythrocyte count	26
3.1.4	Total leucocyte count	27
3.2	Biochemical Investigations	28
3.2.1	Serum glutamic Pyruvic transaminase	29
3.2.2	Serum cholinesterase	32
3.3	Gross and Histopathological examination	36
4	RESULTS	37 - 61
4.1	Toxin consumption	37
4.2	Mortality pattern	39
4.3	Body weights	39

Number	Content	Page No.
4.4	Haematological Investigations	42
4.4.1	Packed cell volume	42
4.4.2	Haemoglobin	47
4.4.3	Total erythrocyte count	47
4.4.4	Total leucocyte count	48
4.4.5	Differential count	48
4.5	Biochemical Investigations	49
4.5.1	Serum glutamic pyruvic transaminase activity	49
4.5.2	Serum cholinesterase activity	50
4.6	Gross changes	50
4.7	Histopathological examination	53
4.7.1	Liver	53
4.7.2	Kidneys	54
4.7.3	Heart	56
4.7.4	Intestine	58
4.7.5	Testis	60
5	DISCUSSION AND CONCLUSIONS	63 - 77
6	SUMMARY	78 - 83
	LITERATURE CITED	84 - 91
	VITA	92

LIST OF TABLES

Table No.	Title	Page No.
1	Experimental Plan ..	22
2	Total amount of toxin consumed by the birds in different groups	38
3	Weekly body weight in kilograms of birds fed malathion for short duration (twentysix to thirtythree days)	40
4	Weekly body weight in kilograms of birds fed malathion for long duration (seventyfive days)	43
5	Haematological changes in birds fed malathion for short duration (twentysix to thirty three days)	45
6	Haematological changes in birds fed malathion for long duration (seventy five days)	46
7	Serum glutamic pyruvic transaminase and cholinesterase activity in birds fed malathion for short duration (twentysix to thirtythree days)	51
8	Serum glutamic Pyruvic transaminase and cholinesterase activity in birds fed malathion for long duration (seventyfive days)	52

LIST OF ILLUSTRATIONS

Figure No.	Description	Page No.
1.	Calibration curve for haemoglobin	28
2.	Calibration curve for pyruvic acid	31
3.	Calibration curve for Acetyl choline	35
4.	Weekly body weights in group I, II, III, IV and V fed malathion for short duration (twentysix to thirtythree days)	41
5.	Weekly body weights in group I, II and III fed toxin for long duration (seventy five days)	44
6.	Liver (Group II 33 days): Hepatic cords disorganisation, congestion of the simusoids, portal triads with focal liver cell necrosis. H & E x 64.	55
7.	Liver (Group V 26 days): disorganisation of hepatic lobular pattern, severe congestion and distortion of the sinusoids focal liver cell necrosis and prominent kupffer cells. H & E x 64.	55
8.	Kidney (Group II 33 days): Extensive interstitial haemorrhages, degenerative changes and exfoliation of the epithelium lining the tubules and increased cellularity of the glomeruli H & E x 64.	57
9.	Kidney (Group V 26 days): Extensive interstitial haemorrhages coagulative necrosis and desquamation of the tubular epithelium and necrosis glomeruli and tubules. H & E x 128.	57

Figure No.	Description	Page No.
10.	Intestine (Group II, 75 days): Epithelium showed necrosis, sloughing and mononuclear cell infiltration the inflamation extended upto muscularis mucosae. H & E x 64.	59
11.	Intestine (Group III, 75 days): The laminar epithelium was extensively affected leading to necrosis sloughing and mononuclear cell infiltration. The inflamation extended upto muscularis mucosae, which showed coagulative necrosis. H & E x 64.	59
12.	Testis (Group II, 75 days): The seminiferous tubules lined by epithelium were normal and spermatogenesis was also normal. Well developed spermatozoa were also found in all the tubules. H & E x 128.	62
13.	Testis (Group V, 26 days): Single layer of spermatogonial cells with degenerative changes, increased interstitial tissue and absence of spermatogenesis. H & E x 64.	62

LIST OF ABBREVIATIONS

@	At the rate of
b.wt.	Body weight
°C	Degrees centigrade
C.C	Cubic Centimetre(s)
E.C	Emulsifiable concentrate
gm.	Grams
hrs.	Hours
i.e.	That is
kg.	Kilograms
µ	Microns
M	Molarity
mg	Milligram(s)
ml	Millilitre(s)
MLD	Minimum lethal dose
mM	Millimoles
µM	Micromoles
MM^3	Cubic millimetre(s)
N	Normality
nm	Nannometer(s)
%	Percentage
PCV	Packed Cell volume
ppm	Parts per million
RBC	Red blood cells
r.p.m.	Rotations per minute
SGPT	Serum glutamic pyruvic transaminase
viz.	Namely
WBC	White blood cells

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ABSTRACT

The environment is constantly exposed to possible hazards of pollution which is inevitable in present day industrialisation and agricultural practices with quarter million of chemicals produced annually and several thousands used to control pests in Agricultural practices. Man and animals stand great risk of toxicity and the toxicity ranges from short term disturbances of homeostasis to diseases, carcinogenesis and finally death requiring continuous monitoring of production of chemicals used for the control of pests.

Malathion was selected to study the short term and long term effects. Fifty male cockrels of five weeks age group were reared in deep litter system and divided into

five groups. Each group consisting of ten birds. The birds in group II were fed with malathion 50 mg/kg b.wt, group III with 100 mg/kg b.wt, group IV 150 mg/kg b.wt. and group V 200 mg/kg b.wt. daily. Five birds from Group I, II and III were sacrified after thirtythree days in the experiment to study the short term effects. The remaining were sacrificed after seventyfive days in the experiment to study the long term effects. Birds in group I served as control. Weekly body weights, mortality pattern, haematological, biochemical investigations and histopathological changes were carried out.

The birds in group IV and V showed nervous symptoms, reduced feed intake, pale comb and started dying from the 1st week and hence these two groups were terminated from the experiment at the end of twentysix days.

The birds in group II fed at 50 mg/kg b.wt. over a period of thirtythree days consumed malathion ranging from 536.50 mg to 820.50 mg and the birds maintained over a period of seventy five days consumed malathion ranging from 2,819.25 mg to 3,322.50 mg.

The birds in group II maintained for thirtythree days and seventyfive days showed an increased body weight, but the gain in weights was not proportional to that of the control group. In general there was a reduction in

PCV ($P < 0.05$), haemoglobin ($P < 0.05$) and total RBC count (P 0.01) only. In birds maintained for seventy five days in addition to these changes an increase in total WBC count ($P < 0.05$), heterophilic count ($P < 0.01$) a decrease in lymphocytic count ($P < 0.05$) and an increase in eosinophilic count ($P < 0.01$) was observed when compared with control group. The serum glutamic pyruvic transaminase and cholinesterase activity was not affected in birds fed with toxin for thirtythree days however, in birds maintained for seventy five days of toxin a significant ($P < 0.01$) increase in SGPT activity and a significant ($P < 0.01$) decrease in cholinesterase activity was noticed.

The histological changes found in different organs in birds of (group II) maintained for thirtythree days were, slight congestion and mild degenerative changes in the hepatic cells. The kidneys were slightly congested associated with interstitial haemorrhages and degenerative changes in the tubular epithelium. The intestine presented a picture of hyperplasia of laminar epithelium with increased goblet cells. The changes in the same organs were prominent in birds maintained on seventyfive days. In addition to this mild degenerative changes in seminiferous tubules and spermatogonial cells of the testis were noticed.

The birds in group III were fed with malathion 100 mg/kg b.wt daily for thirtythree days consumed the toxin ranging from 1,053 mg to 1,599 mg and the birds maintained over a period of seventy five days consumed the toxin ranging from 3,864 mg to 5,316.50 mg.

Weekly body weights did not show any decrease in birds maintained for thirtythree days, however birds maintained over a period of seventyfive days showed significant ($P < 0.01$) decrease in their weekly body weights from sixth week onwards.

Packed cell volume, haemoglobin and total RBC counts in group III were similar to that of birds in group II, whereas the heterophilic and eosinophilic count was significantly ($P < 0.01$) increased lymphocytic count ($P < 0.01$) significantly decrease in birds maintained on toxin for thirtythree days. The PCV, haemoglobin and total RBC counts in group III, were similar to that of group II and the total WBC heterophilic and eosinophilic counts were significantly ($P < 0.01$)increased while the lymphocytic counts, monocytic and basophilic counts were significantly ($P < 0.01$) decreased in birds maintained on toxin for seventy five days. The serum glutamic pyruvic transaminase activity was increased ($P < 0.01$) and the cholinesterase activity was decreased ($P < 0.01$) in birds maintained on toxin for thirtythree days and seventyfive days of toxin feeding.

The histopathological changes which were observed in group II birds were well advanced in group III birds maintained for thirtythree days and seventy five days of toxin feeding. The changes included the distortion of liver lobular pattern followed by proliferation of mesenchymal cells, leading to form an attempt for early cirrhosis. The kidneys showed extensive degenerative changes like cloudy swelling and vacuolation of the tubular epithelium and proliferation of glomerula endothelium. The intestines showed inflamatory changes extending upto submucosa. The testicular changes included well advanced degenerative changes in seminiferous tubules and absence of spermatogonial cells and spermatogenesis.

Thus the administration of a higher dose of 100 mg/kg b.wt. over a period of seventy five days was proved to be definitely toxic than that of 50 mg/kg b.wt. over a period of seventy five days based on haematological biochemical values and also histopathological changes.

The birds in group IV were fed with malathion 150 mg/kg b.wt. daily while the birds in group V were fed with toxin 200 mg/kg b.wt. The birds in group IV and V consumed the total toxin ranging from 489-2,010 mg and 100-2,140 mg respectively during the period of twenty six days in the experiment.

Significant ($P < 0.01$) reduction in weekly body weights was observed at the time of killing in between the group IV and V against the groups I to III. The PCV, haemoglobin total RBC counts lymphocytic counts and cholinesterase activity was significantly ($P < 0.01$) decreased and the total WBC count heterophilic count, eosinophilic count SGPT activity was significantly ($P < 0.01$) increased in group IV and V compared with group I to III.

The histopathological changes noticed in liver of group IV birds were, extensive haemorrhages, congestion, distortion of hepatic cords, and multiple liver cell necrosis. The kidneys showed extensive interstitial haemorrhages, increased cellularity of glomeruli and extensive degenerative changes of the tubular epithelium. The intestinal changes include extensive sloughing of the mucosa and necrosis extending upto muscularis, mucosa. The epithelium lining the seminiferous tubules showed pyknosis and karyorrhexis of the nuclei and the absence of spermatogonial cells and spermatozoa. These changes were much more severe in group V birds. Based on these findings it can be concluded that feeding of malathion 150 mg/kg b.wt and 200 mg/kg b.wt was proved to be toxic within twentysix days only.

CHAPTER - I

INTRODUCTION

Pesticides have brought tremendous benefits to mankind by increasing food and fibre production. It protects crops from damaging pests and weeds. Recent surveys by Food and Agricultural Organisations of the United Nations confirmed the startling fact that even today more than one third of the potential annual world harvest is destroyed by weeds, plant diseases, pests and insects. The financial loss in 1975 was estimated to be one billion dollars. According to pesticide Association of India the annual estimated loss due to pests and other diseases was found to be Rs.5000/- crores (Shanker, 1983).

Pesticides consumption in India has rapidly increased during the past three decades and the actual consumption during 1980-81 was estimated to be about 80,000 tonnes (Sastry, 1983). Pesticides are most effective against a wide variety of Agricultural and animal pests. Use of pesticides is inevitable owing to the increasing damages caused by the insect pests to the Agricultural crops to achieve consistent high yields and high food quality.

The problem of Food stuff pollution by the use of pesticides is the most talked controversy. Infact it is one of the most serious problems of the present era. As

long as pesticides are used in amounts sufficient to control the pest organisms, majority of the existing pesticides have no adverse effect on man, animal or environment. The investigations of Food and drug Administration, U.S.A. showed that the levels of residues of some pesticides in all kinds of food were very low and do not cause any hazard to human health. In excessive doses they may prove harmful. Injudicious use of pesticides by inexperienced hands has resulted in some hazards, health problems and above all the most serious environmental pollution. Synthetic organic pesticides are powerful pollutants. They have many side effects when released into the environment. They possess a high degree of toxicity with regards to wild life and human beings (Jabeen, 1984).

Broadly pesticides can be classified into three groups viz., 1. Chlorinated hydrocarbons, 2. Organophosphorus compounds and 3. Carbamates. Organochlorine pesticides are highly stable in the environment. They are transmitted from contaminated soil to water and plants. They assimilate and are retained in body fat of animals and are transferred to milk in lactating animals. DDT, BHC, Lindane and others belonging to this group are the widely used insecticides and there is evidence that detectable residues will be present

throughout the ecosystem for some time even after their usage is stopped. Organophosphorous and carbamates are mostly used for their selective and high toxicity. The residue of these pesticides dissipates very rapidly from plants and animal tissues.

During recent years, many new organophorous compounds under different trade names are introduced in the field viz., Cythion (Malathion), Neguvon, Thiram etc, For the present study malathion is selected, because it is widely used in Agricultural operations for pests control. Malathion is successfully used on various agricultural crops, ornamental plants, animals and stored grain warehouses. It is claimed by the manufacturers that malathion does not leave any residue on grains or crops and even if residue is present it is non toxic to living organisms (Annonmous, 1960). However, it was found that spraying of grains and agricultural crops, leaves some residues of toxin. This residual toxin was very low in quantity but when this toxin was consumed continuously by the living organisms, it may produce cumulative effect on living system. Hence, it is felt that the evaluation of toxicity of malathion is quiet essential in birds when they continuously consume the residual graded doses over short and long periods.

Review of literature showed that very little information is available on haematological, biochemical and histopathological changes in acute and chronic poisoning of malathion in birds. Therefore this study was carried out to determine the effects of malathion on body weight gains, haematological, biochemical (SGPT and cholinestrase) and histopathological changes at a higher and lower dosage in poultry.

CHAPTER - II

REVIEW OF LITERATURE

2.1 Body Weights

Golz and Shaffer (1955) reported that there were no deaths and chickens appeared normal after consuming feed containing malathion @ 100 and 1000 ppm for ten weeks. They reported that at 5000 ppm level (450 mg/kg/day) the chickens showed definite signs of toxicity such as retardation of growth slow feather development, soft droppings, weakness of legs and paralysis.

Peter et al. (1961) studied the toxic effects of guthion by incorporating guthion in chick starter feed at levels ranging from 9.5 ppm to 2,177 ppm, fed to day old chicks recorded a decrease in weight gain at 306 ppm level within a week and the decrease in weight gain became progressively more severe as the concentration of guthion was increased.

