INVESTIGATION OF PHARMACOLOGICAL PROFILE OF SOME ENDOCRINE DISRUPTORS IN MALE RATS

A THESIS

Submitted in partial fulfilment of the requirements for the award of the degree of

DOCTOR OF PHILOSOPHY

in

BIOTECHNOLOGY

by

SHARAD SHARMA

DEPARTMENT OF BIOTECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY ROORKEE
ROORKEE - 247 667 (INDIA)

MARCH, 2009

INDIAN INSTITUTE OF TECHNOLOGY ROORKEE
ROORKEE

CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **INVESTIGATION OF PHARMACOLOGICAL PROFILE OF SOME ENDOCRINE DISRUPTORS IN MALE RATS** in partial fulfillment of the requirements for the award of the degree of Doctor of Philosophy and submitted in the Department of Biotechnology of the Indian Institute of Technology Roorkee, Roorkee is an authentic record of my own work carried out during the period from January, 2003 to March, 2009 under the supervision of Dr. Partha Roy, Assistant Professor, Department of Biotechnology, Indian Institute of Technology Roorkee, Roorkee, India.

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other Institute.

(SHARAD SHARMA)

This is to certify that the above statement made by the candidate is correct to the best of my knowledge.

(Partha Roy)
Supervisor

Date: 18/3/09

The PhD Viva-Voce Examination of **Mr, Sharad Sharma** Research Scholar has been held on .....

Signature of Supervisor

Signature of External Examiner

ABSTRACT

Endocrine disrupting chemicals (EDC) are the natural or synthetic compounds which mimic or inhibit the actions of endogenous hormones or modulate the synthesis of latter and are said to adversely affect the normal functioning of the endocrine system. They can be broadly categorized as (anti) androgenic, (anti)estrogenic or (anti) progestagenic chemicals depending on their effects on the production or functions of those steroids (i.e. androgens, estrogens, progesterone respectively). EDC may enter into the physiological system of animals/humans through the diet or occupational exposure and have been related with the occurrence of a number of hormone-sensitive disease/disorders like reduced fecundity, abnormal fetal development, delayed onset of puberty, cryptorchadism, abnormal lactation, testicular dysfunction and even various types of cancers

The present work describes action of two major pesticides: Chlordane and Monocrotophos. We investigated chlordane, monocrotophos for endocrine disruptions and reproductive toxicity in animal model (in vivo). Our aim was to evaluate the hazardous effects of chlordane and monocrotophos in order to establish a relationship between the exposure to these insecticides (through any modes) and occurrence of various human diseases.

Results showed that oral administration of chlordane and monocrotophos caused a significant reduction in the overall body weight along with weight of testis and accessory sex organs. However, the weight of the liver was increased significantly. Negative fertility was observed at high dose of chlordane and monocrotophos. Motility of sperms in cauda epididymides was decreased markedly after oral administration of chlordane and monocrotophos at all dose levels. Sperm density in the testis and cauda epididymides was decreased significantly in rats treated with chlordane and monocrotophos at all dose levels. A significant decrease was observed in serum testosterone concentration in the chlordane and monocrotophos treated rats at higher dose levels which was indicative of its anti-androgenic activities. Total erythrocyte count (TEC), haemoglobin concentration and haematocrit values were significantly decreased at various dose levels of chlordane and monocrotophos treatment. Whereas the total leukocyte count (TLC) was increased in chlordane and monocrotophos exposed rats at all the dose levels.

Blood urea and blood sugar, bilirubin concentration, serum protein were significantly increased. Serum phospholipids, Serum cholesterol, HDL-Chol, LDL-Chol and VLDL-Chol levels were increased at all the dose levels. Degenerative changes in the histoarchitecture of testis and reduced amount of secretion in seminal vesicle and prostate was also observed. The histoarchitecture of testis was altered markedly. Inhibition of spermatogenesis, shrunken and damaged seminiferous tubules with increased interstitial spaces, degenerated and vacuolated sertoli cells, presence of vacuoles in the epithelium, disruption of Leydig cells were conspicuous. Epididymes showed reduced number of spermatozoa in the lumen of caput and cauda regions after the treatment with chlordane and monocrotophos. The epithelial lining was regressed. Histopathologically size of the liver was found to be increased (hypertrophy) and various histopathological alterations were observed in the kidney which includes glomerulonephritis, glomeruloscleroses, odenema, pycnotic nuclei and glomerulus deposits.

The results of the present study revealed that the administration of chlordane and monocrotophos insecticides induces reproductive toxicity as well as hepato-nephrotoxicity in male albino rats. In which mating test showed 100% negative fertility that is the state of sterility. Low concentration of spermatozoa in testis and cauda epididymides and reduction in number of spermatogenic elements in testis reflects antispermatogenic nature of chlordane and monocrotophos insecticides. Increase in acid phosphatase, aspartate, alanine transferase and bilirubin indicates the hepatotoxic action of insecticides due to cellular damage. Increase blood urea and pathological alterations further suggests the chlordane and monocrotophos induced intoxication on the structure and function of kidney.

Thus, in this study above results indicated that toxic effect of monocrotophos is more pronounced then that of chlordane on mammals, taking rat as a biological indicator.

Abbreviations-

EDC : Endocrine Disrupting Chemical; cAMP : Cyclic Adenosine Mono Nucleotide Phosphate; ALT: Alanine amino Transferase; AST: Aspartate amino Transferase

Acknowledgment

The flash back view of the period that I spent at the Indian Institute of Technology, Roorkee and thereafter, shows that there are number of people, without their valuable and timely inputs, guidance, cooperation, support, and help at various stages of my work, it would have been practically impossible for me to complete this thesis work. I fall short of words to express my sincere gratitude and appreciation to these personalities.

My Ph.D. supervisor, respectable Dr. Partha Roy, Assistant Professor, Department of Biotechnology, IIT Roorkee is on the top to whom I am deeply indebted and lack short of words in expressing my gratitude and ever lasting gratefulness to his guidance for the completion of this work. His dedication, unflinching zeal, penchant for work, invaluable suggestions and support has been an unforgettable experience for me. With his enthusiasm, inspiration, patience and great efforts to explain things clearly and simply, I was able to transform this PhD venture into a pleasant and memorable journey in spite of all the odds involved in this work.

I owe a duty to pay tribute to my respectable former guide Late Prof. Ben M. J. Periera, the former Head of Department of Biotechnology, IIT Roorkee, who had always been a role model for me during his whole tenureship as my guide and Head of the Department. This thesis is the result of his untiring efforts to give shape to my inclination, ideas and thoughts towards this work. His sudden demise has deprived not only me but others also from his able guidance. It is an irreparable loss for all of us.

I have deep and sincere sense of gratitude towards Prof. Ritu Barthwal, Dr. R.P Singh, Dr.R. Prasad, Department of Biotechnology, Indian Institute of Technology, Roorkee, for providing me the necessary infrastructure for the smoothly submission of

my thesis. I would also like to thank Prof. G. S. Randhawa for providing me in house research facilities and excellent working environment.

I would also like to thank Professor H. S. Dhaliwal and Dr. A.K. Sharma, Department of Biotechnology, Dr. V. K. Gupta, Department of Chemistry, Indian Institute of Technology, Roorkee for their encouragement and providing me the help of research facilities of their respective labs.

It is my great pleasure that I was able to avail the guidance and research facility from Dr. M.M.Ali, Scientist F and Deputy Director, Industrial Toxicological Research Centre (ITRC), Lucknow. I am also thankful to Prof. Ashwani Kumar Tiwari, Former Director, Centre for Biotechnology, and Prof. P.D. Sharma, Head of Department of Chemistry, University of Rajasthan, Jaipur for their valuable guidance and moral support in completion of this work. Words fail to express my gratitude to Dr. R.S.Gupta, Assistant Professor, Department of Zoology, University of Rajasthan, Jaipur for providing cooperation, and assistance in completing the most important work of critical data analysis.

I shall always feel obliged to Prof. Mahesh Chand Sharma, Director, National Institute Of Ayurveda (NIA), Jaipur and Dr. A.K.Srivastava, Director, Institute of Pharmaceutical Research, Jaipur for providing desired liberty to conduct the experiments for this project.

I am also thankful to Dr. B.Lal's Diagnostice Center, Jaipur for carrying out bio chemical examinations of my various samples.

With great pleasure I express my sincere thanks to the departmental staff Department of Biotechnology, IIT, Roorkee for providing me help in all possible ways.

It goes without saying that the contribution of lab mates matters a lot in completing such arduous task. I would like to thank my lab mates Mr. Manish Rana, Mr.

Ashish Das Gupta, Mr.Vikas Pundeer, Mr. Deepak Sharma, Mr. Deepankar Gahlot, Mr.Vijay Tiwari, Ms. Ajanta Chakraborty and Ms. Rajani Salunke for their cooperation, support and providing me with a homelike atmosphere in the lab. .

I owe an immense sense of gratitude to my parents, Mr.N.R.Sharma, a retired District & Sessions Judge and Mrs. Shyama Devi Sharma, who brought me up to be a confident individual and always believed in my abilities to finish the things at my disposal. Their love, support, inspiration and passion for education are highly regarded. Their devotion always kept my motivation up. Words can hardly explain the cooperation extended by my elder brothers Shri Ravi Sharma, Additional District & Sessions Judge, Shri Manvendra Sharma, Pharmacist, Shri Vijay Sharma and Shri Manoj Sharma Advocates who have always been with me having love and cheer all the time. I would also like to thank a lot to my Bhabhi, Dr.Neelam Pancholi, Dr. Rekha Sharma, Anita Sharma (Advocate) and Sarita Sharma who always kept me enthused with new ideas and for all of their cooperation without any complaint for paucity of time.

I would also thank Mr. Hari Singh & Mr. S.N. Joshi for extending their cooperation and support in writing thesis work and Mr. Sawan Kumar for helping me against all odds.

Last but not the least I am thankful to the almighty who gave me the strength and health for completing this work.

I thankfully acknowledge Ministry of Human Resources and Development (MHRD) to grant Fellowship for providing me financial assistance for this thesis.

Date 18-3-09 **(SHARAD SHARMA)**

Contents

Page No.

CANDIDATE DECLARATION
ABSTRACT I-II
ACKNOWLEDGEMENT III-V
CONTENTS VI-IX
LIST OF FIGURES X-XIII
LIST OF TABLES XIV
LIST OF ABBREVIATION XV-XVII

CHAPTER-1 : INTRODUCTION

1.1 Environment. 1
1.2 Natural Environment. 1
1.3 Human body system. 1
1.4 Endocrine system. 1
1.5 Hormones. 1
1.6 Endocrine and reproductive system of animals. 3
1.7 The pituitary. 3
1.8 The pineal. 4
1.9 The thyroid. 4
1.10 The parathyroids. 4
1.11 The thymus. 5
1.12 The pancreas. 5
1.13 The adrenals. 5
1.14 The ovaries. 6
1.15 The testis. 6
1.16 Endocrine disorders. 7
1.17 What are Endocrine disruptors. 7
1.18 Few of the health effects. 8
1.19 List of some major Endocrine Disruptors. 11
1.19.1 Persistent Organohalogens. 11
1.19.2 Pesticides. 11
1.19.3 Phthalates. 11
1.19.4 Others. 11
1.19.5 Metals. 11
1.19.6 Pharmaceuticals. 11
1.20 Scope of work 12

CHAPTER-2 : LITERATURE REVIEW

2.1 Pesticides. 13
2.2 Historical Aspect. 14
2.3 Indian Scenario. 14
2.4 Adverse in vivo effects of EDC to human health. 15
2.5 Endocrine Disruptors: Different Sources, Categories (types) and biological relevance. 16
2.6 Endocrine Disruption. 16
2.6.1 Decent of the concept and definition. 16
2.7 Pesticide Cycle. 18

2.8 Pest Control. 18
2.9 Pharmacodynamics of Insecticides. 19
2.9.1 Organochlorine insecticide. 19
2.9.2 Organophosphate insecticide. 19
2.10 Classification. 20
2.10.1 Aliphatic. 21
2.10.2 Phenyl Derivatives. 21
2.10.3 Heterocyclic Derivatives. 21
2.11 Organophosphates as toxicants. 21
2.12 Practical uses. 23
2.13 Mode of Toxicity. 23
2.13.1 Monocrotophos. 24
2.13.1.1 Chronic toxicity and carcinogenicity. 24
2.13.1.2 Mutagenicity and genotoxicity. 26
2.13.1.3 Reproductive Toxicity. 27
2.14 Key health issues. 30
2.15 Organochlorine as toxicant. 30
2.15.1 Chlordane 33
2.15.1.1 Neuro Toxicity. 34
2.15.1.2 Hepato Toxicity. 34
2.15.1.3. Carcinogenicity. 35
2.15.1.4. Reproductive Toxicity. 35
2.15.1.5. Immuno Toxicity. 36
2.16. (Anti) Androgenic assay. 38

CHAPTER-3 : MATERIALS AND MATHODS
3.1 Insecticides selected for experiment. 39
3.1.1 Organochlorine. 39
3.1.2 Organophosphate. 40
3.2 Mode of Action. 42
3.3 Experimental Model. 42
3.4 Experimental Design. 42
3.5 Median Lethal Dose (LD_{50}). 43
3.6 Treatment Protocol. 43
3.6.1 Chlordane. 43
3.6.2 Monocrotophos. 44
3.7 Mode of administration. 44
3.8 Parameters studied. 44
3.8.1 Body and Organ Weights. 44
3.8.2 Sperm Dynamics. 44
3.8.2.1 Sperm Motility. 44
3.8.2.2 Sperm Density. 45
3.8.3 Histological Studies. 45
3.8.3.1 Histopathology of various tissues. 45
3.8.4 Hematological Studies. 46
3.8.5 Serum Analysis. 46
3.8.6 Statistical Analysis. 46
3.8.7 Serum Alkaline Phosphate, Acid Phosphate, SGPT and SGOT level 47
3.8.7.1 Serum acid phosphatase. 48

3.8.7.2 Serum alkaline phosphatase. 49
3.8.7.3 Serum SGOT 49
3.8.7.4. Serum SGPT. 49
3.8.7.5. Blood Cholesterol. 50
3.8.8 Hershberger Assay. 50
3.8.8.1 Chemicals. 50
3.8.8.2 Animals and Housing. 50
3.8.8.3 Procedure of Hershberger Assay. 51
3.8.8.4 Statistical analysis. 51
3.8.9 Behavioral Toxicity. 51
3.8.9.1 Investigation on sexual behavioral (Libido) on male rats by insecticides. 52
3.8.9.2 Procedure. 52

CHAPTER-4 : OBSERVATIONS AND RESULTS

Insecticides. 59
4.1CHLORDANE 59
4.1.1 Weight response. 59
4.1.2 Sperm dynamics 60
4.1.3 Hematology. 61
4.1.4 Bio-chemical findings. 62
4.1.5 Radio Immuno Assay (RIA) 64
4.1.6 Histology study of chlordane treated rats. 71-102
4.2 MONOCROTOPHOS 108
4.2.1 Weight response. 108
4.2.2 Sperm dynamics. 109
4.2.3 Hematology. 110
4.2.4 Biochemical findings. 111
4.2.5 Radio Immuno Assay (RIA). 113
4.2.6 Histology study of monocrotophos treated rats. 120-167
4.3 Result of Hershberger Assay. 168
4.3.1 Androgen dependent accessory sex organ weights. 168
4.3.2 Liver, kidney, adrenal gland weight. 168
4.4 Result of courtship behavioral study. 169

CHAPTER- 5 : DISCUSSION

5.1 Weight response. 170
5.2 Sperm dynamics. 170
5.3 Sperm density. 171
5.4 Serum testosterone. 171
5.5 Hematology. 172
5.6 Total Erythrocyte Count (TEC). 172
5.7 Total Leucocyte Count (TLC). 173
5.8 Hemoglobin. 173
5.9 Hematocrait Values. 173
5.10 Serum Biochemistry. 174
5.10.1 Asparate amino transferase and Alaninie amino transferase. 174
5.10.2 Acid phosphatase and Alkaline phosphatase. 174
5.10.3 Bilirubin. 175

5.10.4 Total Cholesterol. 175
5.10.5 Blood glucose. 175
5.10.6 Blood urea. 176
5.11 Histopathology. 177
5.11.1 Testis. 177
5.11.2 Epididymides. 178
5.11.3 Seminal vesicle. 179
5.11.4 Prostate gland. 179
5.11.5 Vas deferens. 179
5.11.6 Liver. 179
5.11.7 Kidney. 180
5.12 Discussion of Hersberger Assay. 181
5.13 Discussion of courtship behavioral study. 182

CHAPTER- 6 :SUMMARY AND CONCLUSION
6.1 Weight response. 183
6.2 Effect on fertility. 183
6.3 Antispermatogenic effects. 184
6.4 Antiandrogenic effect. 184
6.5 Hematology. 184
6.6 Serum biochemical analysis. 184
6.7 Histoarchitecture. 185
6.8 Conclusion. 185

CHAPTER- 7 : BIBLIOGRAPHY 187-207

LIST OF FIGURES

Figure No.	Figure Title	Page No.
1	Endocrine organs of human body.	3
2.	Mechanism of endocrine disrupter.	9
3.	A hypothetical model depicting the possible mechanisms of Endocrine disruption.	10
4.	Figure showing sex arousal in male rats treated with chlordane and monocrotophos.	53
Plate No.	**Figures of histology plates of Chlordane**	
1.	Micro-photographs of Testis of rats at various doses of chlordane exposed for 2 weeks.	72
2.	Micro-photographs of Testis of rats at various doses of chlordane exposed for 5 weeks.	74
3.	Micro-photographs of Vas deferens of rats at various doses of chlordane exposed for 2 weeks.	76
4.	Micro-photographs of Vas deferens of rats at various doses of chlordane exposed for 5 weeks.	78
5.	Micro-photographs of Seminal Vesicle of rats at various doses of chlordane exposed for 2 weeks.	80
6.	Micro-photographs of Seminal Vesicle of rats at various doses of chlordane exposed for 5 weeks.	82
7.	Micro-photographs of Cauda Epididymis of rats at various doses of chlordane exposed for 2 weeks.	84
8.	Micro-photographs of Cauda Epididymis of rats at various doses of chlordane exposed for 5 weeks.	86
9.	Micro-photographs of Caput Epididymis of rats at various doses of chlordane exposed for 2 weeks.	88

10.	Micro-photographs of Caput Epididymis of rats at various doses of chlordane exposed for 5 weeks.	90
11.	Micro-photographs of Ventral Prostate of rats at various doses of chlordane exposed for 2 weeks.	92
12.	Micro-photographs of Ventral Prostate of rats at various doses of chlordane exposed for 5 weeks.	94
13.	Micro-photographs of Liver of rats at various doses of chlordane exposed for 2 weeks.	96
14.	Micro-photographs of Liver of rats at various doses of chlordane exposed for 5 weeks.	98
15.	Micro-photographs of Kidney of rats at various doses of chlordane exposed for 2 weeks.	100
16.	Micro-photographs of Kidney of rats at various doses of chlordane exposed for 5 weeks.	102
	Figures of histology plates of Monocrotophos	
17.	Micro-photographs of Testis of rats at 6 mg/kg b.wt/day dose of monocrotophos exposed for 2 and 5 weeks respectively.	121
18.	Micro-photographs of Testis of rats at 14 mg/kg b.wt/day dose of monocrotophos exposed for 2 and 5 weeks respectively.	123
19.	Micro-photographs of Testis of rats at 22 mg/kg b.wt/day dose of monocrotophos exposed for 2 and 5 weeks respectively.	125
20.	Micro-photographs of Cauda Epididymis of rats at 6 mg/kg b.wt/day dose of monocrotophos exposed for 2 and 5 weeks respectively.	127
21.	Micro-photographs of Cauda Epididymis of rats at 14 mg/kg b.wt/day dose of monocrotophos exposed for 2 and 5 weeks respectively.	129
22.	Micro-photographs of Cauda Epididymis of rats at 22 mg/kg b.wt dose of monocrotophos exposed for 2 and 5 weeks respectively.	131
23.	Micro-photographs of Caput Epididymis of rats at 6 mg/kg b.wt/day dose of monocrotophos exposed for 2 and 5 weeks respectively.	133

24.	Micro-photographs of Caput Epididymis of rats at 14 mg/kg b.wt/day dose of monocrotophos exposed for 2 and 5 weeks respectively.	135
25.	Micro-photographs of Caput Epididymis of rats at 22 mg/kg b.wt dose of monocrotophos exposed for 2 and 5 weeks respectively.	137
26.	Micro-photographs of Seminal Vesicle of rats at 6 mg/kg b.wt/day dose of monocrotophos exposed for 2 and 5 weeks respectively.	139
27.	Micro-photographs of Seminal Vesicle of rats at 14 mg/kg b.wt/day dose of monocrotophos exposed for 2 and 5 weeks respectively.	141
28.	Micro-photographs of Seminal Vesicle of rats at 22 mg/kg b.wt dose of monocrotophos exposed for 2 and 5 weeks respectively.	143
29.	Micro-photographs of Ventral Prostate of rats at 6 mg/kg b.wt/day dose of monocrotophos exposed for 2 and 5 weeks respectively.	145
30.	Micro-photographs of Ventral Prostate of rats at 14 mg/kg b.wt/day dose of monocrotophos exposed for 2 and 5 weeks respectively.	147
31.	Micro-photographs of Ventral Prostate of rats at 22 mg/kg b.wt dose of monocrotophos exposed for 2 and 5 weeks respectively.	149
32.	Micro-photographs of Vas deferens of rats at 6 mg/kg b.wt/day dose of monocrotophos exposed for 2 and 5 weeks respectively.	151
33	Micro-photographs of Vas deferens of rats at 14 mg/kg b.wt/day dose of monocrotophos exposed for 2 and 5 weeks respectively.	153
34	Micro-photographs of Vas deferens of rats at 22 mg/kg b.wt/day dose of monocrotophos exposed for 2 and 5 weeks respectively.	155
35	Micro-photographs of Liver of rats at 6 mg/kg b.wt/day dose of monocrotophos exposed for 2 and 5 weeks respectively.	157
36	Micro-photographs of Liver of rats at 14 mg/kg b.wt/day dose of monocrotophos exposed for 2 and 5 weeks respectively.	159
37	Micro-photographs of Liver of rats at 22 mg/kg b.wt/day dose of monocrotophos exposed for 2 and 5 weeks respectively.	161

38	Micro-photographs of Kidney of rats at 6 mg/kg b.wt/day dose of monocrotophos exposed for 2 and 5 weeks respectively,	163
39	Micro-photographs of Kidney of rats at 14 mg/kg b.wt/day dose of monocrotophos exposed for 2 and 5 weeks respectively.	165
40	Micro-photographs of Kidney of rats at 22 mg/kg b.wt/day dose of monocrotophos exposed for 2 and 5 weeks respectively.	167

LIST OF TABLES

Table No.	Table Title	Page No.
1	Effect of various doses of chlordane on body and organ weights.	54
2	Effect of various doses of chlordane on sperm dynamics and fertility.	55
3	Effect of various doses of chlordane on hematology (blood analysis).	56
4	Effect of various doses of chlordane on serum analysis e.g. ALT, AST, Acid and Alkaline phosphatase etc.	57
5	Effect of various doses of chlordane on total protein, phospholipids, triglycerides, cholesterol etc.	58
6	Effect of various doses of monocrotophos on body and organ weights.	103
7	Effect of various doses of monocrotophos on sperm dynamics and fertility.	104
8	Effect of various doses of monocrotophos on hematology (blood analysis).	105
9	Effect of various doses of monocrotophos on serum analysis e.g. ALT, AST, Acid and Alkaline phosphatase etc.	106
10	Effect of various doses of monocrotophos on total protein, phospholipids, triglycerides, cholesterol etc.	107
11	(Anti-) androgenic effect of chlordane and monocrotophos in castrated rats.	168
12	Effect on feed and water consumption in Hershberger assay.	168
13	Courtship behavioral study results for chlordane and monocrotophos treated rats.	169

ABBREVIATIONS USED

ACh	Acetylcholine.
AChE	Acetyl Cholinesterase.
AIIMS	All India Institute of Medical Sciences.
ALT	Alanine Amino Transferase.
AP	Anterior Pituitary.
AST	Aspartate AminoTransferase.
ATP	Adenosine Tri Phosphate.
BBP	Butyl Benzyl Phthalate.
b.wt	Body Weight.
BHC	Benzene Hexa Chloride.
CBS	Central Serum Blank.
CNS	Central Nervous System.
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments On Animals.
Dl	Deciliter.
DBCP	1,2- dibromo -3- Chlorpropane.
DBP	Di-n-Butyl Phthalate.
DCHP	Dicyclohexyl phthalate.
DDT	Dichloro Diethyl Tetrachloro.
DEHP	Di-ethylhexyl Phthalate.
DEP	Diethyl Phthalate.
DES	Diethylstilbestrol.
DHP	Di-hexyl Phthalate.
DNA	Deoxyribo Nucleic Acid.
DPP	Di-n-pentyl Phthalate.
EDC	Endocrine Disrupting Chemical.
EFSA	European Food Safety Authority.
EHP.	Environmental Health Protection.

EPA	Environmental Protection Agency.
FAO	Food and Agriculture Organization.
Fig.	Figure.
FSH	Follicular Stimulating Hormone.
G.	Gram.
GABA	Gamma-Amino Butyric Acid.
Hb	Hemoglobin.
HCH	Hexachlorocyelohexane.
HDL	High Density Lipid.
H & E	Haematoxyline and Eosin.
HPLC	High-Performance Liquid Chromatography.
IARC	International Agency for Research on Cancer.
IDRC	International Development Research Centre.
ILO	International Labour Organisation.
IP	Intraperitoneal.
ITRC	Industrial Toxicology Research Centre.
IU	International Unit.
IUPAC	International Union of Pure and Applied Chemistry.
Kg.	Kilogram.
LD_{50}	Median Lethal Dose.
LDL	Low Density Lipid.
LH	Luteinizing Hormone.
LOAEL	Lowest-Observed-Adverse-Effect Level.
M.	Molarity.
MCV	Mean Cell Volume.
MCH	Mean Cell Hemoglobin.
mg.	Milligram.
ml.	Milliliter.
M.P.	Melting Point.
MT	Metric Ton.
NADP	Nicotinamide Adenine Dinucleotide Phosphate.

NCI	National Cancer Institute (USA).
Ng	Nanogram.
Nm	Nanometer.
NOAEL	No-Observed-Adverse-Effect Level.
NS	Non-Significant.
OC	Organo Chlorine.
OICs	Organochlorine Insecticides.
OP	Organo Phosphate.
PBB	Poly Bromlnated Biphenyls.
PCBs	Polychlorinated Biphenyl.
PIC	Picrotoxin.
PNPP	P-Nitro Phenylphosphate.
PP	Posterior Pituitary.
PPM	Part Per Million.
PVC	Polyvinyl Chloride.
%	Percentage.
RBC	Red Blood Cell.
RIA	Radio-Immuno Assay.
SEM	Standard Error of Mean.
TLC	Total Leckocytes Count.
T-NP	Tartrate Non- Prostatic.
USEPA	United State Environment Protection Agency.
VLDL	Very Low Density Lipid.
v/v	Volume Per Volume.
Wt	Weight.
WHO	World Health Organization.
µ	Micron.

CHAPTER – 1
INTRODUCTION

1.1 Environment

Environment may be defined as the external conditions, resources, stimuli etc. with which organism interacts.

1.2 Natural Environment

It may be defined as all living and non living things that occur naturally on earth. Environmentalism is social moment centered on a concerned for the conservation and improvement of natural environment. Environment preservation is viewed as the stint setting aside of natural resources to prevent damage caused by contact with human activities such as use of hazardous chemicals, fishing, mining, increased industrialization which may lead to damage to environment as well as to human health. Thus public health is directly related to environment.

1.3 Human body system

Human body composed of various biological systems such as respiratory, reproductive, gastro intestinal system, neuronal system, endocrine system, systemic circulatory system, cardio vascular system, lymph system etc.

1.4 Endocrine system

The endocrine system is the network of glands within a human that produces more than 100 hormones to maintain and regulate basic bodily functions. Hormones are chemical substances carried in the bloodstream to tissues and organs, stimulating them to perform some action. The glands of the endocrine system include the pituitary, pineal, thyroid, parathyroids, thymus, pancreas, adrenals, ovaries and testis.

The endocrine system oversees many critical life processes. These involve growth, reproduction, immunity (the body's ability to resist disease), and homeostasis (the body's ability to maintain a balance of internal functions). Endocrinology is science that corelates endocrine glands and the hormones they secrete.

1.5 Hormones

Chemical substances secreted by endocrine glands that are carried in the bloodstream to tissues and organs, stimulating them to maintain and regulate basic bodily functions.

List of hormones, their site of synthesis/release and target organ:-

Hormone	Gland	Target Tissue
Adrenocorticotropic	Pituitary	Adrenal
Aaldosterone	Adrenal	Kidney
Aantidiuretic	Pituitary	Kidney
Atrial naturiuretic	Heart	Kidney
Calcitonin	Thyroids	Bones
Cortisol	Adrenal	Joints or points inflammation
Epinephrine	Adrenal	Medulla of brain
Estrogen	Ovary	Female parts
Fllicle-stimulating or luteinizing	Pituitary	Femeinene parts
Gastrin	Stomach	Digestive system
Glucagon	Pancrease	Pancrease or liver
Growth	Pituitary	Body parts
Insulin	Pancrease	Blood
Melanocyte-stimulating	Pituitary	Skin
Melatonin	Pineal	Femeinene parts
Oxytocin	Pituitary	Uterine wall
Parathyroid	Parathyroid	Bones, Kidney &Intestine
Progesterone	Ovary	Femeinene parts
Prolactin	Pituitary	Mammary gland
Renin	Kidney	Adrenal
Secretin	Intestine	Kidney or Pancrease
Testosterone	Testis	Masculine parts
Thymosin	Thymus	Blood
Thyroid-stimulating	Pituitary	Blood
Thyroxine	Thyroid	Digestive Glands

1.6 ENDOCRINE AND REPRODUCTIVE SYSTEM OF ANIMALS (especially human being are composed of following endocrine organism):

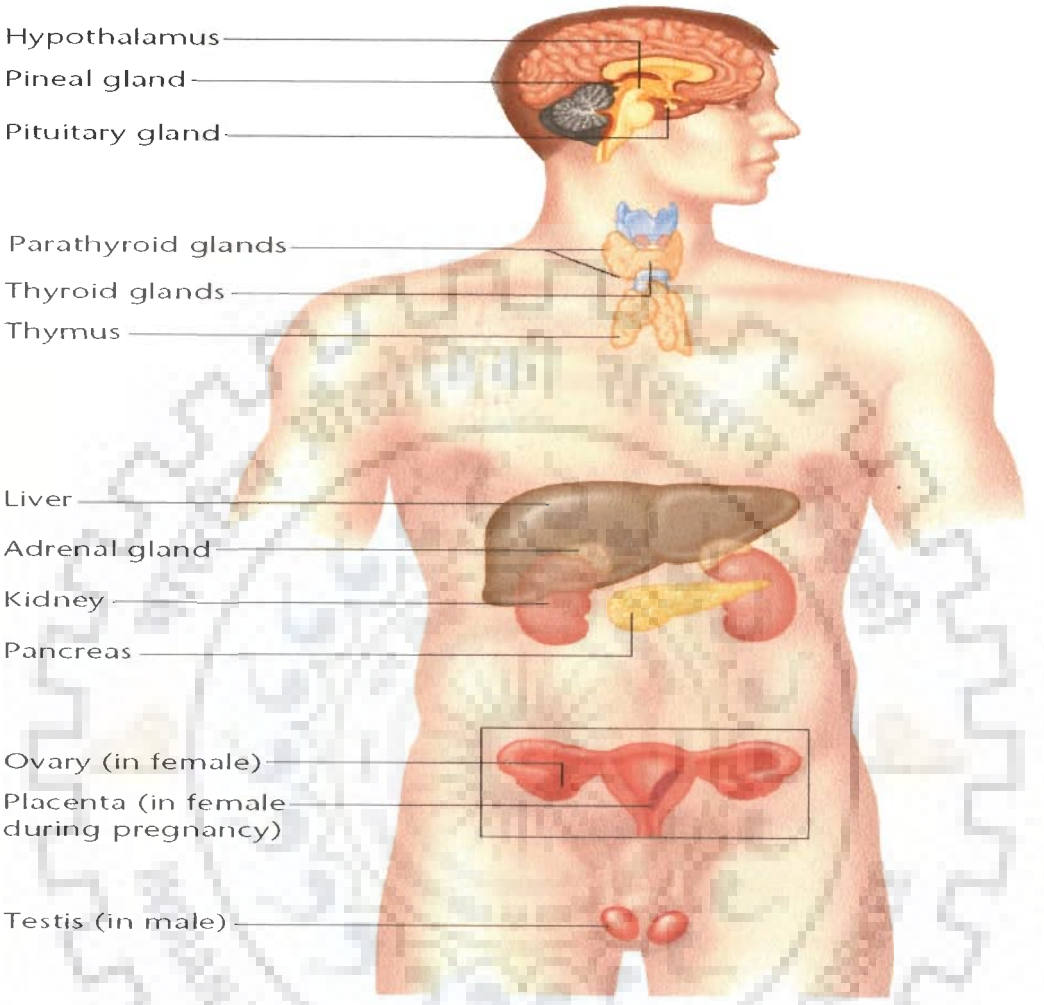


Fig No. 1

1.7 The pituitary

The pituitary gland has long been called the master gland because it regulates many other endocrine glands. It secretes multiple hormones that, in turn, trigger the release of other hormones from other endocrine sites. The pituitary is located at the base of the brain and is separated into two distinct lobes, the anterior pituitary (AP) and the posterior pituitary (PP). The entire pituitary hangs by a thin piece of tissue, called the pituitary stalk, beneath the hypothalamus (the region of the brain controlling temperature, hunger, and thirst).

The pituitary secretes at least five hormones that directly control the activities of other endocrine glands. These are thyrotropic hormone (affecting the thyroid gland), adrenocorticotropic hormone (affecting the adrenal cortex), and three gonadotropic hormones (affecting the reproductive glands).

The pituitary gland also secretes hormones that do not affect other glands, but control some bodily function. These include somatotropic or growth hormone (which controls growth in all tissues) and antidiuretic hormone (which controls the amount of water excreted by the kidneys).

1.8 The pineal

The pineal gland is a small cone-shaped gland believed to function as a body clock. The pineal is located deep in the rear portion of the brain. It secretes the hormone melatonin, which fluctuates on a daily basis with levels highest at night. Scientists are not quite sure of the role of melatonin. Some believe it plays a role in the development of the male and female sex glands.

1.9 The thyroid

The thyroid is a butterfly-shaped gland that wraps around the front and sides of the trachea (windpipe). The thyroid is divided into two lobes connected by a band of tissue called the isthmus. Thyroid hormones play several important roles in growth, development, and metabolism. (Metabolism is the sum of all the physiological processes by which an organism maintains life.) The major hormones produced by the thyroid are thyroxine and calcitonin. Thyroxine controls the metabolic rate of most cells in the body, while calcitonin maintains proper calcium levels in the body.