Udea and Nishimura (1963) found that mice fed with 40 ppm of sumithion had higher weight gain than the controls. The average weight gain was 294 gms in mice for a period of 111 days as against the group fed with normal diet.

Mc.Donald et al. (1964) have shown that upto 100 ppm malathion in the diet of chickens did not affect the growth rate and feed conversion and upto 15 ppm in feed did not affect the hatchability of eggs.

Misu *et al.* (1966) studied the effect of feeding different levels of sumithion in rats viz: 500, 250, 125, 63 and 32 ppm and found that rats fed with 500 ppm of sumithion only showed a significant depression of growth rate when compared to rats in other groups.

Foster (1968) reported that ingestion of powdered standard laboratory ration containing DDT or malathion at 100 and 200 ppm levels for 42 days was neither toxic to rats nor did it effect on weight gains.

Rehfeld *et al.* (1969) showed that malathion when fed at 1000 ppm level to one day old chicks was safe and non toxic. Feeding 2,500 ppm level resulted in decreased growth rate with little mortality and feeding at 5,000 ppm level resulted in mortality in day old chicks within 19 days.

Purushotham (1971) studied the toxic effects of feeding sumithion at a dose levels of 10, 100, 1000 and 5000 ppm for 28, 14, and 10 days in chicks. The studies have shown that day old chicks showed no adverse effects upto 10 ppm. At 100 ppm level chicks showed a significant decrease in weight gain and feed consumption.

Mc.Collister *et al.* (1974) carried out acute and long term oral toxicity studies of chlorpyrifos in dogs and rats. They have found that 0.1 and 0.03 mg chlorpyrifos per kg/day fed in diet for 2 years produced no significant

change in body weights and feed intake. Even animals receiving 3.0 mg/kg/day, did not show any effect other than reversible depression of cholinesterase activity.

Reddy (1975) reported reduced body weight gains in calves fed with 1, 2 and 4 mg/kg body weight ekalux for 21 days.

Uppal and Singh (1981) investigated the toxic effects of various levels of malathion (500, 1000, 2500 and 5000 ppm mixed in feed) fed to local strains of white leghorn chicks for four weeks. The results have shown that a level of 500 ppm in feed was considered safe to chicks without affecting their growth and a concentration of 2,500 and 5,000 ppm were found to be highly toxic and lethal within a week's time of feeding.

Devaney et al. (1982) tested aqueous suspensions of malathion, stirofos, ravap and carbaryl formulations as dips for control of northern fowl mite, Ornithonyssus sylviarum in caged white leghorn hens. No significant change in percent hen day egg production, feed consumption and body weights was observed.

2.2 Haematological Studies

Srivastava et al. (1960) investigated the effects of feeding DDT, BHC, dieldrin and malathion in chicks.

There was significant decrease in hamoglobin and erythrocyte content, which was noticed on fifth day of feeding these insecticides.

Rehfeld et al. (1969) reported that feeding of 10, 100, and 1000 ppm of malathion to chicks for 10 days did not produce any significant change in haemoglobin values.

Petrichev and Lazarov (1970) observed erythrocytopaenia, leucocytosis and decreased packed cell volume in sub-acute organophosphate insecticide toxicity in rabbits.

Earl et al. (1971) investigated the toxicity effects of diazinon at doses of 0, 2.5, 5.0, 10 and 20 mg kg/day and 0, 1.25, 2.5, 5.0 and 10 mg/kg/day respectively for 8 months in dogs and swine. No marked changes were noticed in haemoglobin values, total erythrocyte and total leucocyte counts.

Bello and Torbet (1972) recorded erythrocytopaenia, leucocytosis and decreased packed cell volume in Pony foals which were acutely intoxicated orally with shell SD 15803 (an organophosphorous anthelmentic).

Gupta and Paul (1972) studied the effects of feeding 0.08% (800 ppm) and 0.16% (1600 ppm) malathion sprayed feed to chickens for one month on haematological parameters. They have reported that there was no significant change in

RBC count, packed cell volume, erythrocyte sedimentation rate and blood clotting time.

Hothi and Kwatra (1972) observed decrease in haemoglobin, erythrocytopaenia, leucocytosis and decrease in packed cell volume in buffalo calves fed with malathion and aldrin.

Snow and Watson (1973) reported an increase in haematocrit values from 10-30% in dogs showing moderate to severe toxic symptoms on administration of parathion and dichlorovos.

Mc.Collister *et al.* (1974) studied the acute and long term toxic effects of orally administered chlorpyrifos in dogs and rats and found that 0.1 and 0.03 mg chlorpyrifos/kg/day fed for 2 years produced no significant change in body weights, feed intake and haematological values.

Vadlamudi (1974) recorded erythrocytopaenia, hypohaemoglobinaemia, decreased packed cell volume, leucocytosis and slight accelerated erythrocytic sedimentation rate in sub-acute malathion and sumithion poisoning in buffalo calves.

Reddy (1975) observed that administration of 1, 2 and 4 mg/kg ekalux in buffalo calves for 21 days revealed

that there was no statistically significant adverse effect on total red blood cell count, total white blood cell count, packed cell volume and red cell fragility.

Gupta and Paul (1977) investigated the toxic effects of feeding malathion at 41.66 ppm in buffalo calves for 28 days. They found that at this level of toxin feeding resulted in decreased haemoglobin concentration, decrease in packed cell volume, erythrocytopaenia but no significant change in leucocyte count.

Gupta and Paul (1978) reported that the effect of feeding 0.5 mg/kg/day malathion for one year in buffalo calves resulted in decreased haemoglobin values, packed cell volume and erythrocyte count.

Gupta and Paul (1978) showed that the effect of malathion sprayed at 0.5%, 1.0 and 5.0% in buffalo calves for 28 days did not produce any significant change in packed cell volume erythrocyte count and leucocyte count. But 1.0% and 5.0% levels produced a significant decrease in haemoglobin concentration.

Golbs *et al.* (1978) reported that by feeding parathion and methyl carbaryl either alone or together in various combinations in rats did not show any significant change in haemoglobin values, and packed cell volume.

Kalinowska et al. (1978) observed reduction in PCV and haemoglobin values in sheep administered a single oral dose of 100 mg/kg dermafos directly into the rumen of sheep.

Mitra et al. (1978) showed that calves intoxicated with single oral dose of 180 mg/kg of metasystox died showing typical symptoms of muscarinic and nicotinic action. There was profuse salivation, lacrymation and respiratory rate increased initially followed by respiratory distress, dyspnea bradycardia and decreased pulse rate, while the haematological picture remained unchanged.

Siczek (1980) reported a reduction in haemoglobin values and packed cell volume in rats when administered orally a single dose of chlorfenvinphos at the rate of 0.5 mg/kg body wt (LD 50).

Baimuradov et al. (1981) observed decrease in red cell count in acute toxicity of ethaphos in rabbits.

Chandra et al. (1981) observed decrease in plasma cholinesterase activity and erythrocyte count in one year old fowls by feeding malathion @ 600 mg/kg b.wt.

Buteiko (1983) reported leucocytic changes like lymphopenia, heterophilia, eosinophilia in pheasants following acute toxicity of carbophos.

Gupta et al. (1985) observed that feeding of nitrofen as a drench to bovine calves at a dosage of 0.25 ml, 0.5 ml 1.5 ml/kg b.wt. developed toxic symptoms. The haematological data indicated that nitrofen toxicity produced leucocytosis associated with neutrophilia and corresponding lymphocytophaenia.

Mandal and Lahiri (1985) carried out experimental feeding of fenitrothion in Blue Rock pigeons for more than 2 weeks. They have observed that fenitrothion poisoning had caused progressive reduction in total RBC count, haemoglobin content, ESR, hematocrit and mean corpuscular hemoglobin concentration followed by subsequent increase of total WBC count. Marked heterophilia, eosinophilia together with lymphopenia, monocytopenia, basopenia and gross cellular distortion of heterophils and eosinophils were also noticed.

2.3 Biochemical Studies

2.3.1 Serum Glutamic Pyruvic Transaminase Activity

Wright et al. (1966) reported an elevation in serum transaminase activity in Cattle poisoned with coumaphos.

Earl et al. (1971) investigated the toxicity effects of diazinon at a dosage of 0, 2.5, 5.0, 10 and 20 mg/kg/day and 0, 1.25, 2.5, 5.0 and 10 mg/kg/day for 8 months in

dogs and swine respectively. There was no significant change in serum amino transferase activity.

Increase in transaminase activity was reported by Snow and Watson (1973) in acute toxicity of dichlorovos in dogs.

Michael (1974) reported that feeding of diets containing graded levels of DDT, polychlorinated biophenyl, malathion and mercuric chloride to male quails for 12 weeks resulted in significant increase in amino transferase activity from 2nd week onwards.

Decrease in serum glutamic pyruvic transaminase was noticed in oral administration of fenchlorophos (0.5 - 1 ppm) and heptachlor (1 ppm) to chicks for 4-8 weeks (Maniculea and Ciurgea, 1976).

Moustafa and El-Sherif (1976) observed a decrease in SGPT activity when neguvon was administered orally to sheep at a dosage of 150 and 200 mg/kg b.wt.

Uppal and Ahmad (1977) carried out acute and sub-acute toxicity studies of malathion at dose levels of 100, 50 and 20 mg/kg b.wt. in 12 buffalo calves. An increase in serum transaminases was seen in all the groups. The increase in serum transaminase levels was less marked with the dose of 50 mg/kg b.wt. than with 100 mg per kg b.wt.

Malik *et al.* (1977) investigated the SGPT activity in oral toxicity of hinosan in calves at a dosage of 4 and 8 mg/kg/day for 28 days. No significant change in SGPT activity was detected.

A decrease in SGPT following administration of mecadox 1 mg/kg orally to chicks for 28 days was observed by Maniculea *et al.* (1978).

2.3.2 Serum Cholinesterase Activity

Gupta and Paul (1971) investigated that administration of malathion at a dosage of 200, 400, 600 and 800 mg/kg b.wt. to desi birds, resulted in rapid inactivation of plasma and RBC cholinesterase activity and the degree of inhibition was related to the dose. Maximum inhibition occured in plasma and RBC cholinesterase levels (50 & 67%) respectively during 12-48 hrs.

Purushotham (1971) carried out the investigations of feeding sumithion at a dosage level of 0, 10, 100, 1000 and 5000 ppm in chicks for 28, 14 and 10 days. The cumulative dose of sumithion in groups fed with 10 ppm and 100 ppm for 4 weeks had effectively depressed the whole blood cholinesterase activity.

Srivastava and Parasar (1971) observed that administration of 50 ml of 5% malathion emulsifiable liquid in

birds had resulted in significant reduction in cholinesterase activity.

Vadlamudi and Paul (1973) observed cholinesterase inhibition in both blood and brain in acute toxicity of sumithion in mice.

Michael (1974) reported that male coturnix quails fed diets containing graded levels of DDT polychlorinated biphenyl, malathion and mercuric chloride for 12 weeks, showed a decrease in plasma cholinesterase activity. The decrease was proportional to the log dose of the respective agent.

Chawla et al. (1977) observed 50% inhibition in cholinesterase activity in blood in accidental out break of malathion poisoning in birds due to an inadvertent use of the insecticide for the control of ectoparasites.

Gupta and Paul (1977) carried out subacute toxicity studies of malathion in buffalo calves. They have reported inhibition of cholinesterase activity and the inhibition was more marked in RBC than in plasma. After stopping the administration of toxin the activity of cholinesterase returned to normal levels both in plasma and RBC.

Perry (1977) showed that intraperitoneal injection of 100 and 150 mg/kg b.wt. of malathion in mice produced

significant decrease in blood and brain cholinesterase activity.

Uppal and Ahmad (1977) carried out acute and sub-acute toxicity studies of malathion in buffalo calves. In acute studies with a single dose of 50 and 100 mg/kg b.wt. resulted in 38.7 and 21.3% depression of blood cholinesterase respectively. A daily oral dose of 20 mg/kg b.wt. for 21 days caused 17.6% inhibition in blood cholinesterase activity.

Gupta and Paul (1978) studied the effect of administering malathion as dermal spray for 4 weeks as recommended (0.5 and 1%) and at higher concentration (5%) on various enzymes in Babalus bubalis species. The higher concentration of 5% showed lethal effect after 2-3 exposures. The cholinesterase activity in both RBC and Plasma was inhibited in all the concentrations.

Vadlamudi and Paul (1979) studied the acute oral toxicity of malathion in buffalo calves. They have shown that malathion has produced greater inhibition of plasma cholinesterase activity than red blood cell cholinesterase activity. The extent of either plasma or red cell cholinesterase inhibition was not related to the degree of toxicity.

Chandra *et al.* (1981) showed that one of the toxic symptoms in malathion poisoning in one year old fowls was decreased plasma cholinesterase activity.

Gupta *et al.* (1981) reported that feeding of single minimal lethal dose of malathion to buffalo calves has resulted in significant depression of cholinesterase activity in RBC and Plasma.

Mount (1983) observed that feeding of 5 or 10 mg/kg/day phosmet or imidan for 7 days orally in goats resulted in decrease blood cholinesterase activity.

2.4 Gross and Histopathological Changes

Roger (1955) studied the effects of spraying a mixture of 12 lbs of parathion wettable powder in 300 gallons in water in calves and feeder lambs. Petechiae on heart, lymph nodes and throughout the body was found in necropsy.

Radeleff and Woodard (1957) observed haemorrhages of various sizes on heart, lungs, gastro intestinal tract of cattle and sheep fed with organosphosphorous insecticides.

Shaffer and Bobwest (1960) studied histopathological changes by feeding tetram at 4 ppm for 7-8 weeks in calves. No significant histopathological changes were found in lung, liver, heart, kidney, testis, adrenals, intestine, gall bladder, spleen, pancreas and oesophagus.

Cleveland and Treon (1961) studied the histopathology of different organs in acute phosdrin poisoning in rats. Toxic degeneration of liver, renal tubular epithelium and degeneration of epithelial cells lining ducts and acini of exocrine glands was observed.

Poloz and Poletskii (1965) observed pathological changes in different tissues by administering minimum lethal dose i.e. 65 mg/kg b.wt. demeton in birds. The main post mortem findings were hyperaemia of brain membranes, cerebral Oedema and hemorrhages in brain and sometimes petechial hemorrhages on the epicardium.

Misu et al. (1966) carried out toxicity studies in rats by feeding 32, 63, 125, 250 and 500 ppm of sumithion for 90 days. Histopathological examination of different tissues revealed no significant differences between the control and those fed with sumithion.

Vadlamudi and Paul (1973) conducted acute toxicity studies of sumithion in mice. On Necropsy examination no specific changes except generalised cyanosis was found.