1.10 The parathyroids

The parathyroids are four small glands (each about the size of a pea) located behind the thyroid gland. These glands secrete parathormone, which regulates calcium (and phosphate) levels in the body. Calcium has numerous important bodily functions. It makes up 2 to 3 percent of the weight of the average adult. Roughly 99 percent of the calcium in the body is contained in the bones. Calcium also plays a pivotal role in muscle contraction.

1.11 The thymus

The thymus is located in the upper part of the chest underneath the breastbone. In infants, the thymus is quite large. It continues to grow until puberty, then it begins to shrink. The size of the thymus in most adults is very small. Like some other endocrine glands, the thymus has two lobes connected by a stalk. The thymus secretes several hormones that promote the development of the body's immune system.

1.12 The pancreas

The pancreas is a large gland situated below and behind the stomach in the lower abdomen. The pancreas secretes pancreatic juice into the duodenum (the first section of the small intestine) through the pancreatic duct. The digestive enzymes in this juice help break down carbohydrates, fats, and proteins.

Scattered among the cells that produce pancreatic juice are small groups of endocrine cells. These are called the Islets of Langerhans. They secrete two hormones, insulin and glucagon, which maintain blood glucose (sugar) levels.

Insulin is secreted in response to high glucose levels in the blood. It lowers sugar levels in the blood by increasing the uptake of glucose into the tissues. Glucagon has the opposite effect. It causes the liver to transform the glycogen (a carbohydrate) it stores into glucose, which is then released into the blood.

1.13 The adrenals

The adrenals are two glands, each present like a cap on top of a kidney. The adrenals are divided into two distinct regions: the cortex (outer layer) and the medulla (inner layer). The cortex makes up about 80 percent of each adrenal. The adrenals help the body adapt to stressful situations.

The cortex secretes various steroid hormones. The most important of these are cortisol and aldosterone. Cortisol regulates the body's metabolism and aldosterone regulates the body's water and salt balance. The cortex is extremely important to bodily processes. If it stops functioning, death occurs in just a few days.

The medulla secretes the hormones adrenaline and noradrenaline. Both of these hormones are released during stressful situations. They increase heart rate, blood pressure, blood flow to the muscles, blood sugar levels, and other processes that prepare a body for vigorous action, such as in an emergency.

1.14 The ovaries

In females, the ovaries are located at the end of each fallopian tube and are attached to the uterus by an ovarian ligament. They produce the female reproductive hormones estrogen and progesterone. These hormones work together with the gonadotropic hormones from the pituitary to ensure fertility. They are also important for the development of sexual characteristics during puberty.

Each month after puberty, increased levels of estrogen signal the pituitary gland to secrete luteinizing hormone (LH; a gonadotropic hormone). Once LH is secreted, the ovaries release a single egg (a process called ovulation). While an egg travels down the fallopian tube, progesterone is released, which prevents another egg from beginning to mature. The egg then attaches to the lining of the uterus. If fertilization does not occur, the egg (with the lining of the uterus) is shed outside the body during the monthly process called menstruation.

During pregnancy, high levels of estrogen and progesterone prevent egg from maturing. In addition, progesterone prevents the uterus from contracting so that the developing embryo is not disturbed, and helps to prepare breasts for lactation (the formation and secretion of milk). At menopause, which usually occurs between the ages of 40 and 50, estrogen levels fall dramatically and the monthly cycle of ovulation and menstruation comes to an end.

1.15 The testis

The testis are located in the scrotum, which hangs between the legs behind the penis. In addition to producing sperm, the testis produces testosterone, the principal male sex hormone. At puberty, increased levels of testosterone bring about the development of sexual characteristics (increased genital growth, facial hair, voice change). Testosterone helps sperm to mature and aids in muscular development. After about the age of 40, testosterone levels gradually decline.

1.16 Endocrine disorders

As much as 10 % of the population will experience some endocrine disorder in their lifetime. Most endocrine disorders are caused by an increased or decreased level of particular hormones. Tumors (abnormal tissue growth) in endocrine glands are one of the major causes of hormone overproduction. Hormone underproduction is often due to defective receptors within target cells, which fail to notify an endocrine gland when its level falls below a threshold level. Injury or disease can also result in low hormone levels.

The overproduction of the growth hormone can cause giantism (unusually large structure). Underproduction of the same hormone can lead to the opposite condition, dwarfism. A similar disorder and cretinism occurs when the thyroid does not produce enough calcitonin, which is necessary for bone growth. Addison's disease is a rare condition caused by insufficient hormone production by the adrenal cortex. It is characterized by extreme weakness, low blood pressure, and darkening of the skin and mucous membranes. Low insulin production by the Islets of Langerhans can result in diabetes mellitus, a condition marked by excessive thirst, urination, and fatigue. If left untreated, diabetes can cause death

1.17 What Are Endocrine disruptors -

"Endocrine disruptors are chemicals that may interfere with the body's endocrine system and produce adverse developmental, reproductive, neurological, and immune effects in both humans and wildlife". A wide range of substances, both natural and man-made, are thought to cause endocrine disruption, including pharmaceuticals, dioxin and dioxin-like compounds, polychlorinated biphenyls, DDT and other pesticides, and plasticizers such as bisphenol A. Endocrine disruptors may be found in many everyday products– including plastic bottles, metal food cans, detergents, flame retardants, food, toys, cosmetics and pesticides. The NIEHS supports studies to determine whether exposure to endocrine disruptors may result in human health effects including lowered fertility and an increased incidence of endometriosis and some cancers. Research shows that endocrine disruptors may pose the greatest risk during prenatal and early postnatal development when organ and neural systems are developing.

The synthetic androgens or xenoandrogen or androgen like molecules can mimic the action of endogenous androgen. These chemicals act as the ligand not only for estrogen but also for androgen, progesterone or arylhydrocarbon receptors thereby exerting a combined action (Norgil et al., 2002; Eertmans et al., 2003).

An increasing body of evidences reveals association between various therapeutic/ environmental compounds that act as EDC and many sex hormone-sensitive disease/ disorders (Colborn and Clement, 1992; Satoh et at., 2007). A probable link have been proposed between exposure to EDC and production of a number of diseases like reduced fecundity, abnormal fetal development, delayed onset of puberty, cryptorchadism, abnormal lactation, testicular dysfunction and even various types f cancers (Sharpe and Irvine, 2004; Roy et al., 2005; Darbre,2006; Guillette, 2006; Maffini et al., 2006)

1.18 Few of the health effects.

i. Birth Defects .
ii. Neurologic disorders .
iii. Endometriosis.
iv. Diabetes mellitus .
v. Immunological disorders .
vi. Early Puberty in young girls .
vii. Cancers: breast, colon, vaginal, cervix, testicular, brain and central nervous system .
viii. Non-Hodgkin's lymphoma .
ix. Reduced physical stamina .
x. Genital birth defects: Hypospadias & Cryptorchidism .
xi. Reduced anogenital distance in male.
xii. Reduced sperm counts .
xiii. Enlarged prostates and cancer .
xiv. Developmental, Behavioral and Mental Disorders: Anger, Inattention, Decreased mental capacity.
xv. Learning disabilities, Dyslexia, Attention deficit/hyperactivity disorder (ADHD), Autism
xvi. Propensity to violence.
xvii. Decreases in stamina, gross and fine eye-hand coordination.

Endocrine disruption may take place by following mechanism- (www.whyfiles.org/045env_hormone/main4.html.)

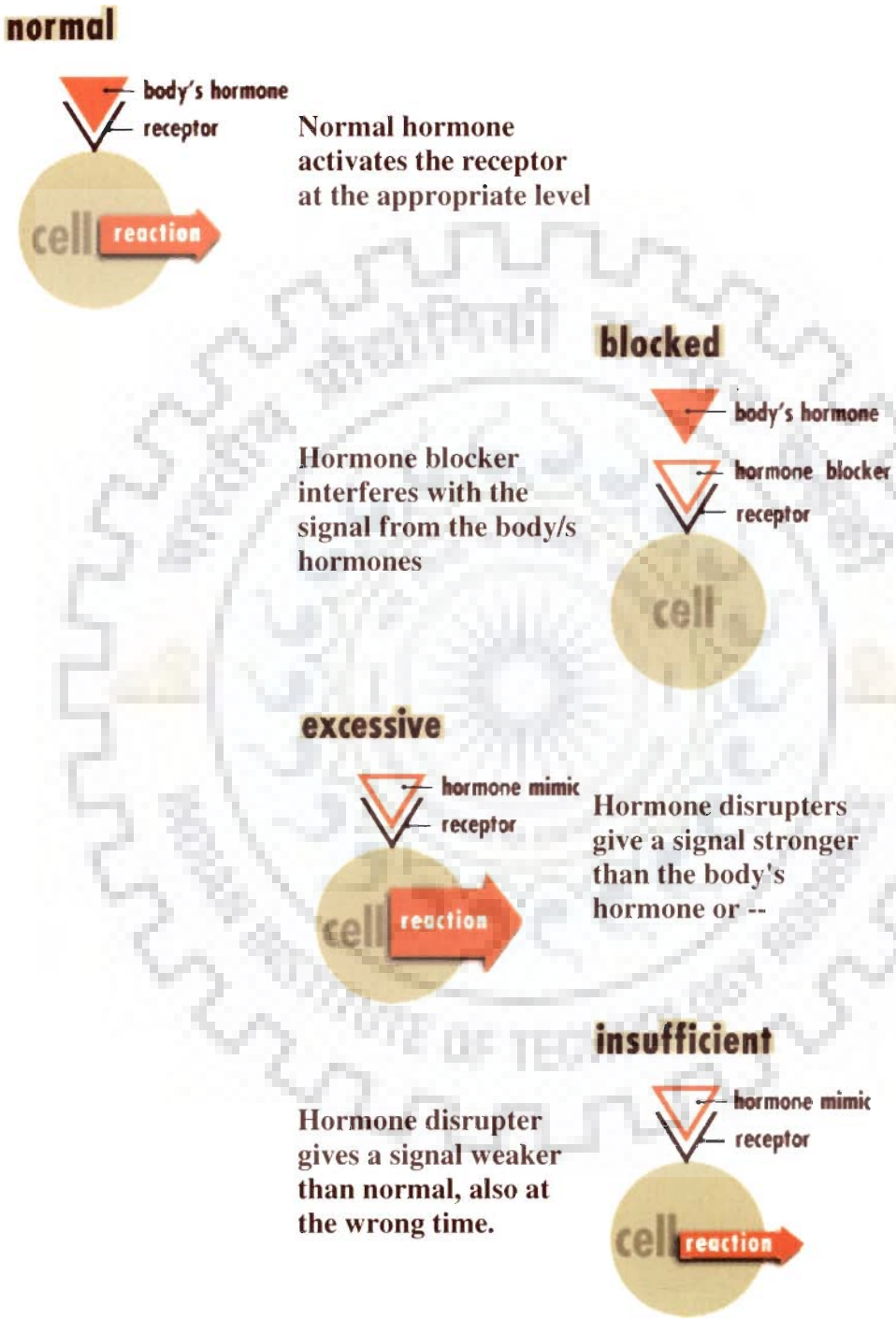


Figure 2: Mechanism of endocrine disrupter

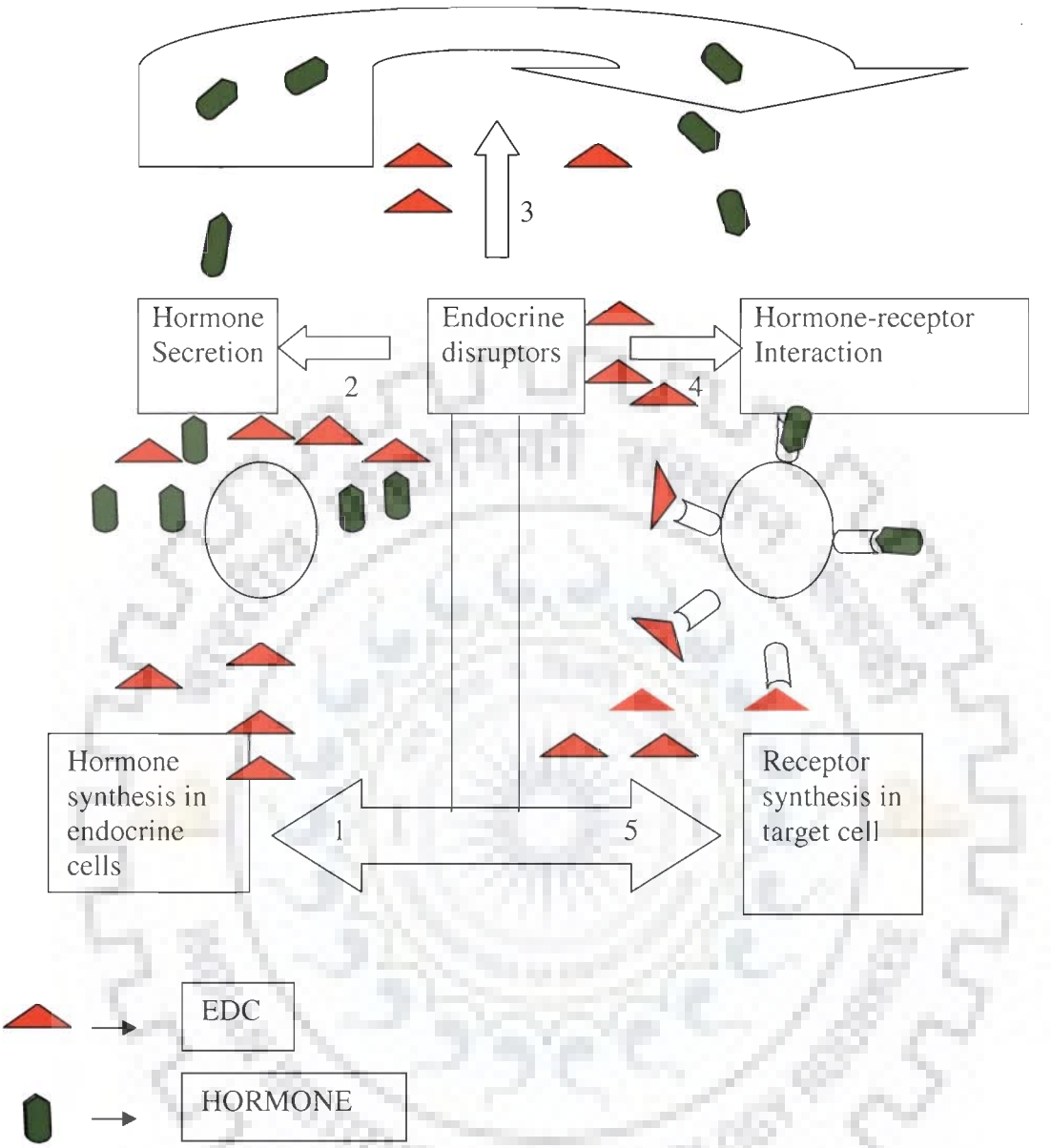


Figure 3: A hypothetical model depicting the possible mechanisms of Endocrine disruption. The disruptor may.:-

1). Inhibit hormone synthesis in the endocrine cells,

2) Change the rate of hormone secretion,

3) Affect the transport of the hormone in circulation.

4) Interfere with the hormone-receptor interaction and

5) Up-regulate or down-regulate receptor levels in target cells

1.19 List of some major Endocrine Disruptors

1.19.1 Persistent Organohalogens:

Dioxins and furans, PBBs, PCBs, Hexachlorobenzene, Octachlorostyrene, Pentachlorophenol.

1.19.2 Pesticides:

Alachlor, aldicarb, d-trans allethrin, amitrole, atrazine, benomyl, beta-HCH, carbaryl, chlordane, chlozolinate, cyhalothrin,cis-nonachlor, cypermethrin, DBCP, DDT, DDT metabolites, dicofol, dieldrin, endosulfan, esfenvalerate, ethylparathion, fenvalerate, h-epoxide,heptachlor, iprodione, kelthane, kepone, ketoconazole, lindane, linurone, malathion, mancozeb, maneb, methomyl, methoxychlor, metiram, metribuzin, mirex, nitrofen, oxychlordane, permethrin, procymidone, sumithrin, synthetic pyrethroids, toxaphene, trans-nonachlor, tributyltin oxide, trifluralin, vinclozolin, zineb, ziram

1.19.3 Phthalates:

Di-ethylhexyl phthalate (DEHP), Butyl benzyl phthalate (BBP), Di-n-butyl phthalate (DBP), Di-n-pentyl phthalate (DPP), Di-hexyl phthalate (DHP), Di-propyl phthalate (DprP), Dicyclohexyl phthalate (DCHP), Diethyl phthalate (DEP),

1.19.4 Other:

Penta- to Nonyl-Phenols, Bisphenol A, Bisphenol F, Styrene dimers and trimers, Benzo(a)pyrene, ethane dimethane, sulphonate, tris-4-(chlorophenyl), methane, tris-4-(chlorophenyl), methanol, Benzophenone, N-butyl benzene, 4-nitrotoluene, 2,4-dichlorophenol, Cyanazine, Diethylhexyl adipate .

1.19.5 Metals:

Arsenic, Cadmium, Lead, Mercury etc.

1.19.6 Pharmaceuticals:

Drug estrogens - birth control pills, DES, cimetidine

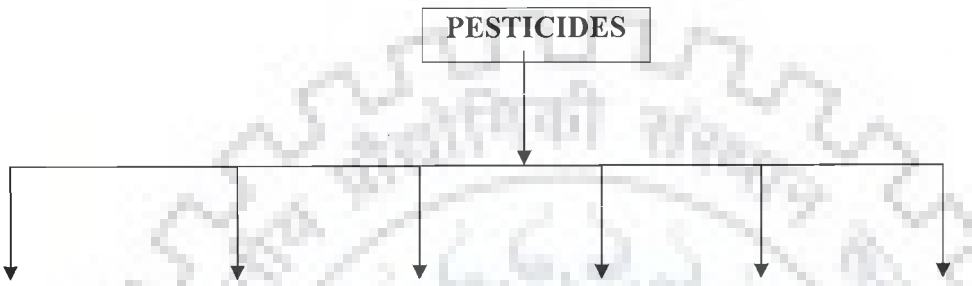
1.20 Scope and objective of work:

As it is evident from previous text, those endocrine disruptors comprises of various class of chemicals. One of the major classes of EDCs is pesticides which includes several insecticides, herbicides, rodenticides, fumigants, fungicides and mollusicicides. Uttarakhand is a state having a major source of revenues from agriculture. It has been reported that for various crop protection, insecticides have been used extensively through out the state including Roorkee. Most of the insecticides being used are of the class organochlorine, organophosphates, pyrethroids and carbamates. The relationship between endocrine disorders by common pesticides and exposure to these chemicals are common poorly understood and scientifically controversial. In order to investigate endocrine disruptions by common insecticides, firstly market survey was carried out in and around Roorkee. It was found that pesticide like endosulfan, chlorpyriphos, monocrotophos, fenvelarate, cypermethrin, chlordane etc. used for crop protection. The present thesis describes action of two major pesticides: Chlordane and Monocrotophos. We investigated chlordane, monocrotophos for endocrine disruptions and reproductive toxicity in animal model (in vivo). Our aim was to evaluate the hazardous effects of chlordane, monocrotophos and to establish a relationship which can occur by getting exposed to these insecticides through any mode for causing various human diseases.

CHAPTER – 2
LITERATURE REVIEW

2.1 Pesticides

The term pesticides includes any substance used for preventing destroying attracting, repelling or controlling any pest including unwanted species of plants and animals during production, storage, transport, distribution and processing of food i.e. agriculture commodities or animal feeds according to Dureja & Parmar (1994) the pesticides are classified as insecticides, herbicides, rodenticides, fumigants, fungicides and mollusicicides.



Insecticides Herbicides Rodenticides Fumigants Fungicides Mollusicicides

Insecticides represent one group of pesticides that are used in large quantities (44%) of total pesticides (ITRC, 1999). At present four major groups of insecticides dominates the market

A) Organo chlorine B) Organo phosphates

C) Carbamate D) Pyrethroids

In the developed countries the consumption of organo chlorine insecticides has been showing a decline trend due to two main reasons. Firstly, due to their persistent character, some of them have resulted in wide spread contamination of the biota, including food commodities and human tissues. Secondly, their indiscriminate use is leading to adverse environmental effects. Similarly organophosphate insecticides are also broad spectrum in nature, few of them are extremely toxic to a particular group of animals and most of them are relatively innocuous to nearly all organisms.

2.2 Historical Aspect

The use of chemicals to control insects possibly dates back to classical Greece & Rome. Homer mentioned the fumigant value of burning sulphur. Pliny advocated the insecticidal use of arsenic and referred use of soda and olive oil for seed treatment of legumes. The middle of 19th century marked the beginning of the first systemic scientific studies for the use of crop protecting by various chemicals. In the period between two world wars, both the number and complexity of crop protecting chemicals increased. During 2nd world war, Dr. Paul Muller discovered the insecticidal potential of dichloro diethyl tetrachloro ethane (DDT). Similarly the insecticidal potential of organo phosphate insecticidal was developed in Germany. In 1950, the first soil acting carbamate herbicide, phenoxy acetic acid was discovered by British workers and organo chlorine insecticides; chlordane was introduced in U.S.A. and Germany in 1950. Since then the use of pesticides and number and type of chemicals have greatly increased. According to the latest information, more than 1000 pesticides are now in use in world market and this number is constantly increasing. Exponential increase in the production, use and chemical disposition of these chemicals have a profound impact on environment and had created unforeseen hazards to human and animals (Desjardins, 1985). In total, almost 40% of pesticides currently in use are linked with at least one adverse health effect (LFO,1988). Most of the evidence on the acute health effects of pesticides exposure worldwide relates to accidental occupational poisoning (WHO, 1990).This also has been proposed to compromise male fertility and offspring development (Sanchez et al., 2004).

2.3 Indian Scenario

Pesticides use in agriculture began in India in 1948-49 by importing DDT for malaria control and BHC (Benzene Hexa Chloride) for locust control. Indigenous production of pesticides began with the establishment of DDT and BHC in 1957. By 1958, India was manufacturing five basic pesticides which are being used till date with addition of fungicides and herbicides. India keeps rank 4th in the world in the use of pesticides and total use of pesticides today is about 1,20,000 million tones every year (FAO, 1988). A wide range of 123 types are being mostly used in India.

The agricultural sector consumes around 67% of the pesticides produced. Two third of the consumption is taken up by just a few crops like cotton, paddy, vegetables

and fruits. Highest consumed pesticides in India include monocrotophos, endosulfan, phorate, chlorpyrifos, methyl parathion, quinalphos, phosphamidon, and chlordane.

According to estimates of International Development Research Centre, every year around 10,000 people die and another 400,000 suffer from various effects of pesticides in developing countries (Times of India, 17th Feb., 1999). As per another estimate, around the world 3 million people suffer from acute pesticide poisoning every year, of which 200,000 die mostly in countries like India (WHO, 1997). India account for one third of the pesticide poisoning in the third world.

The pesticide consumption varies vastly across different states, depending on several factors, including cropping patterns, irrigation facilities, pest resurgence and resistance situations and so on.

2.4 Adverse in vivo effects of EDC to human health

Up to now laboratory experiments with animals and field observations on wild life have strengthened the endocrine disruption hypothesis (Andersson et al., 2008a, 2008b, Kumar et al., 2008). In the recent decade, several instances of environmental chemicals hampering endocrine function in human males have come to light. Epidemiological findings of genital malformations in children of workers exposed to pesticides (Sarmah A.K., 2006) and antihormonal activity of several chemicals (Gray et al,1997), gives credence to the theory of endocrine disruption. In the past few years, an increase in the incidence of poor semen quality, testis cancer, undescended testis and hypospadias has been noticed in several parts of the world, a condition collectively referred to as testicular dysgenesis syndrome (TDS). Experimental and epidemiological studies suggest that TDS is a result of disruption of embryonic programming and gonadal development during fetal life. The increasing incidences of such cases point to the environmental influences as the potential cause rather than the accumulation of genomic structural defects. Relatively few chemicals have so far been closely examined for their bioactivity in disrupting the hormonal balance of the populations. Flutamide and diethylstilbestrol has been shown to have harmful impact on fetal testicular steroidogenesis in the rat (Adamsson et al., 2008). Some herbal/ medicinal drugs induce delay in gastric emptying in rats and thus causes toxicity

(Sharma S.S. et al, 1988). These EDCs can be broadly categorized as (anti) androgenic, (anti) estrogenic (Agrawal S.S. et al 1969).

2.5 Endocrine Disruptors: Different Sources and Categories (types) and biological relevance

The modern life style compels us to use a number of synthetic chemicals, intentionally or unintentionally, for diverse purposes in different sectors of life ranging from the simple household activities like detergents, cosmetics and toilet articles to specialized applications viz. pharmaceuticals, insecticides and pesticides. Besides, during the manufacturing of various commercial products, a number of byproducts are released from the industries as industrial effluent into air and water. All of these chemicals or their byproducts may have various, direct or indirect, adverse consequences on the wildlife and human health. (Babu B.V., 2007). One of the prominent side effects of these chemical is their contrary effect on endocrine system, popularly known as endocrine disruption (Keller et al., 1996; Ternes et al., 1999; Roy et al., 2004 and 2006; Roy and Pereira, 2005; Rogan and Ragan, 2003; Gore, 2008; Kumar et al., 2008). Once any such EDC gets entry into the environment, it may undergo various fates such as: additional distribution between other environmental strata like water, air, and soil/sediment or may be changed into other by products by the action of different environmental agents

2.6 Endocrine Disruption:

2.6.1 Decent of the Concept and Definition

A balanced functioning of the endocrine system, as described above is essential for controlling different pivotal functions in the human or animal body. However, endocrine system is highly sensitive toward factors which leads to partial or complete paralysis of physiological system or may severely impair even the whole development of the organism. In recent years a number of studies have been conducted which indicate that various environmental chemicals may interfere with the normal functioning of the endocrine system of humans and wildlife (Witorsch, 2002; Roy and Pereira, 2005; Roy et al., 2004, 2006; Rogan and Ragan, 2003; Gore, 2008; Kumar et al., 2008a, 2008b). These chemicals have been designated as EDC. In general EDC are the substances that may lead to the generation of the adverse effect

by obstructing the body's hormonal or chemical messengers. Although a chemical can be either a toxic chemical or an EDC, but may just as easily be both (Vogel, 2004, Kumar et al, 2008), however, generally an EDC is different from classical toxicants such as carcinogen, neurotoxin and heavy metals in the sense that it interferes with the normal hormonal functions, but doesn't have a classical toxic effect (Roy and Pereira, 2005). A number of descriptions have been proposed to define EDC and during the Weybridge Conference (1996) the European scientific and regulatory community has agreed on the following definition of a potential endocrine disruptor (Weybridge, UK, 1996).

"An endocrine disruptor is an exogenous substance that causes adverse health effects in an intact organism, or its progeny, consequent to changes in endocrine function." or "A potential endocrine disruptor is a substance that possesses properties that might be expected to lead to endocrine disruption in an intact organism."

In May 1997, the U.S. Environmental Protection Agency (EPA) task force on endocrine disruption (EDSTAC) agreed on the following operational definition:

"An endocrine disruptor is an exogenous chemical substance or mixture that alters the function(s) of the endocrine system and thereby causes adverse effects to an organism, its progeny, or (sub) population."

However, the usage of the word "adverse" effect in the foresaid definition created ambiguity and thus, in order to achieve consensus, the EDSTAC finally agreed to the following general description (EPA Final Report, 1998)-

"The EDSTAC describes an endocrine disruptor as an exogenous chemical substance or mixture that alters the structure or function(s) of the endocrine system and causes adverse effects at the level of the organism, its progeny, the populations, or subpopulations of organisms, based on scientific principles, data, weight-of-evidence, and the precautionary principle."

In a special report, EPA has stated that, "when we consider the existing scenario of the science, we can't consider the endocrine disruption to be an adverse end point per se, instead it can be seen as a mode or mechanism of action which may

lead to other outcomes, for example, carcinogenic, reproductive, or developmental effects" (Crisp et al, 1997).

2.7 Pesticide Cycle

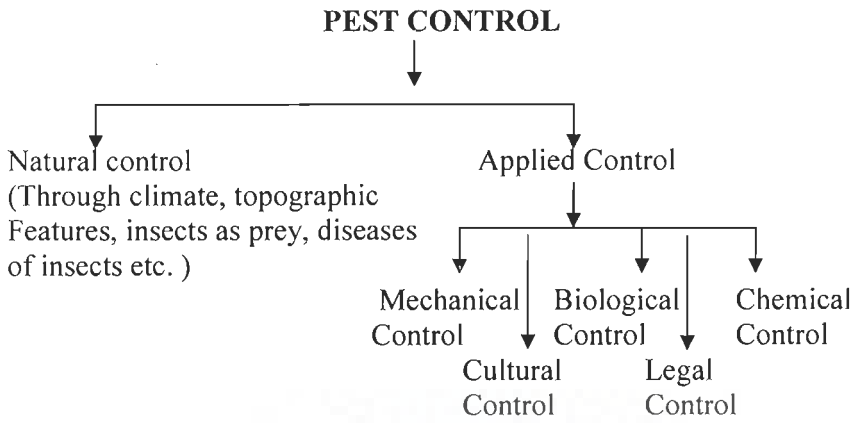
Pesticide intake from food, water and air has long been neglected since the amount of intake was thought to be insignificant. Studies on the subtle effect of chronic exposure to pesticides have begun only recently. Pesticides once entering the body / environment get absorbed by various constituent have and are transported to other places mainly through air and water. They are progressively transported to soil, further to edible crops, grass, herbivorous animals and eventually to humans by food chain.

Insecticides present in water are taken up by planktons and through food chain accumulate in fish and other edible species this result in their accumulation in almost every human being.

Kraybill (1969) estimated that 85-90% of pesticides in human bodies are received through contaminated food (Pesticide Cycle)

2.8 Pest Control

Insects have always been man's most serious enemy, even from the prehistorically days. The cave dwellers or prehistoric men even in the days of plenty of food were not spared from the attacks and nuisance of lice, fleas, ticks etc. Since there was plenty of food all around in earlier days, the loss due to insect damage was not considered to be of great importance. As food was becoming scarcer every day, reduction of losses by the use of pesticides was one of the many attempts made by man. Caffeine and some of its derivatives has potential as pest repellent (Shinjiro Ogita et al, 2005). Degradation of crops due to EDC may be prevented by using some drugs (Eilen Thom, Benckiser G. et al, 1997).



2.9 Pharmacodynamics of Insecticides

A variety of reactions at different levels are involved for the poisoning of organisms with insecticide. The route through which the insecticides enter the body is first step followed by transport to different organs and their biotransformation, resulting in more toxic or equi toxic metabolites.

The active form of insecticides then reaches the target site and exerts its effect on tissue concerned. Most of the insecticides are nerve poisons. However, there could be multiple ways of action for change of behavior and death of organism.

2.9.1 Organochlorine insecticide

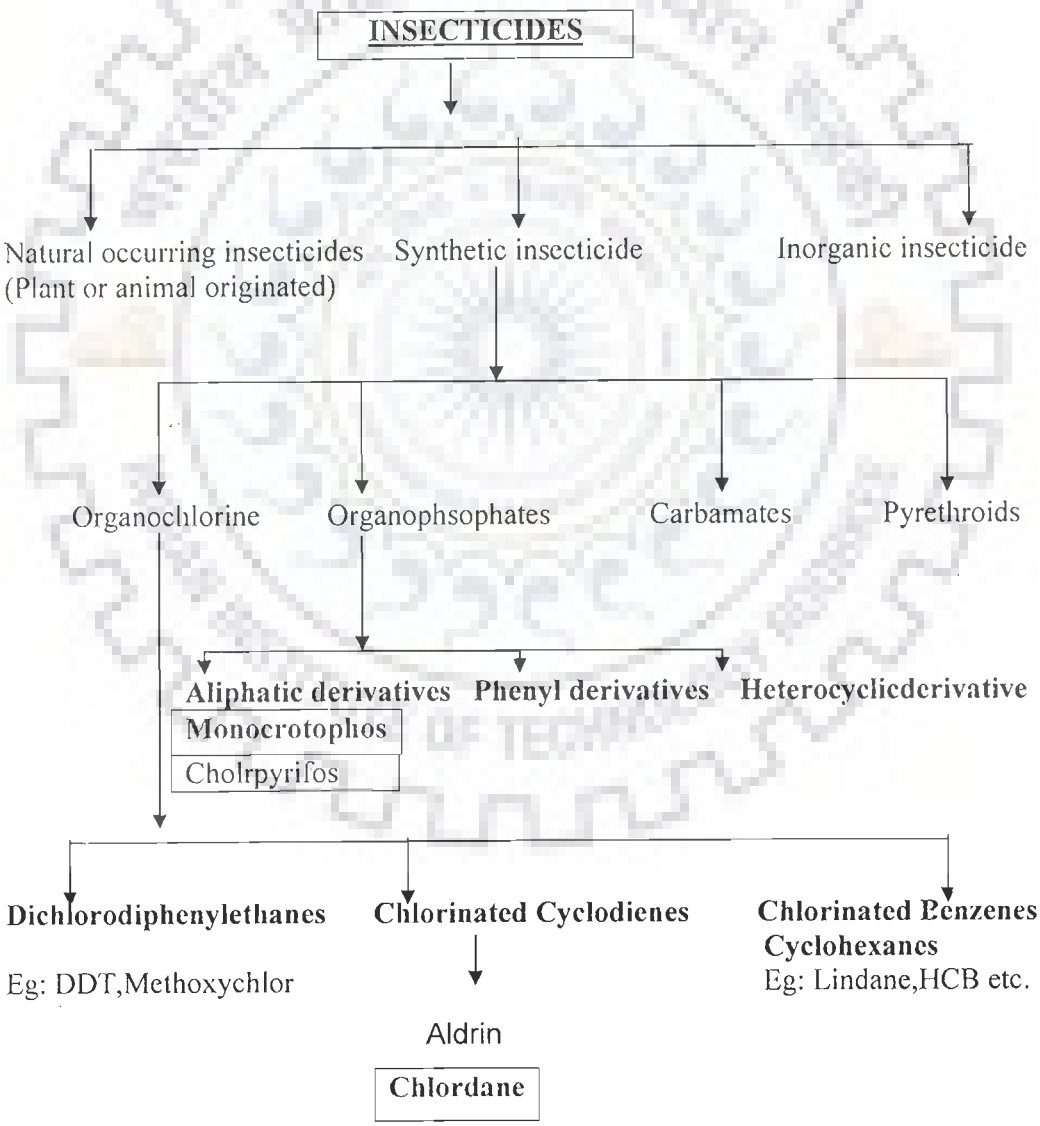
Organochlorine insecticide has a similar method of absorption i.e. ingestion, inhalation and dermal absorption. These insecticides act by inhibition of enzyme acetyl cholinesterase (AChE), which results in accumulation of acetylcholine (ACh.) which is a neuro transmitter in nerve tissue.