Chawla et al. (1977) observed haemorrhages in liver, kidney, trachea, brain and ovary and degenerative changes in liver, kidney and myocardium during an accidental outbreak of malathion poisoning in birds.

Dikshith *et al.* (1978) found that exposure of male rats to parathion (2.6 mg/kg) lindane 17.6 mg/kg through oral intubation daily for a period of 90 days produced histological and biochemical alterations in the liver and testes. They were focal liver cell necrosis, but there were no histological changes in kidneys and epididymis.

Sarin and Saxena (1978) investigated that single intraperitoneal injection of quinalphos (15 mg/kg b.wt) produced severe pathological changes in the testis and liver in Indian desert Gerbil. The degenerative changes included testicular atrophy, reduction in tubular size and enlarged interstitium. In the spermatogenic cells necrosis and pyknosis was observed. Hepatic cells revealed cytoplasmolysis, vacuolation and necrosis. The nuclei of hepatocytes showed karyorrhexis and karyolysis.

Dikshith *et al.* (1979) reported that repeated application of methyl demeton (5 mg/kg/day) on the skin of male rats for a period of 15 days caused pathological changes in liver and there were no changes in skin, testes, epididymis kidney, brain and adrenal.

Chopra *et al.* (1980) studied the acute and chronic toxic changes of metasystox-R and fenthion in male chicks. Both the compounds produced degenerative changes in the nerve fibers of the sciatic nerve and spinal cord and

hyalinization of myofibrils. Degenerative changes in other tissues were also noticed.

Saxena and Sarin (1980) observed mild to severe testicular atrophy in Indian desert gerbil after repeated administration of thimet 0.6 mg/kg/day, intraperitoneally for 7 days. The testis also showed lack of spermatogenesis, sertoli cells were degenerated and lumen of the tubules was filled with oedematous fluid and debris matter. No change in leydig cell complex was observed.

Gupta et al. (1981) carried out histopathological studies in acute malathion poisoning in buffalo calves. The histopathological lesions includes severe congestion of intestinal mucosa and necrosis, degenerative changes in liver, haemorrhages in myocardium, kidney, spleen, epididymis and brain.

Reece (1982) studied the histopathological changes in acute poisoning of malathion in birds. He observed haemorrhagic proventriculus and oesophagitis and in few birds haemorrhages in ovaries and ceacal tonsils.

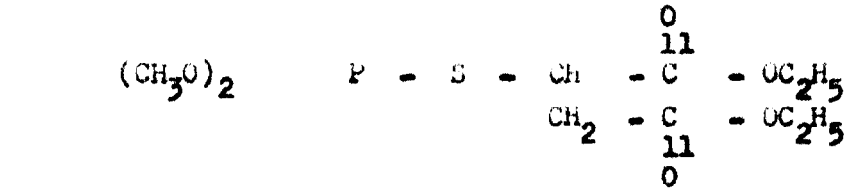
Wilson et al. (1982) reported histopathological lesions in sheep and swine which were acutely intoxicated with triorthocresyl phosphate. They were degenerative changes and focal necrosis of the liver in sheep, however such lesions were not seen in swine.

CHAPTER - III

MATERIALS AND METHODS

Fifty white leghorn cockrels about five weeks age were reared in deep litter system. Their weights were recorded and divided into five groups, with ten birds in each group having approximately equal weights. Malathion obtained from Cyanamid India Limited, was dissolved in distilled water in different dilutions viz: (i) 50 mg/ml; (ii) 100 mg/ml; (iii) 150 mg/ml; (iv) 200 mg/ml. Technical formula of malathion used chemical name: O, O dimethyl/ phosphoro dithioate of diethyl mercapto succinate.

Structural formula:



The birds in group I served as control, while the birds in Group II were fed orally with malathion 50 mg/kg b.wt., Group-III, 100 mg/kg b.wt., Group-IV 150 mg/kg b.wt. and Group-V with 200 mg/kg b.wt. daily (Table-I). All these groups were fed with commercial poultry ration purchased from local market. Weekly body weights were recorded in all the groups. It is proposed to sacrifice five birds after thirtythree days from each group. Blood was collected for haemalogical investigations. Serum was

Table 1. Experimental Plan

Groups	Treatment	Number of birds maintained for 33 days	Number of birds maintained for 75 days
Group I (Control)	Commercial feed	5	5
Group II	Malathion 50 mg/kg b.wt daily	5	5
Group III	Malathion 100 mg/kg b.wt daily	5	5
Group IV	Malathion 150 mg/kg b.wt	5	5
Group V	Malathion 200 mg/kg b.wt	5	5

collected for estimation of SGPT and serum cholinestrase. On postmortem examination liver, intestine, kidneys, Heart and testis were collected for detailed histopathological examination. The remaining birds were sacrificed after seventyfive days of feeding the toxin. Blood, serum and tissues were collected from these birds for carrying out the above mentioned investigations. The haematological and Biochemical data was analysed statistically by employing Anova Table (Pillai and Sinha, 1968).

3.1 Haematological Investigations

3.1.1 Packed cell volume

It was determined with the use of micro haematocrit method. Capillary tubes were filled upto 3/4 length with the blood and they were sealed at one end with the help of sealing wax. These capillary tubes were placed in Haematocrit centrifuge. The centrifuge was runned for 2-3 minutes at 3,000 r.p.m. There was clear separation of erythrocytes, above which was the buffy coat and next to this was the plasma. The column of erythrocytes was measured with the help of the scale and the values were expressed in percentage.

3.1.2 Haemoglobin estimation

To estimate haemoglobin Cyan methaemoglobin method by calibration of haemoglobin curve was followed (Wong, 1928).

Preparation of standard curve

Into a series of test tubes containing 8, 12, 16, 20 and 24 ml of 0.1% sodium carbonate solution, 0.02 ml. blood sample was added and mixed thoroughly. These solutions were equivalent to blood samples containing 1.00, 0.67, 0.50, 0.40 and 0.33 times that of the original sample respectively. Transmission of these solutions was measured at 540 nm in the Klett's Summerson's colorimeter against 0.1% sodium carbonate solution as blank set at zero. A graph was drawn relating to the transmission reading and the known concentration, from which directly the haemoglobin values in g% was read in the test sample (Fig. 1).

Procedure

To 8 ml of 0.1% sodium carbonate solution, 0.02 ml of blood was transferred with the help of haemoglobin pipette and the contents were mixed. The intensity of the colour was read against the 0.1% carbonate solution at 540 nm in a photoelectric colorimeter. The actual amount of haemoglobin was then calculated by refering to the haemoglobin curve.

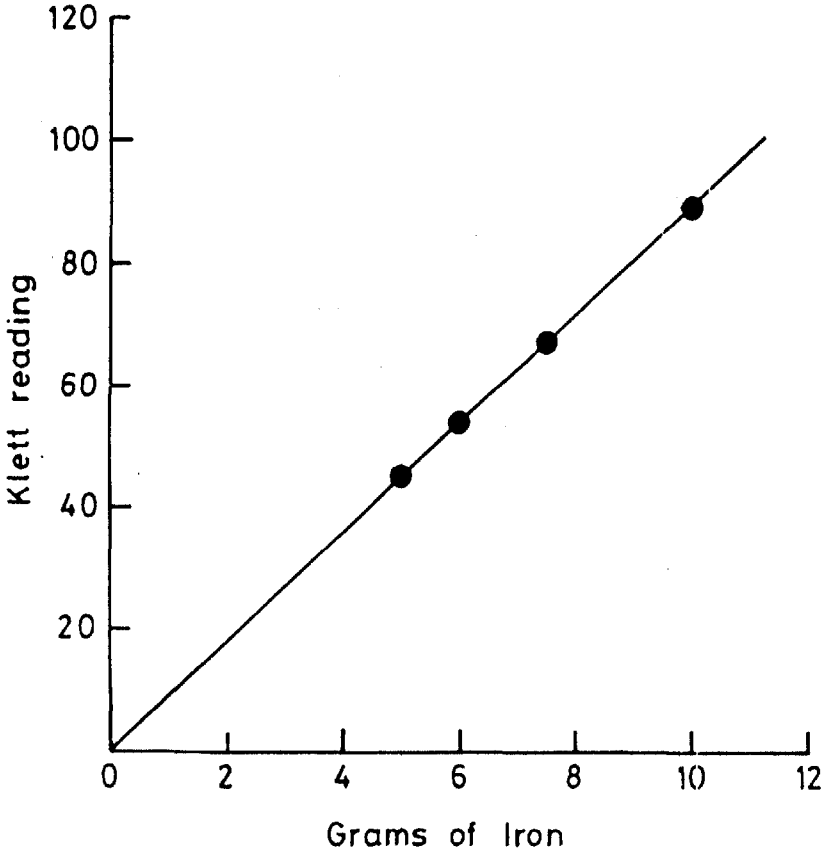


FIG. 1 CALIBRATION CURVE FOR HAEMOGLOBIN

3.1.3 Total Erythrocyte Count

Procedure

Blood was drawn from the vial upto the mark 0.5 in the RBC diluting pipette and the diluting fluid sucked upto 101 mark, and thus making a dilution of 1:200. The diluting fluid and the procedure used for counting erythrocytes in birds was Natt and Herrick's (1952).

Reagents required

1. Sodium citrate 2% solution - 4 ml.
2. Brilliant cresol Blue 0.1% in Ringers solution - 1 ml.
3. Buffered neutral formaline - 5 drops.

All the above 3 solutions were mixed and filtered before use.

Ringer's solution

Sodium chloride - 0.7%

Potassium chloride - 0.03%

Calcium chloride - 0.026%

Sodium bicarbonate - 0.003%.

Buffered Neutral formaline

30-37% Formaline	-	100 ml
Distilled water	-	900 ml
Sodium monophosphate	-	4 gm
Sodium phosphate dibasic anhydrous	-	6.5 gm

3.2 Biochemical Investigations

3.2.1 Serum Glutamic Pyruvic Transaminase (SGPT)

For estimation of SGPT, the method described by Rietman and Frankel (1957) was followed.

Principle

Tonhazy *et al.* (1950) described a procedure for the measurement of transaminase in tissue, whereby Oxaloacetate produced from alpha ketoglutarate, was converted to pyruvate with aniline citrate in a protein free supernatent fluid. The 2, 4 dinitrophenyl hydrazones were produced and the pyruvate hydrazone was extracted in toluene and colorimetrically measured in an alkaline solution. In the procedure described in this paper the precipitation of protein, conversion to pyruvate and the extraction have been eliminated.

Reagents used

1. Phosphate Buffer: 0.1 M pH 7.4: Mixed 420 ml. of 0.1M disodium phosphate and 80 ml of 0.1M potassium dihydrogen phosphate.

2. Pyruvate, 2 mM per liter: Dissolved 22.0 mg of sodium pyruvate in 100 ml. of phosphate buffer.

3. alpha ketoglutarate, 2 mM per liter, dl alanine 200 m M per liter (for GPT substrate). Placed 29.2 mg of

Keto glutaric acid and 1.78 gm. of dl alanine in a small beaker. Added 1 N sodium hydroxide until the solution was complete. Adjusted to a pH of 7.4 with sodium hydroxide, transferred quantitatively with buffer solution to a 100 ml volumetric flask and then diluted to the mark with the buffer solution.

4. 2, 4 dinitrophenyl hydrazine, 1 mM per liter: Dissolved 19.8 mg of 2, 4 dinitrophenyl hydrazine in 100 ml of 1 N hydrochloric acid.

5. Sodium hydroxide 0.4 N: 16 grams of sodium hydroxide was dissolved in 1 liter of distilled water.

Preparation of standard curve

To draw a calibration curve for pyruvic acid, different aliquots of pyruvic acid in seperate glass tubes ranging from 0.2 micromoles to 1 micromoles were taken in quantity 0.1 C.C, 0.2 C.C, 0.3 C.C, 0.4 C.C, 0.5 C.C, and this was made upto 1 ml by the addition of GPT substrate. To this added 0.2 ml of distilled water. Added 1 ml of 2, 4 dinitrophenyl hydrazine reagent immediately there by stopping the reaction. After the tube was permitted to stand at room temperature for a minimum of 20 minutes 10 ml of 0.4 N sodium hydroxide was added and a rubber stopper was inserted and the contents

were mixed by inversion. At the end of exactly 30 minutes the optical density of the solution was measured at 505 nm using water as the blank. The scale reading on Klett's Summerson Colorimeter was recorded (Fig.2).

Procedure

One milliliter of the GPT substrate was pipetted in a test tube, and placed in a water bath at constant temperature (40°C) for 10 minutes. Upon the addition of 0.2 ml serum the contents were mixed and after an incubation period of exactly 30 minutes the tube was removed from the water bath. One milliliter of the 2,4 dinitrophenyl hydrazine reagent was added immediately thereby stopping the reaction. After the tube was permitted to stand at room temperature for a minimum of 20 minutes, 10 ml of 0.4 N sodium hydroxide was added a rubber stopper was inserted and the contents were mixed by inversion. At the end of exactly 30 minutes the optical density of the solution was measured at 505 nm using water as the blank. While the specimens were incubating a control for each serum was prepared. One milliliter of the substrate, 0.2 ml of serum and 1 ml of 2, 4 dinitrophenyl hydrazine reagent were mixed in a test tube. After a minimum of 20 minutes, 10 ml of 0.4 N sodium hydroxide was added. Lower scale reading of both experimental and control samples were noted and change in optical density was measured.