2.9.2 Organophosphate insecticide

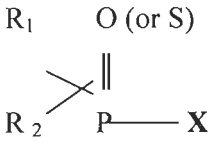
Organophosphate insecticides have a common mode of toxicity as competitive inhibitors of acetyl cholinesterase (AChE), the enzyme responsible for deacetylation of the neurotransmitter acetylcholine (Ruijten et al., 1994). The primary effects of OPs acute exposure are on the parasymphathetic, sympathetic and central nervous system. These pesticides interfere with the metabolism of acetylcholine (ACh) by inhibiting the enzyme that hydrolyzes it, acetyl cholinesterase (AChE) (Ecobichon, 1994). AChE accumulates at the neuronal junctions, resulting in the continued stimulation and the suppression of neurotransmission to organs.

2.10 Classification:

Insecticides vary tremendously in chemical structure and chemical properties (Bloomquist, 1996). These chemicals are classified into groups depending on the positioning of the central phosphorus / chlorine functional groups. All organophosphates are esters of having varying combinations of oxygen, carbon, sulfur and nitrogen attached resulting in six different subclasses: Phosphates, phosphonates, phosphorothioates, phosphorodithioates, phosphorothiolates and phosphoramidates. While Organochlorine insecticides have Dichlorodiphenylethanes, chlorinated cyclodienes and chlorinated benzenes and Cyclohexanes. The insecticides classification is as following -



In spite of the enormous structural diversity of OP insecticides, all the compounds can be represented by the classical hypothetical structure as originally proposed by Schrader.



Where R_1 and R_2 are usually simple alkyl or aryl groups. The group X can be any one of a wide variety of substituted and branched aliphatic, aromatic or heterocyclic groups.

2.10.1 Aliphatic

The aliphatice OPs are carbon chain like in structure eg. Malathion, Monocrotophos, Dichlorvos.

2.10.2 Phenyl Derivatives

The phenyl OPs contain a phenyl ring with one of the ring hydrogens displaced by attachment to the phosphorus moiety and other hydrogens frequently displaced by Cl, NO_2, CH_3, CN or S. eg. Methyl Parathion, Sulprofos, Fenitrothion etc.

2.10.3 Heterocyclic Derivatives

The term "heterocyclic" means that the ring structures are composed of different or unlike atoms, like oxygen, nitrogen or sulfur.e.g. Chlorpyrifos, Azinphosethyl, Phosmet etc.

2.11 Organophosphates as toxicants

From the mid 1970s, when the persistent organ chlorines (OCs) were finally phased out of the agricultural market, they were replaced by highly toxic, but biodegradable, organophosphorus pesticides. Their toxic pedigree was established during World War II, when research was conducted to find potent nerve gases for military purposes. This association of organophosphorus insecticides with war gases was hardly and auspicious beginning for a group of compounds which was destined to become one of the main weapons in our armory against insect pests of importance in

agriculture, public hygiene and medicine. The earliest members of the group, marketed in the immediate post war years, did little to allay these fears, for most of them were toxic to vertebrates.

Organophosphate pesticide kill insect by allowing the neurotransmitter acetylcholine to build up at nerve endings. This short circuits the cholinergic system, which governs involuntary processes (EHP, 2003), in surviving animals, several physiological and behavioral dysfunctions persist after exposure to high dose of OPs (Hall and Clark, 1982). An assessment by the Pesticides Trust revealed Azinophos Methyl, Chlorpyrifos, Monocrotophos, Parathion and Phosphamidon have caused a number of health concerns in a range of developing countries.

Virtually all types of organophosphate pesticides target and depress acetylcholinesterase activity in a dose dependent manner, leading to an excessive acetylcholine (AChE) out put, nerve paralysis and finally death. The body system affected the central nervous system, the autonomic nervous system as well as peripheral muscular pathways (Hassal, 1982).

Organophosphates are efficiently absorbed from the skin, lungs and gastrointestinal tract. All signs and symptoms of acute organophosphate poisoning are cholinergic in nature and effect nicotinic, muscarinic and central nervous system receptors as the whole. All OPs insecticides exert their acute toxic action by their effects on the AChE. Thus, it follows that exposure to more than one such pesticide will usually produce at least and additive effect.

In man, these compounds can lead to a number of behavioral changes for example, spray machine operators have been known to suffer from depression and insomnia and others have lost the power to concentrate on their work to remember things. Occasionally affected worker may suffer a slurring of speech or acquire felling of anxiety or irritability (Levin and Rodnitzky, 1976).

There is increasing evidence that these environmental chemicals may be linked to increase in hormone related cancers, endometriosis, certain behavioral

aberrations and reproductive effects, such as an alarming apparent drop in male sperm counts worldwide in recent years (Flora,S.J.S, Mathur R. et al, 1998).

2.12 Practical uses

Organophosphorus (OP) compounds have overtaken organochlorine compounds as the most used insecticides, whether the criterion of usage is tonnage produced or wholesale value. Among all the pesticides, organophosphate compounds are used predominantly as insecticides and some of them have been misused as war weapons during Second World War. A large number of such pesticides have been introduced in the market and a much greater number have been screened for pesticidal activity with a great variety of pesticides like insecticides, among the organ phosphorus compounds.

OPs are among the most widely used synthetic insect pesticides. The popularity of these compounds is due to their relative non-persistence in the environment and low bio accumulation by biota (Rattner et al., 1982). The use of this class of chemical has resulted in world wide increase in food and fiber production and the control of major disease carrying vectors and damaging insect pests. The majority of organ phosphorus compounds are effective in the control of aphids and similar soft bodied small insects but many of the newer compounds have a wide range of uses. They are used to control coleopteran, diptera, homoptera and lepidoptera in cereals (including stored) various foliar crop pests and also controls boll weevils and mites.

OPs have been in the news because of health concerns following their use in sheep dips and as insecticides in military premises, on equipment and even on personnel during the Gulf War. Some of the compounds marketed for killing the more refractory insects possess persistence or high cost. Guidelines for health surveillance, in collaboration with national occupational health safety commission (1995) and Australian government publishing services, Cannebra, Australia were prepared.

2.13 Mode of Toxicity

The mode of lethality of organophosphorus compound has been determined (Hiroaki et al., 1991), exhibited typical signs of anti ChE poisoning along with marked inhibition of brain and erythrocyte ChE activity. Acetyl cholinesterase is an

enzyme in the nervous system which is inhibited by all organophosphorus insecticides (Wipawan Thangnipon et al, 1994).

The primary biochemical effect associated with toxicity caused by organophosphours pesticides is inhibition of acetyl cholinesterase (AChE). The normal function of AChE is to terminate neurotransmission due to AChE that has been liberated at cholinergic nerve endings in response to nervous stimuli.

In the presence of an inhibitor of AChE, synaptic acetylcholine may increase to abnormally high concentrations, which is postulated to precipitate a "cholinergic crisis" that can be debilitating and possible fatal (Echobichon, 1991). The anti-cholinesterase activity of organophosphates (OPs) results in an excess of acetylcholine activity, primarily in the muscarinic receptors, chronic exposure to OPs have been found to result in a gradual loss of brain stem cholinergic muscarinic and nicotinic receptors (Costa and Murphy, 1983) as well as to increase permeability of blood brain barrier (Dambska et al., 1984). Loss of AChE activity may lead to a range of effects resulting from excessive nervous stimulation and culminating in respiratory failure and death.

2.13.1 Monocrotophos

This non-specific systemic insecticide and acaricide, used to control common mites, ticks and spiders with contact and stomach action, quickly penetrates plant tissue. In common with other OPs, its toxic action is achieved by inhibiting acetyl cholinesterase, an enzyme essential for normal nerve impulse transmission. It is widely used, mainly for foliar application to cotton. Monocrotophos is corrosive, and stable when stored in glass or polyethylene containers.

2.13.1.1 Chronic toxicity and carcinogenicity

In a 2-year study, rats (Charles River; n=25/sex/group) were given technical Monocrotophos (purity: not given) in dietary levels equivalent to 0, 0.05, 0.5, and 5 mg/kg bw/day. However, actual levels may have been considerably lower since diet analysis at week 15 and 40 showed deviations from above presented nominal values of up to 60% due to compound instability. In the high-dose group, signs of toxicity, reduced body weight gain accompanied by reduced food intake in males only, and, in

females only, decreased absolute liver, gonads, thyroid, and pituitary gland weights were observed. No other changes were seen at postmortem gross and microscopic examination. Plasma ChE and red blood Cell AChE activities were inhibited in the mid-dose group by more than 50% from week 6 onwards but were not affected on the low-dose group (no data given for the high dose group). Brain AChE activities measured at the end of the study did not show significant changes in the low-dose group (no data given for the 2 higher dose groups) (Johnston cd, 1966)

Evaluation of mammalian toxicology and metabolism/ toxicokinetics of monocrotophos were carried out with the help of department of health and family welfare (1998), Canberra, Australia.

In a 2-year study with Wistar rats (n=85/sex/group), the animals received technical Monocrotophos (purity: 78.7%) in dietary concentrations equivalent to 0.0005, 0.0015, 0.005, 0.05, and 0.5 mg/kg bw/day. The control group comprised 170 rats of each sex. At 0.5 mg/kg bw, survival, body weight gain, and feed consumption were reduced. No changes were found in haematology and in clinical chemistry test results. There was no evidence of carcinogenic effects, and no gross and microscopic lesions attributable to treatment were found. Brain AChE, red blood cell AchE, and plasma ChE activities were reduced throughout the study by up to 75, 85, and 80%, respectively, at 0.5 mg/kg bw, and by up to 30, 50, and 30, respectively, at 0.05 mg/kg bw. At and below 0.005 mg/kg bw, ChE activities remained within the normal range. The NOAEL for inhibition of brain and red blood cell AChE and plasma ChE activities was therefore 0.005 mg/kg bw/day; the NOAEL for reduced body weight gain and feed consumption was 0.05 mg/kg bw/day (Brown V K et al, 1983).

In summary, long-term studies in mice and rats did not show evidence for a carcinogenic potential of monocrotophos. Brain AChE, red blood cell AChE, and plasma ChE were inhibited by monocrotophos in a dose-dependent manner. The overall 2-year rat oral NOAEL was 0.005 mg/kg bw based on inhibition of brain AChE and red blood cell AChE activities.

2.13.1.2 Mutagenicity and Genotoxicity:

Mutagenicity and genotoxicity assays comprised tests for the detection of gene mutations in bacteria, yeast, and mammalian cells (*in vitro*) and *in vitro* and *in vivo* cytogenicity and other genotoxicity assays.

• ***In vivo* tests**

Male and female Swiss mice treated with single intraperitoneal (I.P.) doses of 1.25, 2.5, and 5.0 mg/kg bw monocrotophos (Nuvacron-400) showed a significantly increased incidence of micronuclei in polychromatic bone marrow erythrocytes at the 2 highest dose levels (Peitl PJ et al, 1996). Increased frequencies of micronuclei and chromosome aberrations were also observed in bone marrow cells of Swiss mice after 2 intraperitoneal doses of 1.5 and 2 mg/kg bw monocrotophos on 2 consecutive days (Vaidya VG et al,1982). However, monocrotophos did not induce micronuclei in bone marrow cells of mice following 2 intraperitoneal doses in the range of 2-8 mg/kg bw (Kirkhart B. et al, 1980) or following 5 intraperitoneal doses in the range of 1.25-5 mg/kg bw. In the latter study, chromosome aberrations were induced by intraperitoneal administration, but no significant effects were detected when monocrotophos was given orally at doses of 5 mg/kg bw (Bhunya SP. et al 1988). No increased frequency of micronuclei was observed in bone marrow cells of mice treated with a single oral dose of 9 mg/kg bw (Hertner T, 1992). In Wistar rats, Monocrotophos induced chromosome aberrations in bone marrow cells following 2 intraperitoneal doses of 2 mg monocrotophos/kg bw (interval 24 hours). At 073-20 Health-based Reassessment of Administrative Occupational Exposure Limits lower doses, no aberrations were induced (Adhikari N. et al. 1988). No increased frequency of chromosome aberrations was observed in bone marrow cells of Chinese hamsters after 2 oral doses up to 5.6 mg/kg bw, 24 hours apart (Strasser F. et al 1986). Dominant lethal effects were not observed in mice fed monocrotophos at levels equivalent to 0.75, 1.5, and 3.0 mg/kg bw, for 7 days or 7 weeks (Simmon V F. et al, 1977). Hens given either intraperitoneal doses of 1.25, 2.5, and 5 mg/kg bw or an oral dose of 5 mg/kg bw showed a significant increased frequency of chromosome aberrations and micronuclei in bone marrow cells and peripheral red blood cells (Bhunya et al, 1993). A significant increase in frequency of micronuclei was also observed in bone marrow cells and peripheral red blood cells of 1-week-old chicks, fed 5 mg monocrotophos/kg for 30 days (Jena GB et al, 1992). A statistically

significant, dose-related increase in mean comet tail length indicating DNA damage was seen in peripheral blood leukocytes 24 hours after treating male Swiss mice (n=6/group) with single (gavage) oral doses of 0, 0.046, 0.093, 0.186, 0. 373, and 0.746 mg/kg bw. At 48 hours post-treatment, mean tail lengths were gradually decreased in all dose groups, but still statistically significantly increased while they returned to control levels at 72 hours post-administration indicating repair of damaged DNA (Mahboob M. et al, 2002). The committee concludes that monocrotophos is mutagenic both in *in vitro* and *in vivo* assays. However, apart from a positive result in a test indicative of DNA damage in mouse, oral genotoxicity studies in either mice or rats were negative.

Comet assay has been used to detect genotoxicity of monocrotophos and other environmental pollutants (B.Saleha Banu et al. 2001).

2.13.1.3 Reproductive toxicity

Male Swiss Albino mice (n=9-10/group) were given monocrotophos (0, 0.9, 1.8, 3.6 mg/kg) by intragastric intubation, for 5 days. The percentage of abnormal sperms increased with the dose from 2.1% in the control and low-dose groups to 3.6 and 5.4% in the 2 higher dose groups (Kumar DV. et al, 1988). When female virgin Swiss albino mice (n=10/group) were given oral doses of technical grade monocrotophos (purity: 75%) of 0, 1.6, 3.3, 6.6, 10, and 13 mg/kg bw/day, for 30 days, dose-dependent decreases in the number of oestrus cycles and in the duration of pro-oestrus, oestrus, and metoestrus with concomitant increases in di-oestrus duration were found reaching statistically significance at doses of 3.3 mg/kg b.w. and above. Morphometric follicular analysis in 4 animals per group, showed dose-dependent decreases in the sizes and number of healthy follicles and increases in the sizes and number of atretic follicles, reaching statistically significance at doses of 6.6 mg/kg b.w. and higher. Statistically significant 073-21 Monocrotophos decreased ovary and uterus weights were seen at 3.3 mg/kg b.w. and above and 10 mg/kg b.w. and above, respectively. At the 2 higher dose levels, body weights were significantly decreased, while treatment did not cause changes in relative weights of liver, kidneys, adrenals, thymus, thyroid, spleen, and pituitary gland (Rao RP. et al, 2002).

A 2-generation reproduction study was carried out in rats (13 male and 26 female rats per dose group) that were given doses equivalent to 0, 0.005, 0.05, 0.15, and 0.5 mg/kg bw via the diet. Parental effects noted before mating were lower body weights in the male rats of the F0 and F1 generation at 0.5 mg/kg bw, and small dark faecal pellets at 0.5 and 0.15 mg/kg bw. At 0.5 mg/kg bw/day, the mating, fertility, and gestation indices were not different among F0 groups, but the mating index of F1 males was lower compared to the control group.

Gestation length was increased, but mean litter size, mean pup weight, and viability and lactation indices were significantly reduced. Three total litter losses were observed in both F1 and F2 generations. At 0.15 mg/kg b.w., one total litter loss was observed in the F2 generation. In addition, mean pup weight and viability index were significantly reduced. F2 female weanlings showed higher kidney and liver weights at 0.15 and 0.5 mg/kg b.w. compared to the controls. A NOAEL of 0.05 mg/kg b.w./day was established for parental and reproduction toxicity (Dix KM. et al, 1981).

In another study, doses of 0, 0.3, 0.6, and 1.2 mg/kg b.wt/day were administered by gavage to female rats (n=10/group), for 2 weeks prior to mating with non-treated males. The female rats had dose-dependent lower body weights, lower resorptions, enlarged ovaries, and reduced fertility and parturition indices. These changes already started at 0.3 mg/kg bw. The gestation index was not affected. Pups showed also a dose-dependent reduction of average birth weight, average crown-rump length, and of viability and lactation indices at 0.3 mg/kg and higher. However, the average litter size was not affected in any of the groups (Adilaxmamma K. et al, 1994).

It has also been observed that some pesticides induced effect on estrous cycle, ovarian and uterine biochemicals parameters in swiss albino mice (T.S. Sreelakshmi and B.B.Kaliwal 2007).

In a developmental study, monocrotophos was given to pregnant Sprague-Dawley rats (number not specified) by gavage at doses of 0, 0.3, 1, and 2 mg/kgbw/day, during days 6 to 15 of pregnancy. Dams were killed on gestation day 20. Maternal toxicity was evident by muscle tremors and twitching, listlessness,

salivation, perianal urine-soaked fur, and crusty eyes at 2 mg/kg bw/day, and maternal body weight was reduced at 1 and 2 mg/kg bw/day. No compound-related effects on maternal reproduction parameters, i.e., number of corpora lutea, of implantations, of resorptions, and number of dead and viable fetuses, were found. At 2 mg/kg bw/day, fetuses had a decreased body weight, a073-22 Health-based Reassessment of Administrative Occupational Exposure Limits decreased crown-rump length, and delayed ossification of sternebrae, and at 1and 2 mg/kg bw, the number of runt fetuses was increased. Fetal visceral examination did not reveal abnormalities. The findings in fetuses were considered to be secondary to maternal toxicity. Brain defects were observed in all groups but were considered unspecific and unrelated to the test compound.