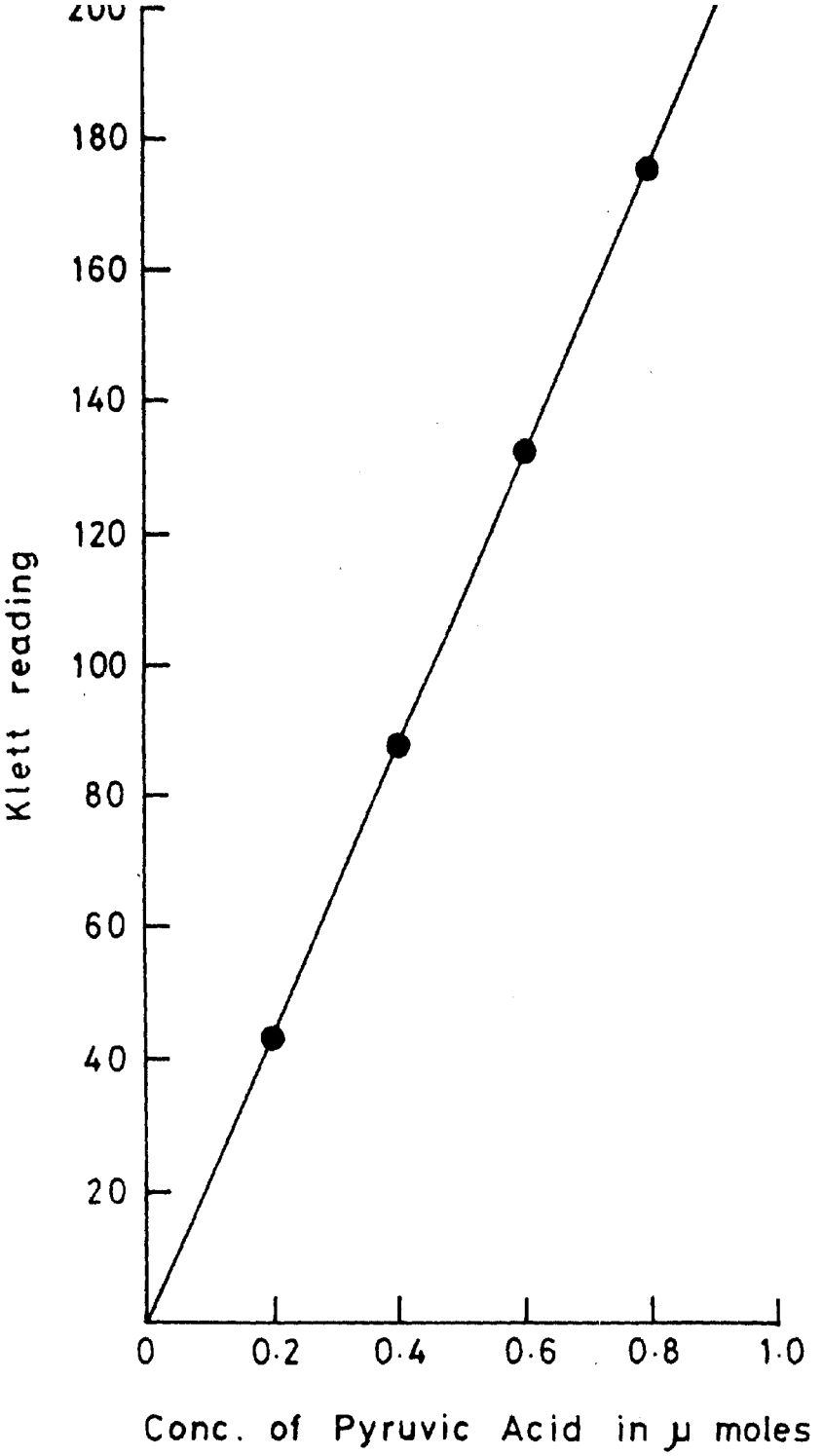


FIG. 2 : CALIBRATION CURVE FOR PYRUVIC ACID

3.2.2 Serum Cholinesterase

Serum acetyl cholinesterase activity was estimated according to the method of Dela Huerga et al. (1952).

Principle: Hydroxylamine in alkaline solution reacts with acetyl choline to give hydroxamic acid which produces a red to violet colour with Ferric chloride. The intensity of this colour is proportional to the concentration of acetyl choline present in it.

Reagents required

1. Buffer solution: Dissolved 10.3 gm of sodium barbitone in about 300 ml of water and slowly added 60 ml of normal hydrochloric acid. Crystals of barbitone were formed. Added 5.3 gm. of anhydrous sodium carbonate, stirred and warmed gently until solution was completely formed. Cooled to the room temperature and made upto 500 ml. with distilled water.

2. Salt mixture: Dissolved 4.2 gm. of anhydrous Magnesium chloride and 0.2 gm of potassium chloride in water and was made upto 1000 ml.

3. Acetylcholine Bromide Salt:- 11.3% . The salt must be recrystallized by dissolving 100 gm. in 600 ml. of ethyl alcohol. After filtering, the solution was placed on the refrigerator for overnight. The crystals that

formed were collected washed with ether and dried in a desicator over sodium hydroxide pellets and under vacuum. The solution was kept frozen.

4. Acetyl choline Buffer salt mixture: Immediately before use mixed 8 volumes of buffer solution and 1 volume each of acetyl choline bromide solution and the salt mixture.

5. Hydroxylamine hydrochloride - 14%

14.0 grams of hydroxylamine hydrochloride was dissolved in water and made upto 100 ml.

6. Acetyl choline Bromide solution (0.5 M)

Dissolved 11.3 gm. of recrystallized acetylcholine bromide salt in water and made it upto 100 ml.

7. Sodium hydroxide - 14%

14.0 grams of sodium hydroxide dissolved in distilled water and made upto 100 ml.

8. Alkaline hydroxylamine:

Mixed equal volumes of 5 and 7 solution.

9. Ferric chloride solution

Dissolved 10.0 grams of ferric chloride ($FeCl_3 . 6H_2O$) in 1 liter of 0.02 N HCl.

10. Hydrochloric acid (0.5 N)

11. Acetyl choline Bromide standard solution

Diluted the 0.5 M solution 1 in 10 which contains 50 micromoles per ml.

Calibration curve for acetylcholine

To draw a calibration curve for acetyl choline bromide, different aliquots of acetyl choline bromide solution in the range of 0.4, 0.8, 1.2, 1.6 and 2.0 ml were taken and made upto 2.2 ml with distilled water. Added 2 ml of alkaline hydroxylamine solution and a minute later added 6 ml of Hcl. Stoppered the tubes and inverted them thrice. Pipetted out exactly 0.5 ml. of each in a seperate tube to add 10 ml. of Ferric chloride solution. The tubes were inverted again to ensure thorough mixing. The tubes were then kept for centrifugation. The supernatant fluids were read at 530 nm in a Klett's Summerson Photoletric colorimeter. The instrument was set with a reagent blank prepared by adding 10 ml. of ferric chloride solution to 0.5 ml Hcl. The lower scale readings were noted and a straight line curve was obtained over the range used (Fig.3).

Procedure: Measured 0.2 ml of serum into one test tube and 0.2 ml. water into another. To each added exactly 2 ml. of acetylcholine bromide buffer salt mixture previously warmed to 37°C allowing an interval of twenty

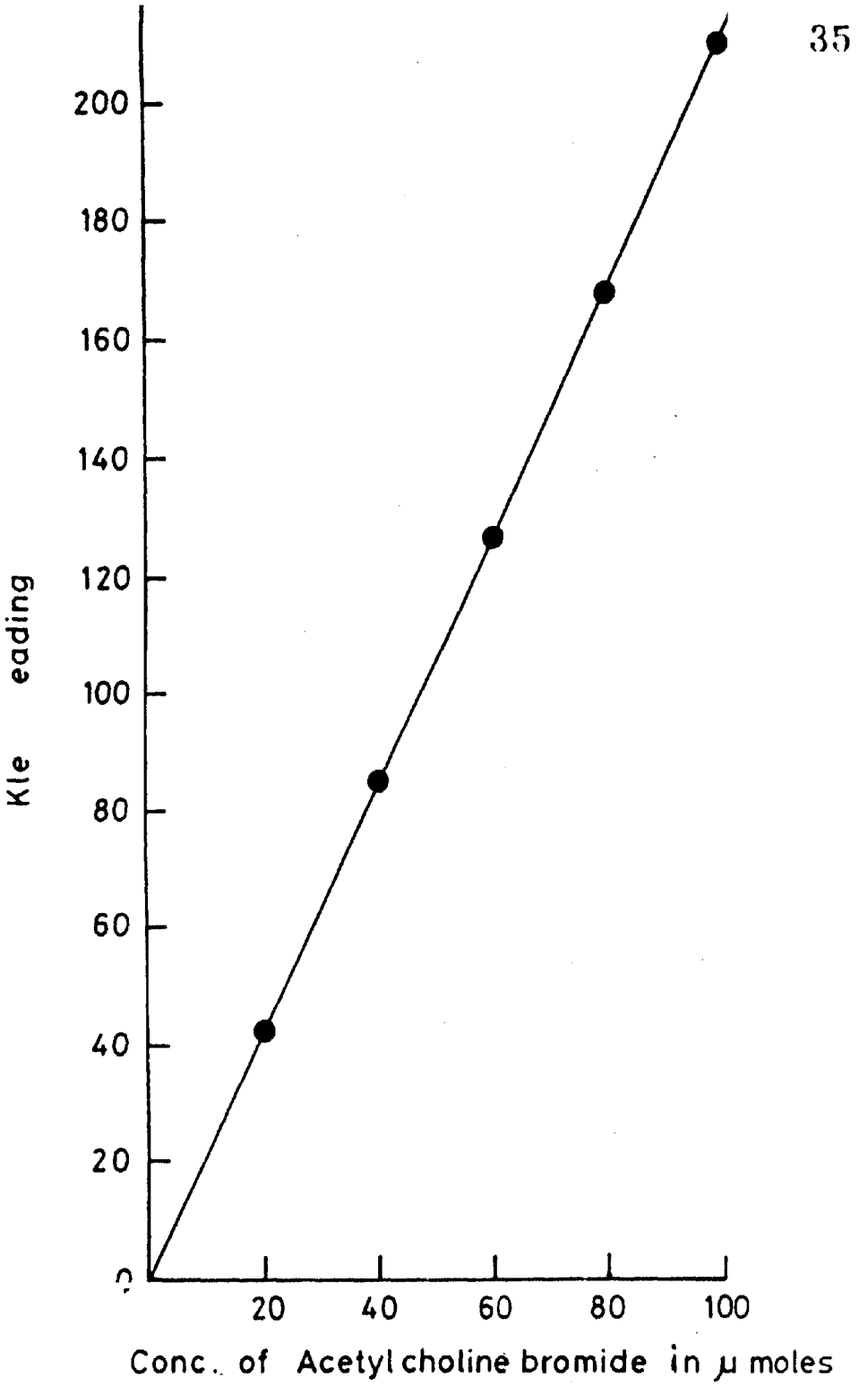


FIG. 3 : CALIBRATION CURVE FOR ACETYL CHOLINE

seconds between the additions. The tubes were then incubated for one hour at 37°C. Then added 2 ml of alkaline hydroxylamine solution. A minute later added 6 ml. of 0.5 N HCl. Stoppered the tubes and inverted them thrice. Pipetted out exactly 0.5 ml, of each into a separate tube to add 10 ml of ferric chloride solution. The tubes were again inverted thrice for proper mixing and then centrifuged. The supernatant fluid was read at 530 nm in a colorimeter. The instrument was set with a reagent blank prepared by adding 10 ml of ferric chloride solution to 0.5 ml of HCl.

3.3 Gross and Histopathological examination

After the slaughter of the birds complete P.M. examination was carried out and then liver, kidney, intestine, heart and testes were collected in 10% buffered formal saline for fixation. The tissues were kept in formaline for 72 hours, then small pieces of the tissues were cut and washed under running water for 24 hours to remove formaline. Tissues were then dehydrated by passing through ascending grades of alcohol and then cleared by passing through Xylol and later embeded in paraffin and blocks were made. Sections were cut from these blocks at 5 μ thickness. These sections were stained with Haematoxylin and eosin for histopathological studies.

CHAPTER - IV

R E S U L T S

The birds in the groups I, II, III consumed normal feed and water, while the birds in group IV and V showed decrease in feed and water intake. The birds in Group IV and V were dull and showed nervous symptoms like incoordination in movement, lying on the floor on one side, opisthotonous, paralysis of wings and legs hence, the birds in group IV and V were sacrificed.

4.1 Toxin Consumption

The birds in group II maintained over a period of thirty three days consumed malathion ranging from 536.50 mg to 820.50 mg, and the birds maintained over a period of seventy five days consumed malathion ranging from 2,819.25 mg to 3,322.50 mg.

The birds in group III maintained over a period of thirty three days consumed malathion ranging from 1,053 mg to 1,599 mg and the birds maintained over a period of seventyfive days consumed malathion ranging from 3,864 mg to 5,316.50 mg.

The birds in group IV maintained over a period of twenty six days consumed malathion ranging from 489 mg to 2,010 mg.

The birds in group V maintained over a period of twenty six days consumed mal thion ranging from 100 mg to 2,140 mg (Table 2).

Table 2. Total amount of toxin consumed by the birds in different groups.

Sl. No.	Short term feeding								Long term feeding			
	Group II (50 mg/kg b.wt.)		Group III (100 mg/kg b.wt.)		Group IV (150 mg/kg b.wt.)		Group V (200 mg/kg b.wt.)		Group II (50 mg/kg b.wt.)		Group III (100 mg/kg b.wt.)	
	No. of days on toxin	Amount of toxin consumed in mg.	No. of days on toxin	Amount of toxin consumed in mg.	No. of days on toxin	Amount of toxin consumed in mg	No. of days on toxin	Amount of toxin consumed in mg.	No. of days on toxin	Amount of toxin consumed in mg.	No. of days on toxin	Amount of toxin consumed in mg.
1.	33	730.00	33	1,599	13	469.0	3	162	75	2,841.85	75	3,864.0
2.	33	536.50	33	1,449	14	1,146.0	8	432	75	2,819.25	75	4,985.0
3.	33	710.75	33	1,410	14	1,257.0	8	512	75	2,920.50	75	5,316.5
4.	33	636.00	33	1,554	20	1,508.0	8	380	75	3,322.50	75	4,378.5
5.	33	820.50	33	1,053	20	1,886.5	18	902	75	2,836.00	75	5,286.5
6.					20	1,050.0	13	766				
7.					26	1,749.0	13	864				
8.					26	1,353.0	26	1,728				
9.					26	1,072.5	26	1,656				
10.					26	2,010.0	26	2,140				

4.2 Mortality Pattern

There was no mortality observed in groups I, II and III during the experimental period. The birds in group IV and V started showing mortality with nervous symptoms and hence the experiment was terminated early in these two groups.

In group IV two birds died in the third week, two in the fourth week and the remaining birds were sacrificed on twenty sixth day of the experiment.

In group V birds started dying early. Two birds died in the first week, another three birds in second week, three birds were sacrificed in third week and the remaining two in the fourth week as they were about to die.

4.3 Body Weights

In general there was proportional increase in the weekly body weights in group I, II and III, while in group IV the increase in weekly body weights was noticed upto third week and there onwards there was a decrease in body weight gain. The birds in group V did not show any gain in weekly body weights. At the time of killing significant difference in body weights was not noticed in Group I II and III, while groups IV and V showed a significant ($P < 0.01$) decrease in body weight as against group I,II and III and no such difference was observed between groups IV and V (Table 3 and Fig.4).

Table 3. Weekly body weights in kgs of birds fed malathion for short duration (Twentysix to Thirtythree days)

Group	Number of weeks toxin fed					
	0 (Initial weight)	1	2	3	4	5 (Killing weight)
Group I (control)	0.295 ± 0.018 (5)	0.345 ± 0.020 (5)	0.470 ± 0.010 (5)	0.523 ± 0.034 (5)	0.625 ± 0.002 (5)	0.663 ± 0.004 (5)
Group II (50 mg/kg b.wt)	0.286 ± 0.040 (5)	0.315 ± 0.020 (5)	0.392 ± 0.027 (5)	0.489 ± 0.024 (5)	0.562 ± 0.004 (5)	0.621 ± 0.0038 (5)
Group III (100 mg/kg b.wt)	0.291 ± 0.055 (5)	0.333 ± 0.020 (5)	0.415 ± 0.043 (5)	0.475 ± 0.040 (5)	0.520 ± 0.035 (5)	0.560 ± 0.033 (5)
Group IV (150 mg/kg b.wt)	0.320 ± 0.015 (10)	0.349 ± 0.025 (10)	0.388 ± 0.024 (8)	0.430 ± 0.0045 (6)	—	0.439** ± 0.006 (10)
Group V (200 mg/kg b.wt)	0.315 ± 0.009 (10)	0.308 ± 0.014 (8)	0.313 ± 0.024 (5)	0.323 ± 0.030 (3)	—	0.351** ± 0.0024 (10)
						Compared between the groups

Mean ± S.E.
(No.) in parenthesis indicates sample size

$P < 0.05$* $P < 0.01$**

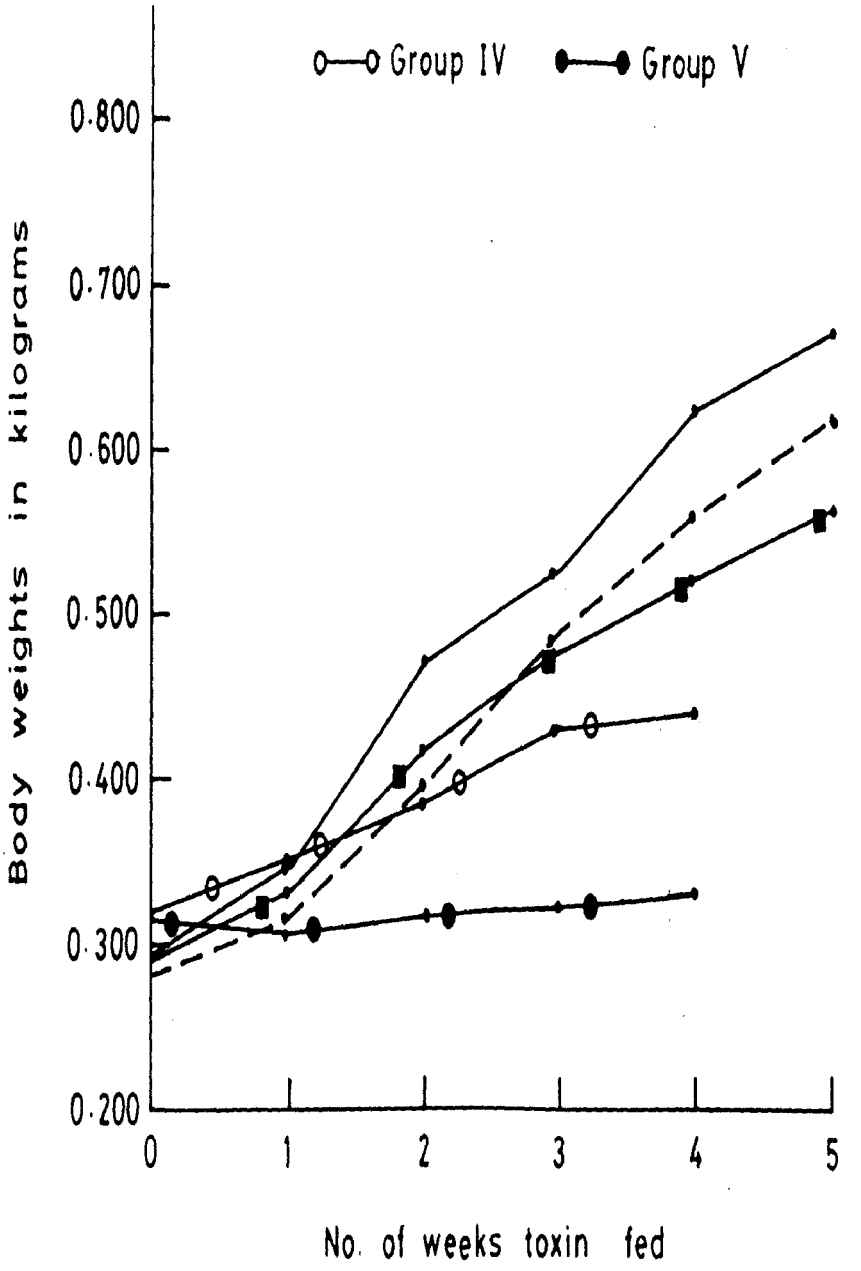


FIG. 4 : WEEKLY BODY WEIGHTS IN GROUP I, II, III, IV AND V FED MALATHION FOR SHORT DURATION (26 TO 33 DAYS)

Weekly body weights recorded in the birds maintained for seventy five days with the same toxin feeding schedule in groups I, II and III, showed a steady gain during the experimental period in groups I and II. The birds in group III showed an increase in the body weight upto fifth week and a slight degrease in their body weight gain for the rest of the experimental period. The decrease was highly significant ($P < 0.01$) from sixth week till they were sacrificed, when compared with group I and II (Table 4 and Fig. 5).

4.4 Haematological Investigations

The haematological values in different groups maintained over a period of thirty three and seventy five days were shown in the Table 5 and 6.

4.4.1 Packed Cell Volume

The mean PCV value in control birds was 33 ± 2.68. In general the PCV values in toxin fed groups (II, III, IV and V) were lowered when compared to control (group I). In groups II and III the decrease in PCV was found to be significant ($P < 0.05$) when compared with group I. Group IV also showed a significant ($P < 0.01$) decrease in PCV values when compared to group I. In group V significant ($P < 0.01$) decrease in PCV was observed when compared to groups I, II, III and IV.

Table 4. Weekly body weight in kgs of birds fed malathion for long duration (Seventy five days)

Group	Number of weeks toxin in fed											
	0(initial weight)	1	2	3	4	5	6	7	8	9	10	11(Killing weight)
Group I (control)	0.336 ± 0.010 (5)	0.401 ± 0.020 (5)	0.511 ± 0.022 (5)	0.638 ± 0.026 (5)	0.720 ± 0.035 (5)	0.838 ± 0.042 (5)	0.897 ± 0.028 (5)	0.997 ± 0.025 (5)	1.006 ± 0.033 (5)	1.147 ± 0.028 (5)	1.275 ± 0.070 (5)	1.398 ± 0.069 (5)
Group II (50 mg/kg b.wt)	0.348 ± 0.010 (5)	0.403 ± 0.020 (5)	0.500 ± 0.024 (5)	0.634 ± 0.028 (5)	0.704 ± 0.038 (5)	0.798 ± 0.024 (5)	0.887 ± 0.026 (5)	0.957 ± 0.021 (5)	1.018 ± 0.024 (5)	1.097 ± 0.031 (5)	1.181 ± 0.068 (5)	1.258 ± 0.040 (5)
Group III (100 mg/kg b.wt)	0.350 ± 0.020 (5)	0.396 ± 0.030 (5)	0.452 ± 0.091 (5)	0.518 ± 0.040 (5)	0.611 ± 0.049 (5)	0.664 ± 0.030 (5)	0.765 ± 0.044 (5)	0.794 ± 0.038 (5)	0.786 ± 0.024 (5)	8.801 ± 0.026 (5)	0.810 ± 0.030 (5)	0.819 ± 0.033 (5)
											Compared between the groups	

Mean ± S.E.

(No.) in parenthesis indicates sample size

P 0.05 * P 0.01 **

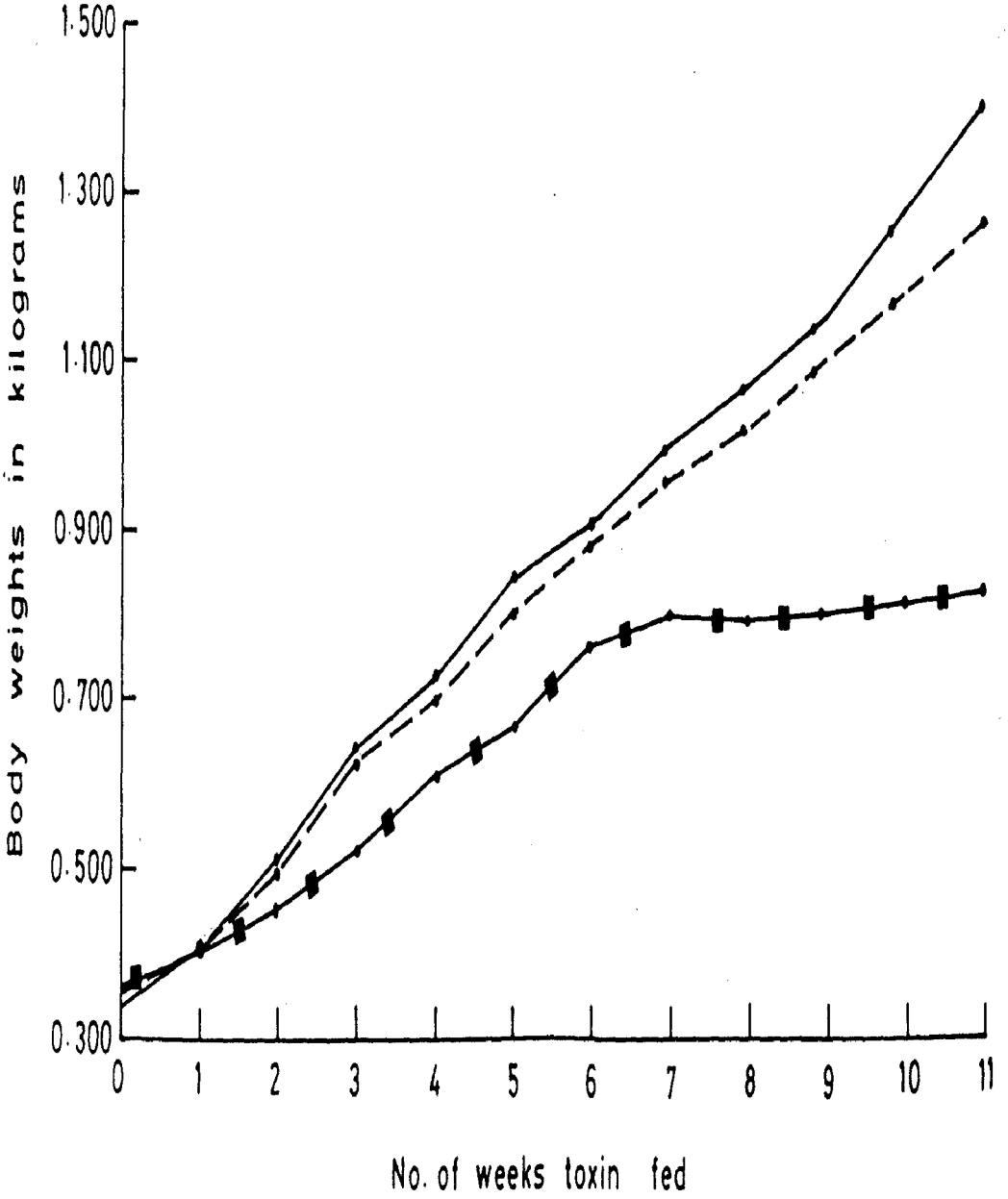


FIG. 5 : WEEKLY BODY WEIGHTS IN GROUP I, II AND III FED MALATHION FOR LONG DURATION (75 DAYS)

Table 5. Haematological changes in birds fed malathion for short duration (twentysix to thirtythree days)

Group	PCV %	Haemo-globin gm/100ml	Total RBC in millions per MM^3	Total WBC in thousands/MM^3	Differential count in percentage				
					Hetero-philes	Lympho-cytes	Mono-cytes	Eosino-phils	Baso-phils
Group I (control)	33.0 ± 2.68 (5)	12.4 ± 0.14 (5)	3.25 ± 0.04 (5)	21,600 ± 430 (5)	21.4 ± 0.51 (5)	71.8 ± 1.28 (5)	4.8 ± 0.8 (5)	0.8 ± 0.8 (5)	1.2 ± 0.49 (5)
Group II (50 mg/kg b.wt)	27.8* ± 0.98 (5)	11.56* ± 0.19 (5)	2.46** ± 0.056 (5)	21,220 ± 340 (5)	26.6* ± 0.81 (5)	70.2* ± 0.86 (5)	2.6* ± 0.51 (5)	— (5)	1.2* ± 0.24 (5)
Group III (100 mg/kg b.wt)	28.0* ± 5.15 (5)	11.52* ± 0.56 (5)	2.32** ± 0.082 (5)	21,120 ± 550 (5)	29.6** ± 0.75 (5)	63.2** ± 1.24 (5)	3.4 ± 0.60 (5)	2** ± 0.44 (5)	1.2 ± 0.49 (5)
Group IV (150 mg/kg b.wt)	25.6** ± 1.28 (6)	11.1** ± 0.11 (6)	1.93** ± 0.06 (6)	21,883** ± 270 (6)	32.3** ± 0.73 (6)	62.3** ± 1.03 (6)	3.5 ± 0.76 (6)	1.6** ± 0.21 (6)	1.1* ± 0.47 (6)
Group V (200 mg/kg b.wt)	22.8** ± 2.54 (6)	9.63** ± 0.33 (6)	1.73** ± 0.041 (6)	22,750** ± 330 (6)	58.1** ± 0.40 (6)	35** ± 0.89 (6)	3.6 ± 0.42 (6)	2.3** ± 0.55 (6)	0.8 ± 0.40 (6)

Mean ± S.E.
(No) In parenthesis indicates sample size
($P < 0.05$) * ($P < 0.01$) ** Compared between the groups

Table 6. Haematological changes in birds fed malathion for long duration (seventy five days)

Group	PCV %	Haemoglobin gm/100 ml	Total RBC in millions per MM3	Total WBC in thousands/MM3	Differential count in percentage				
					Heterophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Group I (control)	31.0± 0.31 (5)	11.94± 0.04 (5)	3.09± 0.04 (5)	21400± 710 (5)	20.0± 0.44 (5)	71.4± 4.75 (5)	6.0 ± 1.12 (5)	0.4 ± 0.13 (5)	2.20± 0.78 (5)
Group II (50 mg/kg b.wt)	29.0* ± 0.70 (5)	11.40* ± 0.15 (5)	1.93** ± 0.17 (5)	23600* ± 1390 (5)	32.0** ± 6.27 (5)	60.2* ± 4.50 (5)	4.4 ± 0.35 (5)	5.0** ± 0.22 (5)	1.4 ± 0.13 (5)
Group III (100 mg/kg b.wt)	27.0* ± 0.44 (5)	9.34** ± 0.22 (5)	1.75** ± 0.59 (5)	27000** ± 640 (5)	42.6** ± 5.06 (5)	52.2** ± 3.45 (5)	3.4** ± 0.13 (5)	3.6** ± 0.13 (5)	0.2** ± 0.8 (5)

Compared between the groups

Mean ± S.E.