The NOAEL for maternal and developmental toxicity was 0.3 mg/kg b.w. (Border CK et al 1983). In order to evaluate the relevance of the brain defects observed in the above study, a new study was performed in female rats that were given doses of 0, 0.1, 0.3, 1, and 2 mg/kg b.w. during days 6 through 15 of gestation. The animals were killed on pregnancy day 20. Clinical signs of intoxication were seen in most animals at 2 mg/kg b.w. and tremors in one female at 1 mg/kg b.w. Body weight gain was reduced at 2 mg/kg b.w. There were no treatment-related necropsy findings. Pre and post-implantation losses, mean litter size, mean fetal weight, and sex ratio were unaffected. No treatment-related external, visceral, and skeletal changes were found in fetuses. This study confirmed the conclusion that the brain defects observed in the preceding study were not related to treatment. This study confirmed the maternal NOAEL value of 0.3 mg/kg b.w, and further, a NOAEL for developmental toxicity of at least 2 mg/kg b.w. was established (Fuchs A.,1992). In a developmental toxicity in rabbits, the animals received oral doses of 0, 0.1, 1, 3, or 6 mg/kg b.w. monocrotophos during days 6 through 18 of gestation. The animals were sacrificed at day 29 of gestation. Maternal effects observed at the top dose were mortality of 13 animals, weight loss, signs of cholinergic toxicity (such as hyperpnoea, tremor, ataxia, salivation, excitation, faecal changes, and constricted pupils). Necropsy revealed gastrointestinal ulceration and pulmonary oedema. At 3 mg/kg b.w, diarrhoea and related faecal changes and transiently reduced body weights were seen. Average numbers of late resorptions were slightly increased, and mean live fetal weights and maternal uterine weights marginally reduced at 6 mg/kg bw.

Average numbers of corpora lutea, implantations, early resorptions, litter size, dead fetuses, and fetal sex ratio were comparable among all groups. Fetuses did not show treatment-related malformations externally, in soft tissues, or in skeletal structures at any dose level. The maternal NOAEL was 1 mg/kg bw and the NOAEL for developmental toxicity 3 mg/kg b.w. (Dearlove GE., 1987).

Toxicity of monocrotophos was observed on tissue, protein and carbohydrate metabolism of esturine crab; Scylla Serrata (SRBCE, 2007).

Biochemical alteration in blood plasma of fish, channa punctatus (Bloch) were also reported (Shewta Agarwal, Kashev C Pandey et al, 2007). Gene expressing profiling of peripheral blood T cells in patients with chronic lymphocyte leukemia (B-cells) were also reported. (Kiaii K., Mashayekhi K. et al. 2007)

In summary, these studies indicate that the overall NOAEL for reproduction toxicity in rats or rabbits is 0.05 mg/kg b.w. monocrotophos/day.

2.14 Key health issues

A concern about the safety of monocrotophos has led to its inclusion in the Prior Informed Consent procedure. Governments are informed that formulations which exceed 600 g/liter of active ingredient may not be safe because of their impact on human health under conditions of use in developing countries. Governments which wish to prohibit these formulations can notify the PIC secretariat. Their decisions will be circulated to exporting countries.

Many incidents in developing countries have been linked to monocrotophos. It is often difficult to trace an incident to a particular active ingredient: frequently a 'block' of pesticides commonly used in a region may be linked to a survey indicating occupational and other health impacts in the area (EPA 1999). Stress-induced changes of plasma antioxidants in aqua cultured sea bass (Guerriero, G., 2002)

2.15 Organochlorine as toxicant

Environment is a dynamic attribute of nature and man. There is always significant correlation of environment on ecological dynamics. In the thrust of modernization and industrialization, man has contributed pollution to the life and

ecology of plants, animals and microbes. Increased demand for food and fiber has led to the chemicalization of agriculture and we have reached on such a stage that modern agriculture is dependent on high yielding varieties which can only be grown under the influence of fertilizers and pesticides. Pesticides are man made chemicals, which are being used to produce enough food. In India 90,000 metric tons of technical grade pesticides are used annually to control pest and plant diseases. The pesticides are classified as insecticides, fungicides, weedicides, herbicides, nematodicides, rodenticides, molluscicides, avicides and algicides of which, insecticides constitutes 77% (ITRC, 1999) of the total pesticides used in different agricultural, animal husbandry practices and in public health operations.

The most widely used modern synthetic insecticides are the chlorinated hydrocarbons, organic phosphorous and carbonate groups of compounds. Although there is considerable toxicity among all these groups, on average, the acute toxicity of the organophosphorus group is some what greater than that of the chlorinated hydrocarbon compounds, due to their greater stability

The indiscriminate and injudicious use of pesticides could cause adverse changes in the biological balance as well as lead to an increase in the incidence of various carcinogenic, teratogenic, mutagenic, immuno toxic, reproductive and neurophatic toxic effects.

Human beings are exposed to pesticides by following ways:

1	Intentional	:	Suicide
2	Accidental	:	Careless handling
3	Occupational	:	In production plants, application in agriculture and public health
4	Contaminated food, air and water		Residues resulting at post application Stage

Organochlorine insecticides (OCIs) which were introduced in the decade following World War II were used extensively in Europe, US, India and other developed countries in the 1970's. However, data began to be accumulated on their

persistence in soils, aquatic sediments and their residues in the various groups of invertebrate and vertebrate animals since 1972.

The persistent organochlorine pesticides such as DDT, dieldrin, chlordane, heptachlor, endosulfan etc. remain in the soil for many years and even for decades after they have performed their job. Human exposure is likely because of its use in some pharmacological preparations and in public health for pest control purpose (Dikshit P. et al., 2006). They are progressively transferred from soil to edible crops, grasses and herbivores and eventually to mass. This has been confirmed by reports that showed contamination of grasses, vegetables, fruits and meat. Recent reports indicated that even drinking water, air, dust sediments, ground water and body tissues of animals and humans are also not free from these toxic chemicals (Foster et al, 1993). These toxins usually pile up in the fatty tissues of vital organs. The scientists of the King George Medical College and the Industrial Toxicological Research Center (ITRC) in Lucknow conducted a series of test on workers spraying DDT and Malathion; half of the workers examined were reported to have developed psychological symptoms like anxiety, sleep disturbance and depression.

Transport of eldrin in the same trucks used for sugar transport was incriminated in an outbreak in Pakistan, 19 out of 194 affected persons died, most of them due to an acute seizure disorder and respiratory arrest (Kowley et al, 1987).

DDT and BHC constitute a major portion of the total pesticide use in India. India is one of the major point source countries in the tropical belt and a reason for the global contamination by organochlorines.

Bokare (1995) analyzed drinking water and wheat flour samples in Jaipur during 1992-94 αBHC, βBHC, heptachlor, aldrin, DDT, endosulfan were found in wheat flour whereas 60 percent of water samples were found to contain aldrin 50 percent with DDT and a few with α-BHC.

An alteration of immune function is an important aspect of environmentally induced chemical toxicity (Gupta and Kanan, 1984) surprisingly; the role of immunologic system in reponse to pesticide has not been widely studied. However in

recent years there has been an increasing interest in the effects of pesticides on the immune system. The available data on immuno suppressive effect of many of the chemicals are inconclusive

2.15.1 Chlordane

Chlordane is extremely lipid soluble, and lipid partitioning of chlordane and its metabolites have been documented in both in humans and animals. Concentrations of chlordanes (cis- chlordane, trans-chlordane, oxychlordane, and trans-nonachlor) detected in human liver samples were 17-fold higher when expressed on a fat rather than a wet-weight basis (Mlussalo-Rauharmaa, 1991). It was reported that the mean fat/blood ratio of oxychlordane to be 290/1 among workers who had been exposed to technical grade chlordane (Adeshina and Todd, 1991). This value is concordant with the results of Khasawinah (1989b), who reported this ratio to be 200-300/1 in rats and monkeys after 90-days of inhalation exposure to technical chlordane. Taguchi and Yakushiji (1988) measured chlordane residues (including the epoxide metabolites oxychlordane and hepatchlor epoxide) in the milk of 15 mothers who had lived in chlordane-treated residences for an average of 1.8 years, and the investigators evaluated these measurements with those for the milk of unexposed mothers. In a subgroup of these women who had been exposed for approximately 2 years in treated residences, the overall chlordane residues in milk (0.254 mg/kg milk fat) were similar to those of PCBs (0.389 mg/kg milk fat). Because of its long retention time in adipose tissue, oxychlordane is believed to be more toxic than its parent isomers, which are eliminated relatively rapidly from the body (Satoh and Kikawa, 1992) and, therefore, may be a major contributor to chlordane toxicity. High levels of organoclorines residue were found in milk of mothers (Subramanian A, et al 2007).

Available occupational studies, although limited, give no indication that the liver is a target organ in humans as a consequence of chronic exposure to low levels of chlordane. Way back in 1953 Alvarez and Hyman found no hepatic abnormalities after thorough physical examinations and liver function tests administered to 24 male workers who had worked for 2 months to 5 years at a chlordane manufacturing plant. Liver function tests were also normal in 15 workers at a chlordane manufacturing plant, 14 of whom had been employed 9-16 years (Fishbein et al., 1964).

2.15.1.1 Neuro Toxicity

Recent epidemiological findings indicate that neurotoxicity may be a relevant human toxicological endpoint as a consequence of chronic as well as acute chlordane exposure. Neurotoxicity and possibly hematotoxicity (Fleming and Timmeny, 1993) are the principal endpoints of acute chlordane toxicity in both experimentally poisoned animals and accidentally poisoned humans, with tremors and convulsions being common interspecies symptoms (Grutsch and Khasawinah, 1991). These results indicate that neurological effects are a relevant endpoint in humans exposed to chlordane. Chronic exposure of pesticides has lethal effect on human health in term of cancer and neuro toxicity (Michael C.R. et al, 2004).Neurobehavioral toxicity of deltamethrin exposure was also reported. (Dayal M, Parmar D. et al, 2003)

2.15.1.2 Hepato Toxicity

Other oral studies confirm the liver as the target organ of chlordane in rodents.In a 30-month oral study (Khasawinah and Grutsch, 1989b), Fischer 344 rats (80/sex/group) were exposed to 0, 1, 5, or 25 ppm technical chlordane in the diet (0, 0.055, 0.273, or 1.409 mg/kg-day for females). Because symptoms of age-related leukemia, necrosis, swelling, hypertrophy, and fatty degeneration. (Solleveld et al., 1984; Stromberg and Vogtsberger, 1983) confounded exposure-related liver lesions, incidence of nonneoplastic liver lesions only in nonleukemic rats were evaluated. A review of these data (ICF-Clement, 1987) indicated that exposure-related lesions were found only in the liver. Absolute liver weight was increased significantly ($p < 0.05$) in 5- and 25-ppm males at 130 weeks and in 25-ppm females at 26 and 52 weeks. The livers of nonleukemic female rats in the 25-ppm (15/44) and 5-ppm (9/40) groups [but not the livers of animals exposed to 1 ppm (4/43)] had statistically significant increases in regional hepatocellular hypertrophy relative to controls (2/37). No such effects were noted in the nonleukemic males, although group sizes were smaller (n = 16-23). Because of the confounding of leukemia and the nonadverse nature of hepatocellular hypertrophy, no effect levels were assigned to this study.

The study indicates that rats may be more insensitive to the hepatotoxic effects of chlordane than are mice, which manifested adverse hepatic effects (necrosis and fatty degeneration) at comparable exposure levels.

It was investigated that alterations of fetal liver colony formation can take place by prenatal chlordane exposure (Bame H J B et al 1990).

2.15.1.3 Carcinogenicity

Groups of 50 male and 50 female Osborne-Mendel rats were fed low- or high-dose diets for 80 weeks and then observed for 29 weeks (NCI, 1977). Average doses were 10.2 and 20.4 mg/kg-day for male rats and 6.0 and 12.1 mg/kg-day for female rats. Surviving rats were sacrificed at 109 weeks. Statistically significant differences between exposed groups and control groups were restricted to observations that average body weight of high-dose male and female rats were consistently lower (by about 10%) than that of control rats throughout the study, and that obvious signs of toxicity (rough and discolored hair coats, palpable masses) occurred "frequently" in treated rats during the first year and increased in frequency during the second year. The lower dose (6-10.2 mg/kg-day) is a LOAEL for both sexes. This same study also reported cancer but no cancer effects in the livers of B6C3F1 mice (50/sex/group) that were fed diets with analytical chlordane for 80 weeks and then observed for 10 weeks. Average doses were 4.3 and 8.0 mg/kg-day for male mice and 4.3 and 9.1 mg/kg-day for female mice. Surviving mice were sacrificed at 90-91 weeks. Survival of male mice in both exposed groups was decreased significantly relative to control males.

Occupational exposures to pesticides and risk of hematopoietic cancers have also been reported (M.Merhi, M. Raynal., E. Cahuzac et al 2007).

2.15.1.4 Reproductive Toxicity

No multi-generational reproductive studies, by any route, exist for technical chlordane. Several items within the current chlordane database suggest that reproductive effects could be a relevant endpoint for chlordane. The study of Cassidy et al. (1994) indicates alterations in reproductive- related behavior in male rats as a consequence of chlordane exposure. Data on tissue distribution of chlordane or its metabolites also indicate the potential for reproductive consequences. Rani et al. (1992) reported accumulation of a major component of technical chlordane (heptachlor) in ovary, uterus, and adrenals in non-pregnant rats within 30 minutes after an oral dose of 120 mg/kg heptachlor. In pregnant rats, levels were markedly elevated in the uterus compared to non-pregnant rats; the higher accumulation is believed to be a result of a slower metabolic turnover of heptachlor. These results

indicate that chlordane or some of its components/metabolites have an increased affinity towards reproductive organs during pregnancy and may have potential to adversely affect reproductive processes. Reproductive/ developmental toxicity in male rats exposed to chlordane was observed (EFSA journal 2007).

Toxicity of chlordane matabolite oxychlordane in female rats was also investigated (Genevieve bondy. et al 2003).

Testicular cancer is often quoted as the most common type of cancer in young men. The secular trends across Europe and USA show that it is increasing in incidence in Caucasian men (Bergstrom et al, 1996; 2003; Fisher, 2004). The obvious regional differences in incidence and the association with birth cohort suggest a possible role of environmental factors in the development of testicular cancers (Mills NC, 1990). Testicular cancer arises from the carcinoma in situ (CIS) cells, which should have their origin in fetal life, whereby subnormal androgen and/or an increased estrogen exposure are potentially important factors (Eertmans et al, 2003). The main risk factor for testicular cancer is cryptorchidism, followed by hypospadiasis (Sharpe, 2003). A study conducted on workers of the plastic industry exposed to polyvinyl chloride (PVC) demonstrated a significantly increased risk of seminoma (cancer of semniferous tubules) (Ohlson & Hardell, 2000).

2.15.1.5 Immuno Toxicity

Several investigations have assessed the effects of chlordane on the immunological system of offspring exposed during gestation and found that chlordane may affect cell-mediated immunity. Pregnant BALB/c mice (6/group) were fed 0, 0.16, or 8 mg/kg-day chlordane during the 19-day gestation period (Spyker-Cranmer et al., 1982). At birth, pups were randomized within treatment groups (45 pups/group), weaned at 28 days, and fed normal diets. Immunological tests performed at 101 days of age showed a marked decrease ($p < 0.01$) in cell-mediated immune response (contact hypersensitivity assay) in the 8-mg/kg-day group. The lower dose of 0.16 mg/kg-day is a NOAEL for this endpoint. Barnett et al. (1985a) found that in utero exposure to either 8 or 16 mg/kg-day chlordane (in peanut butter) in pregnant BALB/c mice during gestational days 1-18 resulted in a significant decrease ($p < 0.05$) in virus-specific delayed-type hypersensitivity response in the offspring (degree

of footpad swelling), but only at 48 hours post injection of an influenza type A virus. No differences were apparent at 72 hours postinjection. The LOAEL is 8 mg/kg-day. In another mice study by Barnett et al. (1985b), delayed hypersensitivity response in offspring, as well as depressed mixed lymphocyte reactivity of spleen cells in male offspring, occurred after in utero exposure to 4 or 16 mg/kg-day during gestational days 1-19. The LOAEL is 4 mg/kg-day. For both of these studies, offspring also were exposed to chlordane via milk.

Estmation of apoptosis and necrosis caused by pesticides *in vitro* on human lymphocytes using DNA diffusion assay were also carried out (Das GP et al 2006).

Fleming and Timmeny (1993) reviewed case studies of aplastic anemia associated with acute exposure to chlordane and other chlorinated pesticides, implicating these pesticides in bone marrow toxicity. In an effort to understand the effects of prenatal chlordane exposure on adult bone marrow expansion potential, Barnett et al. (1990b) exposed female mice orally to chlordane at 0, 4, or 8 mg/kg-day for 18 days during pregnancy. Offspring were nursed, which would provide some postnatal chlordane exposure. Bone marrow hematopoietic activity, as measured by the ability of bone marrow cells to undergo clonal expansion in response to stimulating factors, and spleen colony forming units (after irradiation) both were evaluated in offspring of these mice at 100 and 200 days of age. Results showed a significant dose-related depression ($p < /= 0.05$) of both measures at both 100 and 200 days postexposure. In a subsequent study in which these same measures were evaluated at 18 days gestational age (and without chlordane exposure via milk), these results were confirmed, indicating that damage to stem cells occurred during the fetal period (Barnett et al., 1990b). A LOAEL of 4 mg/kg-day is indicated by these results.