(No) in parenthesis indicates sample size

$P < 0.05^{*}$ $P < 0.01^{**}$

The birds in group II maintained for seventy five days on toxin feeding showed a significant ($P < 0.05$) reduction in PCV values when compared to group I. Group III also showed a significant ($P < 0.01$) reduction in PCV values as against group I and II.

4.4.2 Haemoglobin

The mean haemoglobin value in control group was 12.4 ± 0.14. In toxin fed groups the values were significantly lowered. Group II and III showed a significant ($P < 0.05$) reduction in haemoglobin when compared to group I. Group IV and V also showed a significant ($P < 0.01$) reduction in haemoglobin values when compared to group I. Group V also showed a significant ($P < 0.01$) decrease in haemoglobin values when compared to group III, IV and V.

Haemoglobin values were also lowered in group II and III, maintained for seventy five days on toxin feeding. Group II showed a significant ($P < 0.05$) reduction in haemoglobin values and in group III the reduction was significant ($P < 0.01$), when compared with group I.

4.4.3 Total erythrocyte count

Total erythocyte count in control birds was 3.25 ± 0.04. In general a decrease in erythrocyte count was seen in all the toxin fed groups. The decrease in total erythrocyte count was found to be significant

($P < 0.01$) in group II. A significant reduction ($P < 0.01$) in total RBC count was noticed in group III, when compared with group I and II, in group IV when compared with Group I and III and in group V as against group I to IV.

Birds maintained for seventy five days on toxin feeding showed significant ($P < 0.01$) reduction in total RBC count in groups II and III as against group I and in between group II and III.

4.4.4 Total leucocyte count

The total WBC count in control birds was 20,600 ± 430. Increase in total WBC count was found to be significant ($P < 0.01$) in group IV and V as against groups I, II and III. However, there was no such difference in between the group IV and V.

The total WBC count was significantly increased in group II ($P < 0.05$) and in group III ($P < 0.01$) maintained for seventy five days when compared with group I and II birds maintained for seventy five days on toxin feeding.

4.4.5 Differential count

In birds maintained for thirty three days a significant ($P < 0.01$) increase in heterophilic and eosinophilic count was found in group V when compared with group I to IV,

in group IV as against group I to III and in group III when compared with group I, however there was no such difference in between group II and III. Lymphocyte count was found to be decreased significantly ($P < 0.00$) in group V as compared to group I to IV, in group IV as against group I to III and in group III when compared with group I. There was no such difference in basophilic and monocytic count in any of the groups.

The birds in group II and III fed toxin for seventy five days showed a significant ($P < 0.01$) increase in heterophil and eosinophil count when compared to group I. Group III also showed a significant ($P < 0.01$) increase in heterophil count as against group II. A significant decrease was noticed in lymphocytic count ($P < 0.05$) in group II and ($P < 0.01$) in group III and monocytic count ($P < 0.01$) and basophilic count ($P < 0.01$) in group III, as compared to group I.

4.5 Biochemical Investigations

4.5.1 Serum Glutamic Pyruvic Transaminase Activity

SGPT activity was estimated at the time of sacrifice in all the groups. There was no difference in activity in birds of group I and II. However a significant ($P < 0.01$) increase in activity was noticed in group III when compared with group I and II, and in group IV as against I to III,

and in group V when compared to group I to IV (Table 7). In the birds maintained over a period of seventy five days of toxin feeding in group II and III showed a significant ($P < 0.01$) increase in the activity as ~~xxx~~ against group I and was much higher ($P < 0.01$) in group III when compared with group II (Table 8).

4.5.2 Serum Cholinesterase activity:

Serum cholinesterase activity was estimated at the time of sacrifice in all the groups. A decreasing trend in the activity was noticed in group III to V. The activity decreased significantly ($P < 0.05$) in group III birds when compared with group I and II, in groupIV ($P < 0.01$) whencompared to groups I to III and in group V ($P < 0.01$) as against groups I to IV.

In birds maintained over a period of seventy five days the activity was further decreased significantly ($P < 0.01$) in group II and III when compared with group I and the values in group III were significantly ($P < 0.01$) low as against group II (Table 7 and 8).

4.6 Gross Changes

In general the birds in groups I, II, III did not show any body changes while the birds in group IV and V showed a decrease in the carcas weight. The internal

Table 7. Serum glutamic pyruvic transaminase and Cholinestrase activity in birds fed malathion for short duration (twentysix to thirtythree days).

Group	SGPT micro-moles/liter	Cholinesterase micromoles/ml.
Group I (Control)	3.63 ± 0.14 (5)	99.2 ± 0.8 (5)
Group II (50 mg/kg b.wt)	4.29 ± 0.29 (5)	97.4 ± 0.81 (5)
Group III (100 mg/kg b.wt)	$5.48 \pm 0.22^{**}$ (5)	$96.8 \pm 0.73^{*}$ (5)
Group IV (150 mg/kg b.wt)	$6.32 \pm 0.27^{**}$ (6)	$83.0 \pm 1.00^{**}$ (6)
Group V (200 mg/kg b.wt)	$7.26 \pm 0.28^{**}$ (6)	$77.6 \pm 0.92^{**}$ (6)

Compared between the groups

Mean $\pm$ S.E. (No) in parenthesis indicates sample size.

$P < 0.05^{*}$ $(P < 0.01)^{**}$

Table 8. Serum glutamic pyruvic transaminase and Cholinesterase activity in Birds fed malathion for long duration (Seventy five days).

Group	SGPT in micro moles/ liter	Cholinesterase micromoles/ ml.
Group I (Control)	$3.65 \pm \underset{(5)}{0.04}$	$100.4 \pm \underset{(5)}{0.75}$
Group II (50 mg/kg)	$4.96 \pm \underset{(5)}{0.11}^{**}$	$92.0 \pm \underset{(5)}{1.38}^{**}$
Group III (100 mg/kg)	$6.66 \pm \underset{(5)}{0.30}^{**}$	$82.4 \pm \underset{(5)}{1.12}^{**}$
	Compared between the groups	

Mean $\pm$ S.E (No) in parenthesis indicates sample size.

$P < 0.05^{*}$ $P < 0.01^{**}$

organs viz., liver, kidney, intestine, showed slight congestion while there was no such changes in heart. The degree of these changes were prominent in birds of group III, IV and V. Testis were atrophied and intestines were empty and showed haemorrhages in groups IV & V. In addition to these changes, the blood was thick and brick red in colour. The comb and wattles were slightly pale when compared to birds in groups I to III.

4.7 Histopathological Examination

4.7.1 Liver

The histology of the liver in control group of birds was essentially normal. The birds maintained in group II over a period of thirty three days showed in general a slight congestion in the sinusoids and portal triads. The hepatic cells also revealed mild degenerative changes and kupffer cell proliferation was prominent. There was loss of cytoplasmic basophilia and ocassionally focal liver cell necrosis and infiltration with mononuclear cells was noticed (Fig.6).

In birds maintained over a period of seventy five days of toxin feeding, the above mentioned changes in the liver were much advanced. Congestion was severe in sinusoids, portal triads and in central veins. There was loss of liver architecture with an attempt to form

pseudolobulation. Areas of multiple focal liver cell necrosis infiltrated with mononuclear cells were also noticed.

The livers in the birds of group III maintained for a period of thirty three days showed liver changes which included disortion of liver lobular pattern, severe congestion of sinusoids, portal triads and central veins. There were areas accumulated with pooled blood. Proliferation of Kupffer cells was prominent. The liver also showed distortion of lobular pattern and in few areas the cells were surrounded by a delicate fibrous tissue, an attempt for early cirrhosis. These changes were well advanced in the group III birds maintained for seventy five days.

Livers in the birds of group IV maintained over a period of twenty six days, showed a severe and extensive congestion of sinusoids, portal veins and central veins. Distortion of hepatic cords and multiple areas of liver cell necrosis were also noticed. In group V the changes were similar to that of group IV, but were much advanced (Fig.7).

4.7.2 Kidneys

The kidneys showed slight congestion associated with interstitial haemorrhages in the birds of group II

Fig.6. Liver (Group II,33 days): Hepatic cords disorganisation, congestion of the sinusoids, portal triads with focal liver cell necrosis. H&Ex64.

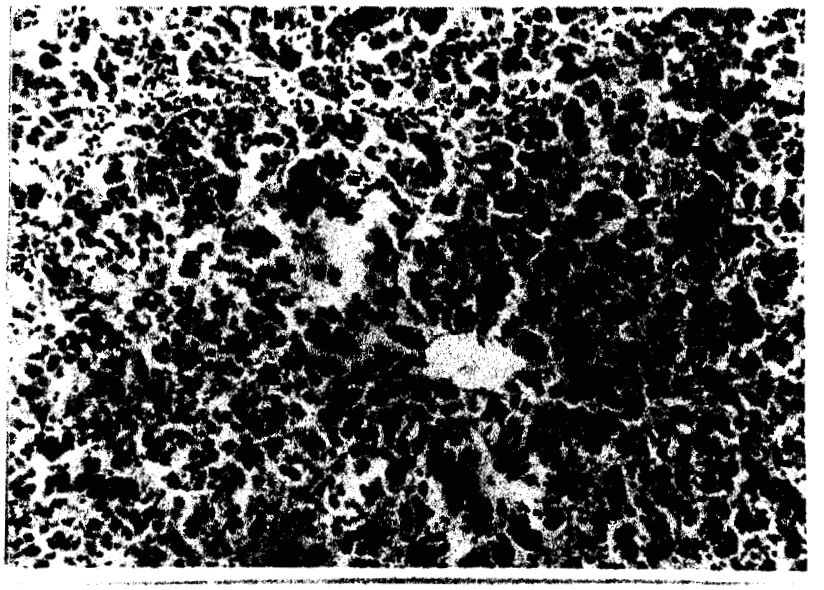


Fig.7. Liver(Group V 26 days): Disorganisation of hepatic lobular pattern, severe congestion and distortion of the sinusoids, focal liver cell necrosis and prominent kupffer cells. H&E x 64.

maintained for thirty three days. The tubular epithelium showed degenerative changes like cloudy swelling and vacuolation. At places hyper cellularity of glomerular endothelium was noticed.

These changes were extensive and diffuse in the birds maintained over a period of seventy five days (Fig.8). The kidneys in group III birds maintained over a period of thirtythree days showed interstitial haemorrhages and in few areas extensive localised haemorrhages. The tubular epithelium showed diffuse degenerative changes and casts were found in the lumen due to the desquamation of epithelial cells. In the birds maintained over a period of seventy five days these changes were well advanced.

In group IV the kidney changes include extensive interstitial haemorrhages, increased cellularity of glomeruli and extensive degenerative changes of tubular epithelium. Vacuolation of tubular epithelial cells was very much conspicuous.

These changes were well advanced and much more extensive in group V birds. In addition few areas showed loss of tubular epithelial cells upto the basement membrane (Fig.9).

4.7.3 Heart

The hearts did not show any specific histopathological

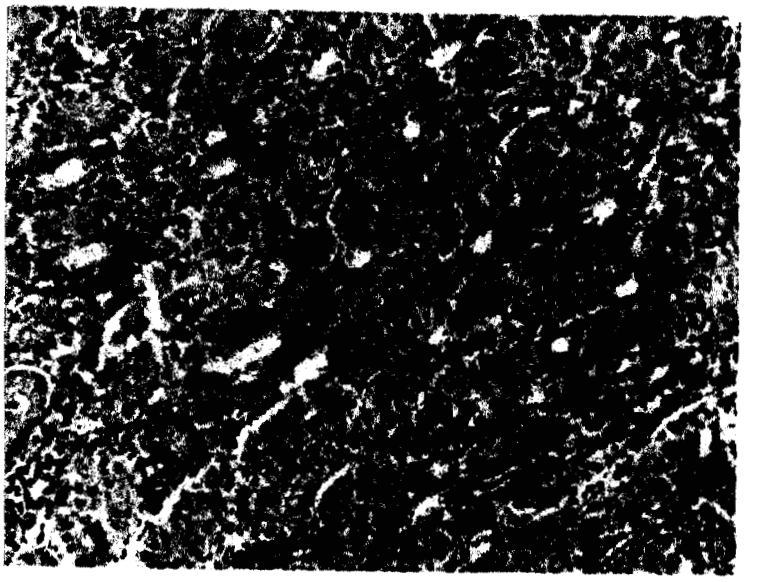


Fig.8. Kidney(Group II, 33 days): Extensive interstitial haemorrhages, degenerative changes and exfoliation of the epithelium lining the tubules and increased scellularity of the glomeruli. H&E x 64.

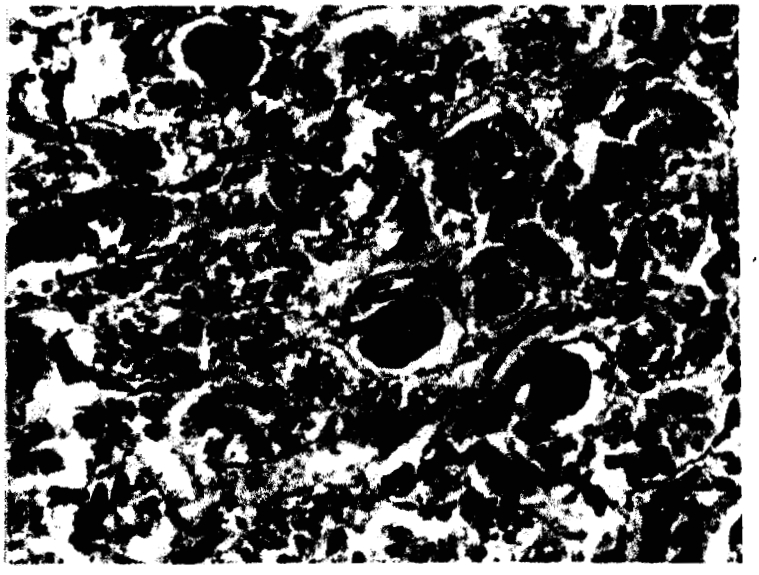


Fig.9. Kidney (Group V, 26 days): Extensive interstitial haemorrhages coagulative necrosis and desquamation of the tubular epetholium and necrosis glomeruli and tubules. H&E x 128.

changes in birds of group I to III, however group IV and V showed slight congestion and oedema.

4.7.4 Intestine

Intestines in group II showed hyperplasia of laminar epithelium associated with increase in goblet cells. There was also increase in enterochromaffin cells. However there was no congestion of muscularis mucosa and submucosa.

In group II birds which were maintained for seventy five days, showed similar changes described in group II, in addition to that the sloughing of laminar epithelium and necrosis was also observed. The necrotic zones were infiltrated with mononuclear cells. Enterochromaffin cells and goblet cells were markedly increased (Fig.10).

In group III birds fed toxin for thirty three days the laminar epithelium was extensively affected and infiltrated with mononuclear cells. There was congestion of muscularis mucosae and submucosa. The birds maintained over a period of seventy five days, showed extensive necrosis of epithelium extending upto submucosa (Fig.11).

In group IV and V the intestine showed similar changes but the lesions were more severe and extensive.

Fig.10. Intestine (Group II, 75 days): Epithelium showed necrosis, sloughing and mononuclear cell infiltration The inflamation extended upto muscularis mucosae. H & E x 64.

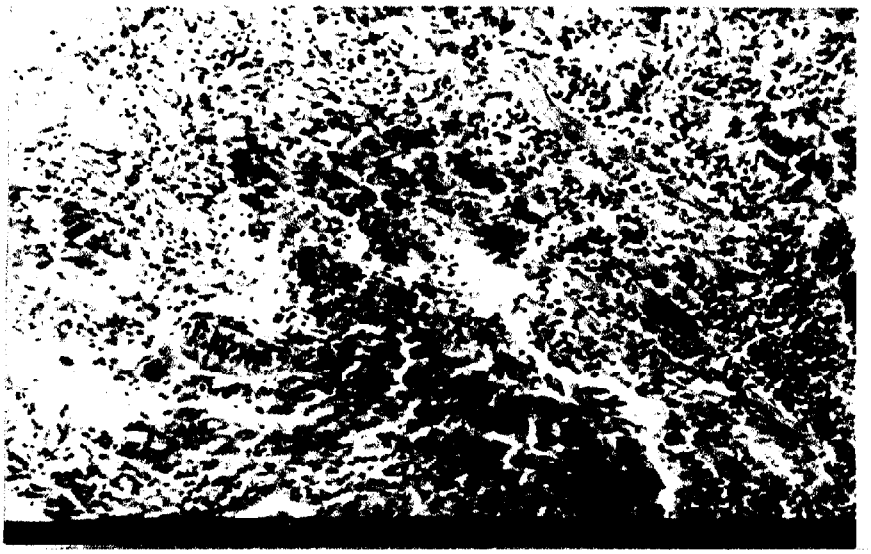


Fig.11. Intestine (Group III, 75 days): The lamina epithelium was extensively affected leading to necrosis sloughing and mononuclear cell infiltration. The inflamation extended upto muscularis mucosae, which showed coagulative necrosis. H&E x 64.

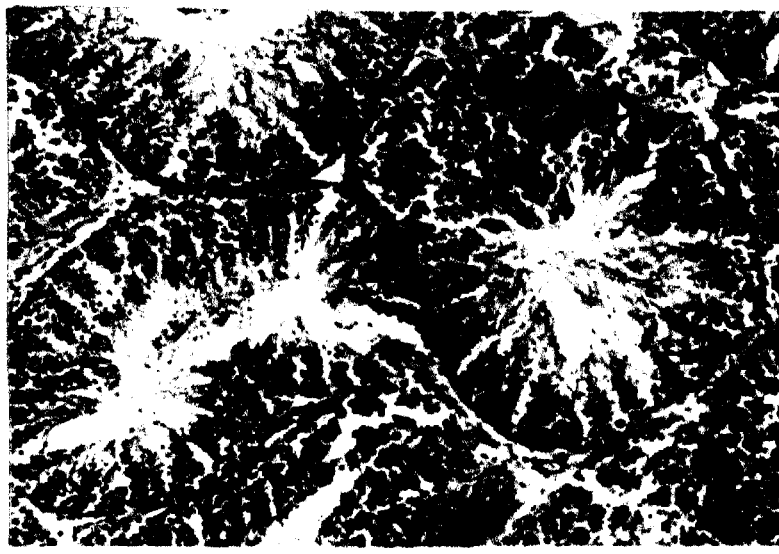


Fig.12. Testis(Group II, 75 days): The seminiferous tubules lines by epithelium were normal and spermatogenesis was also normal. Well developed spermatozoa were also found in all the tubules. H&E x 128.

Fig.13. Testis(Group V, 26 days): Single layer of spermatogonial cells with degenerative changes, increased interstitial tissue and absence of spermatogenesis. H&E x 64.

CHAPTER - V

DISCUSSION AND CONCLUSIONS

Organophosphorous pesticides are widely used to control plant pests, flies and parasites of livestock and poultry. The principal exposures of livestock to these pesticides are through careless handling, using higher concentration than those recommended, accidental mixing with the feed and use of pesticide treated grains or sprayed crops in livestock and poultry feeds.

Malathion is selected for our study because it is successfully and widely used in Andhra Pradesh for various agricultural crops, ornamental plants, animal and stored grain ware houses. It is claimed by the manufacturers that malathion does not leave any residue and if present it is not toxic to living organisms. However, it was found that the use of these contaminated grains and crops in the rations of livestock and poultry were not safe since they contain residual toxins and produced cumulative effects on the living system. Hence, it was proposed to study the effects of different levels of malathion for short and long term duration in poultry. The birds in group IV and V fed with malathion 150 mg and 200 mg/kg b.wt respectively started dying during the second week of the experiment due to acute toxicity and hence the two groups were terminated by sacrificing the birds in twenty six days.

The birds in control group I showed gradual increase in weekly body weights during the experimental period. The birds fed with malathion 50 mg and 100 mg/kg b.wt. (group II & III) over a period of thirty three days showed a gradual increase in their weekly body weights as against its initial body weight, however the gain in body weight was less with that of control (group I). The weekly body weights in birds fed malathion 150 mg/kg b.wt (group IV) and 200 mg/kg b.wt (group V) over a period of twentysix days showed a significant ($P < 0.01$) reduction as against group group I, II and III and in between groups IV and V at the time of killing. Feeding of malathion 100 mg/kg. b.wt. for seventy five days (group III) resulted in highly significant ($P < 0.01$) loss in weekly body weights from sixth week onwards however feeding of malathion 150 mg/kg b.wt (group II) for seventy five days did not have similar effect on weekly body weight gains. The reduction in weeklybody weights were proportional to the toxin fed, lowest with 100 mg/kg b.wt. and highest with 200 mg/kg b.wt. which is attributable to the effect of malathion toxicity. Decrease in weekly body weights has been reported in rats in sub-acute poisoning with sumithion (Misu *et al.*, 1966); in chicks fed with guthion (Peter *et al.*, 1961); in sub-acute and chronic malathion (Rehfeld *et al.* 1969) and sumithion (Purushotham, 1971) poisoning in day

old chicks. Reddy (1975) also reported a decrease in weight in calves fed with ekalux 1, 2, 4 mg/kg b.wt. for twentyone days. Feeding of 2,500 and 5,000 ppm malathion to birds for ten weeks caused a decrease in body weights (Uppal and Singh, 1981).

The haematological changes viz., PCV, Haemoglobin, Total RBC; WBC and differential counts showed significant changes in malathion fed birds. The birds fed with malathion 50 mg/kg.b.wt (group II) and 100 mg/kg.b.wt. (group III) for thirtythree days showed a significant decrease ($P < 0.05$) in PCV value, and the reduction was highly significant ($P < 0.01$) in birds fed malathion 150 mg/kg b.wt (group IV) and 200 mg/kg b.wt (group V) as against the control values. In the birds maintained for seventyfive days on toxin the PCV values decreased significantly ($P < 0.05$) in group II and the decrease was highly significant ($P < 0.01$) in group III. Similar decrease in PCV values has been reported in sub-acute organophosphorous insecticide toxicity in rabbits (Petrichev and lazarov, 1970), in buffalo calves fed with malathion and aldrin (Hothi and Kwatra, 1972), in pony foals intoxicated with shell SD 15803 (Bello and Torbet, 1972), in sub-acute malathion and sumithion poisoning in buffalo calves (Vadlamudi, 1974; Gupta and Paul, 1977, 1978), in sheep given a single oral dose of

100 mg/kg b.wt. dermafos (Kalinowska et al. 1978), in rats administered LD 50 dose of chlorfenvinphos (Siczek, 1980) and in pigeons intoxicated with fenitrothion (Mandal and Lahiri, 1985). The decrease in PCV values is attributable to the decreased feed intake associated with internal haemorrhages in the parenchymatous organs resulting in anaemia. A similar view has also been expressed by Sharma and Saxena (1983).

In group II and III maintained for thirtythree days showed a significant ($P< 0.05$) reduction in haemoglobin values while the reduction was highly significant ($P< 0.01$) in group IV and V as compared to group I and in between the groups. In group II and III maintained for seventyfive days on malathion feeding the decrease in haemoglobin values were ($P < 0.05$) and ($P < 0.01$) respectively. The decrease in haemoglobin values has been reported in chicks fed with DDT, BHC, dieldrin and malathion (Srivastava et al., 1960), in buffalo calves fed with malathion and aldrin (Hothi and Kwatra, 1972) in sub-acute malathion and sumithion poisoning in buffalo calves (Vadlamudi, 1974), in buffalo calves fed 41.66 ppm malathion sprayed fodder for 28 twentyeight days (Gupta and Paul, 1977), in buffalo calves fed malathion 0.5 mg/kg b.wt for one year and when malathion was sprayed at 0.5, 1 and 5% for twentyeight days (Gupta and

Paul, 1978 , 1978), on administration of dermafos 100 mg/kg b.wt into the rumen of sheep (Kalinowska et al., 1978), in rats administered a single oral dose of chlorfenvinphos (Siczek, 1980) and in fenitrothion poisoning in pigeons for 2weeks (Mandal and Lahiri, 1985). The reduction in haemoglobin values is associated with reduction in the total number of RBC leading to anaemia was thought to be due to the effect of pesticide (Mandal and Lahiri, 1985).

A decrease in total RBC count was found to be significant ($P < 0.01$) in group II, III, IV and V birds maintained on malathion feeding for thirtythree days. Significant ($P < 0.01$) reduction in total RBC count was also observed both in group II and III maintained for seventyfive days on malathion feeding. A similar reduction in total RBC count was also reported in chicks fed with DDT, BHC, dieldrin and malathion (Srivastava et al., 1960), in organophosphorous pesticide toxicity in rabbits (Petrichev and Lazarov, 1970), in buffalo calves fed with malathion and aldrin (Hothi and Kwatra, 1972), in pony foals which were acutely intoxicated orally with shell SD 15803 (Bello and Torbet, 1972), in sub-acute malathion and sumithion poisoning in buffaloe calves (Vadlamudi, 1974), in buffalo calves fed 41.66 ppm of malathion sprayed fodder (Gupta and Paul, 1977), in

chronic toxicity of malathion in buffalo calves (Gupta and Paul, 1978), in acute toxicity of ethaphos in rabbits (Baimuradov et al., 1981), in fenitrothion poisoning in pigeons (Mandal and Lahiri, 1985). The increase in total WBC count was significant ($P < 0.01$) only in group IV and V birds maintained for twentysix days on malathion feeding. The increase in total WBC count was significant ($P < 0.05$) in group II and highly significant ($P < 0.01$) in group III birds maintained for seventyfive days on malathion feeding.

A similar increase in total leucocyte count in a variety of toxic conditions was reported by Wintrobe (1961), Florey(1962), Petrichev and Lazarov (1970) in sub-acute organophosphorous pesticide toxicity in rabbits, Hothi and Kwatra (1972) in buffalo calves fed with malathion and aldrin, Bello and Torbet (1972) in pony foals acutely intoxicated with shell SD 15803, Vadlamudi (1974) in sub-acute malathion and sumithion poisoning in buffalo calves, Gupta et al. (1985) on feeding nitrofen to bovine calves, Mandal and Lahiri (1985) in fenitrothion poisoning in pigeons. The increase in WBC count may be due to the degenerative and inflamatory reactions produced in different organs by the pesticide (Gupta et al., 1985).

Heterophilic and eosinophilic counts were significantly ($P < 0.05$) increased in group III, IV and V when

compared with group I and within the groups during thirtythree days and seventy five days of feeding the toxin. Heterophilic and eosinophilic counts were also increased significantly ($P < 0.01$) in group II birds maintained for seventyfive days on toxin. Lymphocyte count was significantly ($P < 0.01$) decreased in group III, IV and V as compared to group I and within the groups and also in group II and III maintained for seventy five days. There was no change in monocytic and basophilic counts in groups II to V maintained for thirty three days however, a decrease in monocytic and basophilic count was noticed in group III maintained for seventy five days. Similar changes in heterophilic, eosinophilic, lymphocytic, monocytic and basophilic counts were observed in pheasants during acute toxicity of carbophos (Buteiko, 1983), in calves fed with nitrofen (Gupta et al., 1985), and in pigeons fed with fenitrothion (Mandal and Lahiri, 1985).

Significant ($P < 0.01$) increase in SGPT activity was noticed in group III, IV and V birds fed malathion for thirtythree days. In group II and III which were fed malathion over a period of seventyfive days also showed a significant ($P < 0.01$) increase in the activity. The increase in SGPT activity has been reported to be due to the damage in liver, kidney and heart (Rouiller, 1964) associated with increased permeability of cell

membrane or increased synthesis or decreased catabolism of aminotransferase (Dinman et al., 1963). The increase in SGPT activity in malathion toxicity is in agreement with the observations reported by Wright et al. (1966) in cattle poisoned with coumaphos, Snow and Watson (1973) in acute toxicity of dichlorovos in dogs, Golbs and Kunhart (1973) in rats, Michael (1974) in sub-acute toxicity of DDT, PCB malathion and mercuric chloride in quails, Uppal and Ahmad (1977) in acute and sub-acute toxicity of malathion in buffalo calves.

A decrease in the cholinesterase was noticed in group III ($P < 0.05$) and group IV and V ($P < 0.01$) maintained for thirtythree days on malathion feeding. In birds maintained over a period of seventyfive days on malathion feeding the decrease in cholinesterase activity was highly significant ($P < 0.01$) in group II and III. Decrease in cholinesterase activity has been reported in organophosphorous pesticide toxicity by O'Brien (1960), Srivastava and Parasar (1971) on feeding 50 ml of 5% malathion emulsifiable liquid, Purushotham (1971) on feeding sumithion @ of 10, 100, 1000 and 5000 ppm for 28, 14, 10 days, Gupta and Paul (1971) in malathion toxicity to birds, Vadlamudi (1973) in acute toxicity of sumithion in mice, Michael (1974) in quails fed DDT, PCB malathion and mercuric chloride for 12 weeks,

Chawla et al. (1977) in accidental outbreak of malathion poisoning in birds, Gupta and Paul (1977) in sub-acute toxicity studies of malathion in buffalo calves, Perry (1977) when intraperitoneal injection of malathion was given to mice, Uppal and Ahmad (1977) in acute and sub-acute toxicity studies of malathion in buffalo calves, Gupta and Paul (1978) in Bubalus bubalis when malathion was given as dermal spray @ 0.5-1%, Gupta et al. (1981) on feeding single MLD of malathion to buffalo calves, Vadlamudi and Paul (1979) in acute oral toxicity of malathion in buffalo calves, Chandra et al. (1981) in malathion poisoning in fowls.