Acute toxicity bioassay using paramecium caudatum, a key member to study the effects of monocrotophos, on swiming behaviour and morphology was done (Rao VJ et al, 2007).

Some evidances were found for a novel endocrime disruptors : the pesticides propanil (a herbiside) requires the ovaries and steroid synthesis to enhance humoral immunity. Cytogenetic effects of commercial formulation of cypermethrin in root meristem cells of Allium sativum (P. N. Saxena. et al 2005)

2.16 (Anti) Androgenic assay:

Assays to detect androgenic properties of different chemicals have been developed since 1930s, but Hershberger et al. demonstrated the best among them (Hershberger et al., 1953). They analyzed in castrated rats, the response of the ventral prostrate, seminal vesicles and coagulating glands to exposure of several chemicals, mostly androgenic and some estrogenic and even progesterogenic. In this assay, among all parameters tested the capacity of the exogenously administered chemicals to restore the weight of the accessory sex organs of the castrated male rats was determined and validated. It has been used widely to screen different (anti) androgenic compounds (Yamada et al., 2004; Freyberger, 2007; Tinwell et al., 2007). Anti androgenic activity in diesel exhaust particles were also reported (Chun Mei Li et al,2006).

In contrast to the situation for reproductive effects, a number of studies have examined the potential of chlordane or its metabolites to affect developmental processes through investigations of a variety of endpoints.

CHAPTER – 3

MATERIALS AND METHODS

Ever increasing needs to increase food production have resulted in the accelerated use of herbicides, pesticides, fungicides and other agrochemicals in modern agriculture. Though these are biologically active chemicals, used for control of weeds and pests, but their chemical activity quite often extends beyond their target, resulting in serious problems in the biosphere.

Due to their lipophilic nature organochlorine pesticides accumulate up in the food chain and cause serious health problems. Similarly organophosphates also have direct effect on the biota. The present study was, therefore, planned to assess the effects of organochlorine and organophosphate pesticides, upon reproductive function of the mammals.

3.1 Insecticides selected for experiments

Following insecticides have been used for the experimentation:-

(1) Chlordane : an organochlorine

(2.) Monocrotophos : an organophosphate

3.1.1 Organochlorine

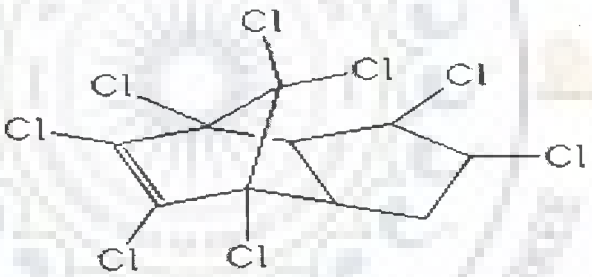
Organochlorine insecticides are lipophilic and due to their slow biodegradable nature they persist in the environment. They are usually termed as chlorinated hydrocarbon. Insecticides of technical grade were obtained from Hoechst India Ltd. Mumbai, India. The purity of Chlordane insecticide is as follow.

Pesticide Standards

Pesticide	:	Chlordane
Common Name	:	Chlordane
IUPAC Name	:	1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-exahydro-4, 7-methanoindene
Mol. Formula	:	$C_{10}H_6Cl_8$
Mol. Weight	:	409.76 g/mol
Form	:	Thick, liquid man-made chemical whose color ranges from colorless to amber.

Solubility	:	Chlordane is not soluble in water and is either odorless or has a mild, irritating odor. Lipid Soluble.
Acute oral LD_{50}	:	150-700 mg/kg b.wt. for rat.
Mode of action	:	Chlordane mimics the action of nerve excitement of Picrotoxine. Chlordane in neuro transmitter Gamma Aminobutyric acid (GABA) found in C.N.S. GABA Inhibits results in partial repolarization of the neuron and a state of uncontrolled excitation.
Use	:	Chlordane is also used as a pesticide on agricultural crops, lawns, and gardens and as a fumigating agent.

Structure :



CHLORDANE

3.1.2 Organophosphate

Organophosphate insecticides possess phosphorus atoms and therefore, also termed as phosphate insecticide or organic phosphate. They (OPs) inhibit the cholinesterase enzyme of the nervous system.

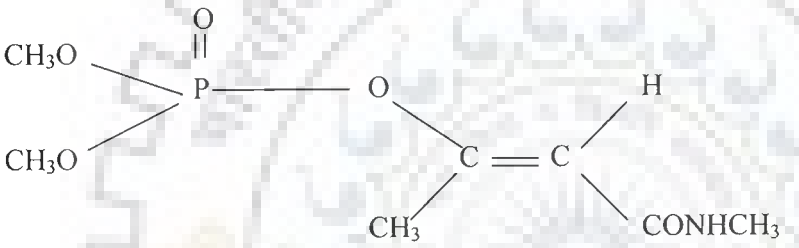
Pesticide Standards

Pesticide: Monocrotophos

Synonyms: phosphoric acid, dimethyl [1-methyl-3-(methylamino)-3-oxo-1-propenyl] ester; (*E*)-phosphoric acid dimethyl ester, ester with 3-hydroxy-*N*-methylcrotonamide; -(dimethoxyphosphinyloxy)- *N* - methyl - *cis*crotonamide; dimethyl 2-methylcarbamoyl-1-methylvinyl phosphate, Azodrin, Nuvacron.

Molecular formula : $C_7H_{14}NO_5P$

Structure



Monocrotophos

CAS number: 6923-22-4

Physical and Chemical Properties:

Molecular weight	:	223.2
Boiling point	:	At 0.07 Pa: 125°C
Melting point	:	54-55oC
Vapour pressure	:	3 x 10-4 Pa
Solubility in water	:	miscible
Log Poctanol/water	:	-0.22
Conversion factors	:	not applicable

3.2 Mode of Action

It inhibits the cholinesterase enzyme of the nervous system. Monocrotophos is a systemic insecticide and acaricide belonging to the vinyl phosphate group. It controls pests on a variety of crops, such as cotton, rice, and sugarcane. It is used to control a wide spectrum of chewing and sucking insects and also mites.

3.3 Experimental Model

The present study was carried out on inbreed proven fertile adult male albino rats (*Rattus norvegicus*) of wistar strain. Healthy animals weighing 150-200 g. were selected for the experiments.

The animals were housed individually in polypropylene cages (Measuring 12" X 10" X 8") with stainless steel wire lids and heat-treated hard-woods chips as bedding. The animals were maintained at a room temperature (21-24°C±3°C) and uniform light dark cycle (14:10: L: D) with relative humidity 55 ±5%. The rats had a diet of standard commercial pelleted feed (Ashirwad food industries Ltd., Chandigarh, India) and water ad libitum thought out the study. The weight of animals was measured weekly to see any change in the body weight.

3.4 Experimental Design

All the experiments reported in this thesis had prior approval of IAEC and performed accordingly .The experimental protocol met the national guidelines on the proper care and use of animals in laboratory research. The animals were classed according to their body weights and allocated randomly to groups. Two and five weeks treatment periods were chosen to investigate the toxicity of pesticides.

At the end of the experimental period, the animals were weighed and euthanized under light ether anesthesia and blood samples were collected by cardiac puncture in preheparinized tubes. Serum was separated by centrifugation at 3000 rpm and stored at-20°C until biochemical and testosterone assay.

The reproductive organs-testis, epididymides, seminal vesicle, ventral prostate, vas deferens and vital organs-liver and kidney were dissected out and weighed after removing the adherent tissue and were frozen for the biochemical

estimations. They were also fixed with Bouin's fixative for at least 48 hrs., processed by the paraffin wax impregnation method, and sections (5 μm thick) were cut using a rotary microtome. The sections were stained with haematoxylin and eosin (H&E) and examined by light microscopy for histopatholigical changes. Photomicrographs of sections were taken. We performed various biochemical test.

3.5 Median Lethal Dose (LD_{50})

The LD_{50} is statistically derived single dose of a substance that can be expected to cause the death of 50% animals. In a probit analysis method of LD_{50} the selected dose levels should bracket the expected LD_{50} value with at least one dose level higher than the expected LD_{50} but not causing 100% mortality and one dose level below the expected LD_{50} but not causing 0% mortality.

In the present investigation, various calculated doses (mg./kg. b.wt.) of insecticides were given orally with the help of hypodermic syringe having pearl point needle. Six animals were tested for each dose level. Control rats were given equivalent amount of vehicle. Poisoning symptoms and mortality were observed daily for three days following the treatment. Results of the toxicity were analyzed statistically (Finney, 1971) for the determination of LD_{50} values of insecticides.

3.6 Treatment Protocol

Weight matched fertile male rats of similar size and ages were assigned to groups of five animals each for both two insecticides as follows:

3.6.1 Chlordane

Group I	:	Control received vehicle (Olive oil) only
Group II	:	Animals received 200 mg/kg. b.wt./day of chlordane orally for 2 and 5 weeks.
Group III	:	Animals received 400 mg/kg. b.wt. / day of chlordane orally for 2 and 5 weeks.
Groups IV	:	animals received 600 mg/kg. b.wt. / day of chlordane orally for 2 and 5 weeks.

3.6.2 Monocrotophos

Group I	:	Control received vehicle (Peanut oil) only.
Group II	:	Animals received 6 mg/kg. b.wt./day of monocrotophos orally for 2 and 5 weeks.
Group III	:	Animals received 14 mg/kg. b.wt. / day of monocrotophos orally for 2 and 5 weeks.
Groups IV	:	Animals received 22 mg/kg. b.wt. / day of monocrotophos orally for 2 and 5 weeks.

3.7 Mode of administration

Forced Oral Feeding

Various calculated doses of technical grade of insecticides; Chlordane and Monocrotophos were administered forcibly through mouth by pearl point needle. The diet was withdrawn before the oral intubation, after those animals were allowed to take normal rat feed and water ad libitum.

On the day next to final dosing, animals from each group were autopsied for detailed hematological, histological, radioimmunoassay, serum and tissue biochemical investigation.

3.8 Parameters Studied

3.8.1 Body and Organ Weights

Initial and final body weights and weights of testis, epididymis, seminal vesicle, ventral prostate, adrenal gland, liver, heart and kidney were recorded.

3.8.2 Sperm Dynamics

3.8.2.1 Sperm Motility

Sperm motility was assayed by the method of Prasad et al., (1972). The epididymides were removed immediately after anesthesia and weight of cauda epididymis was gently teased in a specific volume of physiological saline (0.9% NaCl) to release the spermatozoa from the tubules. The sperm suspension was examined within five minutes after their isolation from epididymis. The results were determined by counting both motile and immotile sperm in at least ten separate and randomly selected fields. The results were finally expressed as percent motility.

3.8.2.2 Sperm Density

Total number of sperm were counted using haemocyto meter after further diluting the sperm suspension from cauda epididymis and testis. The sperm density was calculated in million per the dilution (Prasad et al., 1972).

3.8.3 Histological Studies: -

3.8.3.1 Histopathology of various tissues

For histopathological staining, the basic protocol by Mukherjee et al. (2003) was followed with modifications according to our laboratory conditions. Briefly, the liver, kidney, testis, ovary, SATs and uterus were separated and fixed in Bouins solution (saturated aqueous solution of picric acid, 75%; formalin, 25%; glacial acetic acid, 25%) for 4 hrs. Following the fixation, sections were washed thoroughly in 30% alcohol until all the color of picric acid disappeared. This was followed by gradual dehydration of the samples by placing them in ascending series of alcohols (each change for 1 hr.) viz. 30% - 50% - 70% - 80% (2 changes) - 95% (2 changes) - 100% (3 changes) – xylene (3 changes). The tissues were then placed in wax, made into blocks and finally cut into sections of 5 micron thickness. Sections were adhered on the glass slide treated with Mayer's solution (per 100 ml. having 50 ml. egg albumin, 50 ml. glycerin, 1.0 gm sodium salicylate,), stretched at approximately 40 ^{0}C temperatures and preserved. The sections were then stained in haematoxylin and eosin by placing the slide in different solutions in a sequential manner as described below: xylene (2 changes, each 5 min.) followed by 1 min. change each in 1:1 (v/v) xylene and 100% alcohol - 100 % alcohol (2 changes) – 90% alcohol – 80% alcohol – 70% alcohol – washing in tap water followed by gentle tapping of slide – haematoxylin solution (5min.) – washing in running water – 3 to 4 dippings of slide in 0.5% HCl – wash in distilled water (1 min.) – several dippings in ammonia water (section changes to blue color) - wash in distilled water (1 min.) – 80% alcohol (1 min.) – 95% alcohol (1 min.) – eosine staining (2 to 3 min.) – 70% alcohol – 95% alcohol– 100% alcohol (1 min. each) – xylene (two changes, 1 min. each). This was followed by the mounting of tissue with canada balsam and preservation with cover slip.

The reproductive organs (testis, epididymis, seminal vesicle, ventral prostate and vas deferens) and vital organs (liver and kidney) were fixed in Bouin's fixative

and cut into sections and processed through ethanozylene series. The tissues were embedded in paraffin and bee wax (3:1) (m.p.-55-62° C). Sections were cut at 5 μ and stained with Harris haematoxylene and eosin.

3.8.4 Hematological Studies

Following haematological parameters were estimated in blood:-

1 Haemoglobin
2 Haematocrit
3 Total erythrocyte count
4 Total leukocyte count
5 Blood Sugar
6 Blood Urea

3.8.5 Serum Analysis

Blood collected from heart was followed to clot at room temperature and serum was separated by centrifugation and stored at -20° C for following parameters:-

1 Total Cholesterol
2 Total Protein
3 Serum Phospholipid
4 Serum Triglyceride
5 HDL Cholesterol
6 LDL Cholesterol
7 VLDL Cholesterol
8 Acid- Phosphatase
9 Alkaline Phosphatase
10 Aspartate Amino Transferase
11 Alanine Amino Tranferase
12 Bilirubin

3.8.6 Statistical Analysis

The data obtained from the above experiments were subjected to statistical analysis. All the values of body weights, hematological and biochemical estimations were averaged, standard error of the mean values were calculated and compared. Student't' test was applied for standard comparison (Ipstein and Poly, 1970).

Standard error

$$\delta x = \sqrt{\frac{n\Sigma x^2 - (\Sigma x)^2}{n^2 (n-1)}}$$

Significance 't'test

$$\text{'t' value} = \frac{\bar{X}A - \bar{X}B}{\sqrt{(\delta XA)^2 + (\delta XB)^2}}$$

Percent deviation

$$\frac{\bar{X}B - \bar{X}A}{\bar{X}A} X\ 100$$

Degree of freedom = n_1+n_2-2

Where,

n = number of variables in each group

$\sum X$ = Sum of independent variables

$\bar{X}A$ = Mean value of control group

$\bar{X}B$ = Mean value of treated group

$\delta\bar{X}A$ = Standard error of control group

$\delta\bar{X}A$ = Standard error of control group

n_1 = number of variable of control group

n_2 = number of variable of treated group

Significance between the values of different groups was judged at 0.0, 0.01 and 0.001 levels.

3.8.7 Serum Alkaline Phosphatase, Acid Phosphatase, SGPT and SGOT Level

Serum from all the animals was subjected to assay of the four enzymes present in serum viz. Alkaline phosphatase (ALP), acid phosphatase (ACP), serum aspartate aminotransferase (AST) or glutamate oxaloacetate transaminase (SGOT)

and alanine aminotransferase (ALT) or serum glutamate pyruvate transaminase (SGPT) by the methods as described in Mukherjee, 2003.

3.8.7.1 Serum acid phosphatase

First of all following reagents were prepared-

- Citrate buffer (0.09 gm/L)- 18.9g of citric acid monohydrate, 500 ml H_2O + 180 ml 1N NaOH + 100 ml 0.01 N HCl, adjust the pH to 4.85 and dilute to 1000 ml.
- Tartrate – Citrate buffer (pH 4.85), 0.09 mole citrate and 0.04 mole tartrate/L- 1.5 g of L-tartrate in 250 ml of citrate buffer (1).
- Stock substrate of PNPP (4 mg/ml)
- Working buffered substrates- mix equal volumes of acid buffers and substrate:
  (a) Citrate buffer and stock substrate (solution 1 and 3)
  (b) Tartrate – Citrate buffer and stock substrate (solution2 and 3)
- Store both solutions in 1 ml aliquots in test tubes (15 ml) Sodium hydroxide (0.1N).

After the reagents are prepared frozen substrate solutions were taken -2 citrate substrate tubes and one tartrate substrate tube. They were marked as "CT" (citrate total), "CSB" (citrate serum blank) and "T-NP" (tartrate non-prostatic). Two more tube of one each citrate substrate and one tartrate were taken and labeled as CB and TB. They were used as blank and all the tubes were incubated at 37^0C for 5 min. After exact 5 min., 0.2 ml. serum was added to CT and T-NP test tubes and continues incubation at 37^0C for 30 min. after which 4 ml. of 0, 05 M NaOH was added to both test tubes and mixed well to stop the reaction. Tubes were removed from water bath and 0.2 ml. serum was added to the tube marked as 'CSB' and 0.2 ml. water was added to the substrate blank tubes marked as 'CB' and 'TB'. The solution in "CB" tube was used to zero "CT" and "CSB" tube and "TB" tube was used to zero"T-NP" tube. Calibration curve was referred for reporting the enzyme activity in international units (U/L)

3.8.7.2 Serum Alakline Phosphatase

Two test tubes were taken and were labeled as T and B for test and blank respectively. 1 ml. working substrate (equal volume of p-nitrophenyl disodium phosphate, 4 mg/ml. and glycine buffer) was taken and incubated at 37^0C for 5 min. After exact 5 min., 0.5 ml. serum was added to test tube marked as 'T' and incubation was continued at 37^0C. After 30 min., 10 ml. of 0, 05 M NaOH was added to both test tubes and was mixed well to stop the reaction. Tubes were removed from water bath and 0.5 ml. serum was added to the tube marked as 'B' (for blank). Absorbance was recorded against water at 405 nm and determines the change in absorbance by subtracting the absorbance of tube 'B' from 'T'. Calibration curve was referred for reporting the enzyme activity in international units (U/L).