The internal organs viz: liver, kidney, Intestine showed slight congestion while there was no such change in heart in group II birds. The degree of these changes were prominent in birds of group III, IV and V. In addition the testes were atrophied and intestines showed haemorrhages and were empty in the birds of group IV and V. The comb and wattles were slightly pale when compared to birds in group I to III. The gross lesions noticed in malathion toxicity were similar to those described by different workers Koger (1955) in acute parathion toxicity in calves and lambs, Jolly (1957) in acute malathion toxicity in birds, Radeleff and Woodard (1957) in organophosphorous toxicity in cattle and sheep, Poloz and Poletskii (1965) in acute poisoning of demeton in birds,

Hothi and Kwatra (1972) in buffalo calves fed with malathion and aldrin, Chawla *et al.* (1977) in accidental outbreak of malathion poisoning in birds, Gupta *et al.* (1981) in buffalo calves fed with MCD of malathion.

The histopathological changes observed in liver in group II birds were in general congestion of the sinusoids and portal triads and Kupffer cell proliferation which was quite prominent. The hepatic cells revealed mild degenerative changes with loss of cytoplamic basophil: and ocassionally few focal areas of necrosis infiltrated with mononuclear cells. These changes were more advanced in birds maintained over a period of seventyfive days of toxin feeding. These changes were severe congestion of the sinusoids, portal trials and central veins. The architecture of the liver was lost with an attempt to form pseudolobulation. Areas of multiple focal liver cell necrosis with infiltration of mononuclear cells were also noticed. The livers in the birds of group III maintained for thirtythree days showed liver changes which included a more severe congestion of sinusoids, portal triads and central veins. There were areas of accumulation of pooled blood. Proliferation of Kupffer cells was very prominent. The liver also showed distortion of lobular pattern and this distorted lobule was surrounded by a delicate fibrous

tissue, an attempt to form early cirrhosis. These changes were well advanced in group III birds maintained over a period of seventyfive days. Livers in group IV birds maintained over a period of twentysix days showed a severe and extensive congestion of sinusoids, portal veins, central veins, distortion of hepatic cords and multiple areas of liver cell necrosis. Group V birds also showed similar changes. The histopathological changes studied in liver were similar to those described by Cleveland and Treon (1961) in acute phosdrin poisoning in birds, Chawla et al. (1977) during an accidental outbreak of malathion poisoning in birds, Sarin and Saxena (1978) on giving intraperitoneally single dose of quinalphos to Indian desert gerbil, Chopra et al. (1980) in acute and chronic toxicity of metasystox-R and fenthion in male chicks, Gupta et al. (1981) in acute malathion poisoning in buffalo calves, Wilson et al. (1982) in sheep acutely intoxicated with triorthocresyl phosphate.

Birds maintained for thirty three days in group II the kidneys showed slight congestion associated with interstitial haemorrhages. The tubular epithelium had degenerative changes like cloudy swelling and vacuolation, At places the glomerular endothelium showed proliferation, These changes were extensive and diffuse in group II birds maintained over a period of seventyfive days.

In group III birds which were maintained over a period of thirty three days the kidneys showed interstitial haemorrhages and few pockets of localised haemorrhages. The tubular epithelium showed diffuse degenerative changes. Epithelial casts were found in tubules as a result of desquamation of epithelial cells. These changes were still advanced when fed toxin for seventyfive days. In group IV the changes in kidneys were extensive interstitial haemorrhages, increased cellularity of the glomeruli, degenerative changes and vacuolation of tubular epithelial cells. Similar changes but well advanced were observed in group V birds. Similar changes were reported by Cleveland and Treon (1961) in acute phosdrin poisoning in birds, Gupta et al. (1981) in acute malathion poisoning in buffalo calves.

Congestion and Coagulative necrosis of heart was noticed in group V birds only and in other groups the heart appeared normal. Petechiae and hemorrhages have been reported in heart in acute parathion toxicity to calves and lambs (Koger, 1955), in organophosphorous toxicity in cattle and sheep (Radeleff and Woodard, 1957), in acute poisoning of demeton to birds (Polozˇ and Poletskii, 1965), in accidental outbreak of malathion poisoning in birds (Chawla et al., 1977) and in acute malathion poisoning in buffalo calves (Gupta et al., 1981).

Intestine in group II maintained for thirtythree days on toxin showed hyperplasia of laminar epithelium associated with increase in goblet cells and increase in enterochromaffin cells. However, there was no congestion of muscularis mucosa and submucosa. Group II birds maintained for seventyfive days, similar changes were noticed with sloughing of laminar epithelium and necrosis. Group III maintained for thirtythree days, the changes were similar to that noticed in group II birds but they were markedly increased. In group III birds maintained for seventy five days similar changes were observed and also the congestion extended upto muscularis mucosae and submucosa. Group IV and V presented similar hitopathologic changes but were more extensive. The changes include severe congestion, necrosis and sloughing of mucous membrane extending upto muscularis mucosae. Similar histopathological changes in the intestines were reported by Chopra *et al.* (1980) in acute and chronic poisoning of metasystox-R and fenthion in male chicks; Gupta *et al.* (1981) in buffalo calves fed with minimum lethal dose of malathion; Reece (1982) in acute poisoning of malathion in birds.

Testes in group II birds maintained upto thirtythree days presented essentially a normal histological structure, where as the birds in group II maintained over a period of

seventyfive days showed slight degenerative changes of the seminiferous tubules and the spermatogonial cells. In group III birds maintained for thirty three days the testicular changes were more advanced which included degenerative changes in seminiferous tubules, with many spermatogonial cells showing pyknotic and karyorrhexis of nuclei and spermatogenesis was absent in some tubules. The birds which were fed toxin for seventyfive days, showed degenerative cells, tissue debris and oedematous fluid in the lumen of the tubules. Spermatogenesis was completely absent. Basement membrane of the tubule was thickened with a slight increase in the interstitial tissue. The testicular changes noticed in group IV and V were also much similar to the changes observed in group III but were more advanced and there was complete loss of spermatogenesis and the basement membrane of the tubule was more prominent and thick due to increase in interstitial tissue. The histopathological changes noticed corroborates with that reported by Sarin and Saxena (1978) in Indian desert gerbil injected intraperitoneally with single dose of quinalphos; Dikshith *et al.* (1978) in rats given parathion and lindane orally for 90 days; Saxena and Sarin (1979) in Indian desert gerbil on repeated administration of thimet 0.6 mg/kg b.wt. intraperitoneally for seventyfive days.

Uppal and Singh (1981) reported that feeding of 500 ppm level of malathion was safe to chicks and feeding of 1000 ppm, 2,500 and 5,000 ppm were found to be highly toxic and lethal. In this study the dosage ranged from 50 mg to 200 mg/kg b.wt. or 800 ppm to 3,200 ppm and it was observed that feeding of 800 ppm for thirtythree days did not show any signs of toxicity but it could produce slight haematochemical and histopathological changes. Thus it can be concluded from the studies undertaken that feeding of malathion 50 mg/kg b.wt. for a short duration in nontoxic, whereas administration of 50 mg/kg body weight for seventyfive days, and 100 mg, 150 mg and 200 mg/kg body weight for short and long term definitely brought about haemotological, biochemical and histopathological changes in the birds.

CHAPTER - VI

SUMMARY

Fifty white leg horn cockrels of five weeks age were reared in deep litter system. Initial weights of these birds were recorded and divided into five groups with ten birds in each group, having approximately equal weights. Malathion obtained from Cyanamid Company was dissolved in distilled water to make concentrations of 50 mg; 100 mg; 150 mg and 200 mg/ml. The birds in group II were fed with malathion 50 mg/kg b.wt, group III with 100 mg/kg b.wt, group IV with 150 mg/kg b.wt. and group V with 200 mg/kg b.wt., while the birds in group I did not receive any toxin and served as control. Weekly body weights of the birds were recorded, and toxin was fed orally daily according to their body weights. All the birds in the five groups were maintained on a commercial ration purchased from a private poultry feed company. These birds were fed adlibitum. Five birds from grpup I, II and III were sacrificed after thirtythree days of feeding malathion to determine the short term effects while the remaining birds were continued in their feeding schedule for seventyfive days to find out the long term effects. The birds in group IV and V started dying within 1st week of the experiment and hence all the birds in group IV and V were sacrificed by twentysixth day. There was no mortality in group I, II and III during

the experiment while in group IV two birds died in 3rd week and also in fourth week and in group V, two birds died in the first week and three in second week.

The birds in control group showed a gradual increase in their weekly body weights. The birds fed with malathion 50 mg/kg b.wt and 100 mg/kg b.wt. showed a gradual increase in their weekly body weights as against their initial body weights, the gain in body weights were less when compared to that of group I birds. The weekly body weights were reduced significantly ($P < 0.01$) at the time of killing in group IV (150 mg/kg b.wt) and group V (200 mg/kg b.wt) during thirtythree days of feeding the toxin, when compared to group I, II and III. Significant reduction ($P < 0.01$) in weekly body weights was observed from sixth week onwards in group III maintained for seventyfive days as compared to group I and II. The reduction in their body weight is proportional to the level of toxin fed.

Haematological investigations viz., PCV, Haemoglobin total RBC, WBC and differential counts were determined in the birds of all the groups. Feeding of malathion 50 mg (group II) and 100 mg/kg b.wt (group III) orally for thirtythree days resulted in a decrease in PCV, haemoglobin ($P < 0.05$) and total RBC count ($P < 0.01$) while there was an increase ($P < 0.01$) in neutrophilic,

eosinophilic count and a decrease in lymphocytic count ($P < 0.01$) in group III only. In birds maintained with these levels of toxin over a period of seventyfive days in addition to these changes, there was significant increase ($P < 0.01$) in total WBC, heterophilic and eosinophilic counts. Thus feeding of malathion 100 mg/kg b.wt (1600 ppm) over a period of seventyfive days produced more significant haematological changes than that could be produced by feeding the same dose for thirtythree days. Feeding of malathion 150 mg/kg b.wt (2,400 ppm) and 200 mg/kg b.wt. (3,200 ppm) for twentysix days resulted in a significant reduction in PCV, Hb, total RBC ($P < 0.01$) count and an increase in total WBC count, heterophils, eosinophils ($P < 0.01$) and a reduction in lymphocytic ($P < 0.01$) count. Hence the effects of these two dose levels were more severe than the other two levels in a short period of twentysix days.

An increase ($P < 0.01$) in SGPT activity was noticed in group III, IV and V which were fed with 100 mg, 150 mg, and 200 mg/kg b.wt. over a period of twentysix to thirtythre days as against groups I to IV. Thus feeding of 100 mg (1600 ppm), 150 mg (2,400 ppm) and 200 mg (3,200 ppm)/kg b.w over a period of thirtythree days brought about an increase in the SGPT activity. Groups II & III birds maintained over a period of seventyfive days also showed a significant increase ($P < 0.01$) in the SGPT activity.

Serum cholinesterase activity was markedly decreased ($P < 0.01$) in group IV and V which were fed with 150 mg and 200 mg of malathion respectively over a period of twentysix days, while the reduction in activity was ($P < 0.05$) in birds fed malathion 100 mg/kg b.wt over a period of thirtythree days. There was no change in the activity in birds fed 50 mg/kg b.wt (group II) and in control (group I) birds. The birds (group II) maintained over a period of seventyfive days also showed a significant ($P < 0.01$) decrease in the activity. Further, feeding of 50 mg/kg b.wt. over a period of seventyfive days brought about a decrease ($P < 0.01$) in cholinesterase activity.

Gross changes of the internal organs were more prominent in group IV and V than the other groups. The gross changes include varying degrees of congestion and enlargement of liver and kidneys. Intestines showed slight congestion. Heart and adrenals did not show any change. Testes were atrophied in group IV and V only.

Feeding of malathion 50 mg/kg b.wt over a period of thirtythree days showed slight congestion in sinusoids and portial triads. Degenerative changes in the hepatic cells viz. loss of basophilia cloudy, swelling and few areas of focal liver cell necrosis were observed. The kupffer cells showed proliferation. In birds maintained for seventyfive days these changes were severe and there

were areas of multiple focal liver cell necrosis. The group III birds fed 100 mg/kg b.wt. of malathion for thirtythree days revealed loss of liver lobular pattern, congestion and severe haemorrhages and an attempt to form early cirrhosis. These changes were well developed in birds maintained for seventyfive days on toxin. In group IV and V these changes observed were similar but more advanced and severe.

Kidneys in group II birds maintained for thirtythree days showed a slight congestion with interstitial haemorrhages. Tubular epithelium showed degenerative changes like cloudy swelling and vacuolation, at places glomerular endothelium was proliferated. These changes were more severe and diffuse in birds maintained for seventyfive days. Group III maintained for thirtythree days showed interstitial haemorrhages degenerative changes of tubular epithelium and casts in the lumen. These changes were much advanced in birds maintained for seventyfive days. Extensive interstitial haemorrhages, degenerative changes of tubular epithelium, vacuolation and hypercellularity of the glomerular tufts were observed in group IV and V birds maintained for twentysix days. Heart did not show any specific histopathological changes in group I, II and III, however slight congestion and few focal areas of coagulative necrosis of cardiac musculature was noticed in group IV and V.

Intestines in group II showed hyperplasia of laminar epithelium with increase in goblet cells and enterochromaffin cells. These changes were severe in birds fed toxin for seventyfive days. All the changes described in group II were also seen in group III birds maintained for thirtythree days and seventyfive days but these changes were severe and extended upto submucosa. Similar changes were also noticed in birds fed malathion 150 mg/kg b.wt (Group IV) and 200 mg/kg b.wt (group V).

The testicular changes were noticed in group II birds maintained for 75 days only. Testicular changes were also noticed in group III, IV and V. The changes noticed were degenerative changes in spermatogonial cells, accumulation of tissue debris and oedematous fluid in the lumen of the seminiferous tubules and absence of spermatogenesis. An increase in interstitial tissue with thickening of the basement membrane was also noticed.

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* Originals not seen.

V I T A

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