3.8.7.3 Serum SGOT

Two test tubes were taken and were labeled as T and B for test and blank respectively. 1 ml substrate (200mM/L DL-aspartate + 2mM/L alpha-ketoglutarate in 0.1M phosphate buffer, pH 7.4) was taken and incubated at 37^0C for 5 min. After exact 5 min., 0.2 ml. serum was added to test tube marked as 'T' and incubation was continued at 37^0C After 60 min., 1.0 ml. of hydrazine was added to both test tubes (1mM/L prepared in 1N HCl) and mixed to stop the reaction and develop the colour. Tubes were removed from water bath and 0.2 ml. serum was added to the tube marked as 'B' (for blank). After 20 min., 10 ml. of 0.4N NaOH was added and mixed by inversion and reaction was continued for 5-20 min. Absorbance was recorded against water at 505 nm and the change in absorbance was determined by subtracting the absorbance of tube 'B' from 'T'. Calibration curve was referred for reporting the enzyme activity in international units (U/L).

3.8.7.4 Serum SGPT

Two test tubes were taken and labeled them as T and B for test and blank respectively. 1 ml. substrate was taken (200mM/L DL-alanine + 2mM/L alpha-ketoglutarate in 0.1M phosphate buffer, pH 7.4) and incubate at 37^0C for 5 min. After exact 5 min., 0.2 ml. serum was added to test tube, marked as 'T' and incubation was continued at 37^0C. After 30 min., 1.0 ml. of hydrazine was added to both test tubes (1mM/L prepared in 1N HCl) and mixed to stop the reaction and develop the colour.

Tubes were removed from water bath and 0.2 ml. serum was added to the tube marked as 'B' (for blank). After 20 min., 10 ml. of 0.4N NaOH was added and mixed by inversion and reaction was continued for 5-20 min. Absorbance was recorded against water at 505 nm and the change were determined in absorbance by subtracting the absorbance of tube 'B' from 'T'. Calibration curve was referred for reporting the enzyme activity in international units (U/L).

3.8.7.5 Blood Cholesterol

Placed 10 ml. of the alcohol-acetone solvent in a centrifuge tube and added 0.2 ml. of serum or blood. Immersed the tube in a boiling water bath with shaking until the solvent began to boil and than removed the tube and continued shaking the mixture for further 5 minutes. Cooled to room temperature and centrifuged. Decanted the supernatant fluid into a test tube and evaporated to dryness on a boiling water bath. Cooled and dissolved the residue in 2 ml. of chloroform. At the same time, set up a series of standard tubes containing cholesterol and a blank with 2 ml. of chloroform.

Than added 2 ml. of acetic anhydride-sulphuric acid mixture to all tubes and thoroughly mixed. Left the tubes in the dark at room temperature and read the extinction at 680 nm.

3.8.8 Hershberger Assay

For evaluation of androgenic activity of chlordane and monocrotophos in castrated rats.

3.8.8.1 Chemicals

Chlordane, Monocrotophos and olive oil, propylene glycol were purchased. The routine chemicals used in this thesis were purchased from the SRL, Mumbai; SD fine Chemicals, Mumbai.

3.8.8.2 Animals and Housing

Male wistar albino rats were provided by All India Institute of Medical Sciences (AIIMS), New Delhi. Rats were provided with tap water and a commercial diet ad libitum.The animal room was maintained at temperature

24± 2° C, relative humidity of 50±20% and a 12-h light/dark cycle. 53 days adult male rats were allocated to each test group. Body weight and clinical signs were recorded daily throughout the study. All animals were cared according to the guidelines for good laboratory practices prepared by committee for the purpose of control and supervision on experiments on animals (CPCSEA).

3.8.8.3 Procedure of Hershberger Assay

Chlordane was dissolved in olive oil and diluted to dose of 200 mg/kg, while Monocrotophos was diluted with propylene glycol to dose of 10mg/kg body weight and forty two days old rats were castrated and administered via gavages to 51 days of castrated rats. Approximately 24 hrs. after final treatment androgen-dependent accessory sex organs or glands; vas deference, seminal vesicle, epididymis, prostate gland were carefully removed and weighed. Liver, kidney, adrenal gland were also weighed.

3.8.8.4 Statistical analysis

Body weight gain, organ weights were analyzed by one-way analysis of variance with post hoc comparison between the control group and each treatment group, followed by Duncan's multiple comparison using statistical program. A p-value ‹ 0.05 was taken as a statistically significant difference between two groups.

3.8.9 Behavioral Toxicity

Sex arousal (Libido) capacity of chlordane and monocrotophos treated male rats were investigated.

Behavioral toxicology is the study of the behavioral pattern of organisms under exposure to toxicants. Behavioral study is the final common pathway for all neural and non neural activity and as such represents an integrated response to an environmental stimulus or insult. Behavioral pattern offers a sensitive pathway to monitor subtle damage to the functioning of central nervous system (CNS). A critical assertion in behavioral toxicity is that the behaviour of organisms represents the net result of various sensory, monitor and associative functions of the nervous system. It is the endpoint of the functional integrity of the nervous system. Human and

experimental studies have shown that behavioral aberrations are amount of the earliest of the toxicity symptoms and often occur at exposure levels insufficient to produce histological detectable damage in the CNS or other organs. Thus, deviations from normal behavioral pattern may comprise an early warning system, presaging more severe damage induced by an agent at higher doses or after longer exposure. In Japan, France and U.K. Government regulations mandate that behavioral evaluations be done on all new drugs during pre market testing. In the USA the Toxic Substances Control Act of 1976 (TOSCA) was enacted in an attempt to consolidate the regulation of chemical industry and it specifically states that chemicals should be evaluated for their behavioral effects.

Warner et al. (1966) commented, "The behavioral (or activities) of an organism represents the final integrated result of a diversity of biochemical and physiological processes. Thus, a single behavioral parameter is generally more comprehensive than a physiological or biochemical parameter." They also considered that "Behavioral patterns are known to be highly sensitive to changes in the steady state of an organism." Hence in their search for a rapid, biological method for detecting the sub lethal effects of pesticides alterations in the behavioral was used as the diagnostic tool for identifying the ecological effects of the release of a toxicant in to an environment.

3.8.9.1 Investigation on sexual behavioral (Libido) on male rats by insecticides:

Administration of insecticides may alter the sex arousal capacity in male rats. To investigate it, we fabricated a shuttle box of wooden (80x40cm) .This shuttle box consists of two adjacent chambers connected through a perforated plastic partition having three pores of 3X3 cm with a sliding manual door.

3.8.9.2 Procedure

Initially in one chamber, control rat was placed and second chamber was kept vacant. Number of approaches of normal male rats towards female rats of control group was recorded and then chlordane and monocrotophos administered rats were placed in another chamber respectively. Then adult female (in estrous cycle) rat was

kept in second chamber and numbers of approaches of test male rats were counted for 60 minutes for investigating the alterations in libido.

Figure No.4: Showing sex arousal in male rats treated with chlordane and monocrotophos.

CHAPTER – 4
OBSERVATIONS AND RESULTS

[a] Chlordane

Table 1: Body and Organ Weight

Treatment	Body weight		Testis	Epididymides	Seminal Vesicle	Prostate Gland	Liver	kidney	Adrenal Gland
	Initial	Final							
	Gm		mg/100 gm body wt .						
Group I Control (Vehicle treated)	195.67 ±9.10	195.75 ±7.60	1275.25 ±22.79	431.35 ±20.67	405.30 ±26.17	148.53 ±16.25	3420.16 ±84.18	695.12 ±31.16	19.12 ±0.96
200 mg/kg.b.wt./day of Chlordane									
Group IIA (2Week)	202.60 ±19.00	271.20 ±22.60	1115.35** ±30.72	355.24* ±20.79	288.65* ±18.75	165.30 ±18.20	3438.20* ±240.50	731.27 ±33.14	25.12 ±4.13
Group IIB (5Week)	271.10 ±19.00	286.2 ±19.00	1201.04* ±39.32	416.41 ±11.23	360.18 ±46.20	176.20 ±10.75	3810.10** ±62.60	746.15 ±21.40	21.25 ±2.80
400 mg/kg.b.wt./day of Chlordane									
Group IIIA (2Week)	176.50 ±9.30	154.00 ±8.00	1110.29** ±20.28	378.52 ±19.15	271.85* ±29.08	145.05 ±21.22	4212*** ±175.67	715.71 ±22.17	33.90* ±1.61
Group IIIB (5 Week)	182.25 ±10.00	181.25 ±10.00	1055.65*** ±20.65	330.67** ±16.26	238.19** ±31.18	177.60 ±17.40	4789.16*** ±210.11	738.15 ±17.63	51.50 ±3.10
600 mg/kg.b.wt./day of Chlordane									
Group IVA (15 Days)	183.00 ±10.20	165.50 ±7.70	1015.56*** ±32.10	372.17* ±13.60	260.20** ±66.40	187.11* ±11.85	4040.21*** ±61.60	772.62 ±19.11	34.16* ±3.62
Group IVB (30 Days)	180.00 ±8.60	168.00 ±6.00	910.00*** ±20.18	313.23** ±12.05	292.51** ±11.60	227.15* ±13.12	4413.15*** ±108.07	790.12 ±21.51	34.78* ±4.78

Group IIA, B,; IIIA, B; IVA, B; compared with Group I

(Mean ± SEM of 5 animals)

* = $p \leq 0.05$

** = $p \leq 0.01$

*** = $p \leq 0.001$

Table 2 : Sperm Dynamics and Fertility

Treatment	Sperm Motility (%) (Table 2.1)	Sperm Density (Million/ ml) (Table 2.2)		Fertility (%) (Table 2.3)
		Testis	Cauda Epididymides	
Group I Control (Vehicle treated)	69.19 ±3.75	4.16 ±0.07	21.25 ±0.42	100% (+)tve
200 mg/kg.b.wt./day of Chlordane				
Group IIA (2Week)	56.10* ±2.35	2.69** ±0.32	20.82 ±0.35	20%(-)ve
Group IIB (5 Week)	44.27** ±2.16	0.82 ±0.08	12.46** ±1.92	20%(-)ve
400 mg/kg.b.wt./day of Chlordane				
Group IIIA (2 Week)	52.28** ±3.17	0.88*** ±0.12	14.42*** ±1.39	40%(-)ve
Group IIIB (5 Week)	38.40*** ±1.25	0.70*** ±0.04	11.52*** ±1.69	60%(-)ve
600 mg/kg.b.wt./day of Chlordane				
Group IVA (2 Week)	17.15*** ±1.40	1.30*** ±0.21	7.24*** ±1.31	80%(-)ve
Group IVB (5 Week)	16.64*** ±2.17	0.85*** ±0.27	6.41*** ±0.47	100%(-)ve

Group IIA, B,; IIIA, B; IVA, B; compared with Group I

(Mean ± SEM of 5 animals)

* = $p \leq 0.05$

** = $p \leq 0.01$

*** = $p \leq 0.001$

Table 3 : Blood Analysis (Hematology)

Treatment	Total erythrocyte Count (TEC)	Total leukocyte Count (TLC)	Hemoglobin	Haematocrit	Blood Sugar	Blood Urea
	------ million /mm^3 ----------		gm %	%	----- mg/dl------	
Group I Control (Vehicle treated)	6.64 ±0.24	5420 ±240	15.60 ±0.30	49.45 ±1.49	85.15 ±2.10	47.75 ±3.50
200 mg/kg.b.wt./day of Chlordane						
Group IIA (2 Week)	6.47 ±0.18	6770* ±521	12.16*** ±0.58	52.10 ±1.10	105.31* ±5.62	81.60*** ±3.20
Group IIB (5 Week)	6.02* ±0.28	5688 ±127.10	11.74*** ±0.36	45.25 ±2.65	130.81*** ±5.20	77.50** ±4.65
400 mg/kg.b.wt./day of Chlordane						
Group IIIA (2 Week)	5.78** ±0.04	6560* ±375.20	9.60*** ±0.78	43.00 ±4.40	102.48* ±5.50	73.10** ±2.50
Group IIIB (5 Week)	5.58** ±0.36	7260** ±345.14	9.08*** ±0.98	40.15* ±6.20	128.10* ±11.00	90.51*** ±1.50
600 mg/kg.b.wt./day of Chlordane						
Group IVA (2 Week)	5.41** ±0.28	6891.50* ±612.10	10.58*** ±0.61	33.50* ±4.75	135.99*** ±6.01	85.20*** ±4.50
Group IVB (5 Week)	5.22** ±0.13	7410** ±487.10	10.02*** ±0.19	36.60** ±2.1	113.30** ±5.31	89.60** ±8.60

Group IIA, B,; IIIA, B; IVA, B; compared with Group I

(Mean ± SEM of 5 animals)

* = $p \leq 0.05$

** = $p \leq 0.01$

*** = $p \leq 0.001$

Table 4 : Serum Analysis

Treatment	Alanine amino transferase (ALT)	Aspartate amino transferase (AST)	Acid Phosphatase	Alkaline Phosphatase	Bilirubin
	--------- units/ml ----------		----------KA units----------		mg %
Group I Control (Vehicle treated)	131.28 ±7.12	71.80 ±1.80	4.51 ±.07	65.70 ±2.20	0.22 ±0.02
200 mg/kg.b.wt./day of Chlordane					
Group IIA (2 Week)	173.50** ±8.60	114.00*** ±1.35	4.57 ±0.18	47.15** ±2.29	0.28 ±0.05
Group IIB (5 Week)	161.10** ±1.35	110.00*** ±1.20	4.86 ±0.15	52.27** ±0.32	0.55 ±0.04
400 mg/kg.b.wt./day of Chlordane					
Group IIIA (2 Week)	147.50 ±6.20	111.60*** ±6.28	4.98* ±.0.09	50.60** ±4.12	0.78** ±0.18
Group IIIB (5 Week)	162.75 ±10.15	128.00*** ±2.40	5.82 ±1.01	53.07** ±3.08	0.70** ±0.28
600 mg/kg.b.wt./day of Chlordane					
Group IVA (2 Week)	196.5*** ±5.56	116.75*** ±1.55	5.78** ±0.40	50.42** ±3.90	2.85** ±0.89
Group IVB (5 Week)	173.5** ±6.25	124.65*** ±1.93	5.74** ±0.51	40.50** ±3.78	3.95*** ±0.14

Group IIA, B,; IIIA, B; IVA, B; compared with Group I

(Mean ± SEM of 5 animals)

* = $p \leq 0.05$

** = $p \leq 0.01$

*** = $p \leq 0.001$

Table 5 : Serum Analysis

Treatment	Total protein	Phospholipid	Triglyceride	Total – Chol.	HDL- Col.	LDL – Chol.	VLDL- Chol.
	---------- mg/dl ----------						
Group I Control (Vehicle treated)	16950.15 ±621.19	146.12 ±4.10	99.16 ±1.21	96.72 ±4.9	41.10 ±5.70	37.50 ±6.20	18.80 ±2.60
200 mg/kg.b.wt./day of Chlordane							
Group IIA (2Week)	35411.87*** ±522.50	136.40 ±5.01	94.52 ±2.72	88.70 ±3.50	36.70 ±3.22	32.70 ±3.27	17.70 ±4.85
Group IIB (5 Week)	41280.10*** ±846.60	161.20 ±12.81	139.00 ±9.60	140.20* ±13.02	59.91* ±6.60	53.20 ±13.90	28.00 ±2.20
400 mg/kg.b.wt./day of Chlordane							
Group IIIA (2 Week)	39810.10*** ±401.10	258.50** ±6.10	114.45 ±13.60	85.26 ±11.90	44.40 ±6.80	22.15 ±6.40	21.75 ±2.50
Group IIIB (5 Week)	43510.42*** ±450.15	288.60** ±17.15	103.25 ±8.95	122.00* ±11.78	52.15 ±9.80	18.00 ±1.82	20.20 ±1.90
600 mg/kg.b.wt./day of Chlordane							
Group IVA (2 Week)	36817.95*** ±680.10	178.80* ±5.89	176.00** ±15.25	128.00** ±5.60	51.05 ±5.70	62.00* ±4.10	36.20** ±3.10
Group IVB (5 Week)	37320.16*** ±485.15	176.00* ±7.12	200.00*** ±10.15	148.20** ±5.10	66.00** ±4.98	60.00* ±2.10	42.00*** ±2.20

Group IIA, B,; IIIA, B; IVA, B; compared with Group I

(Mean ± SEM of 5 animals)

* = $p \leq 0.05$

** = $p \leq 0.01$

*** = $p \leq 0.001$

INSECTICIDES

4.1 CHLORDANE

The exposure of rat with technical grade of chlordane was carried out at three dose levels 200 mg, 400 mg and 600 mg/kg b.wt./day for 2 weeks and 5 weeks respectively.

4.1.1 WEIGHT RESPONSE

Body weight (Table 1)

The treatment with chlordane at all the three dose levels for 2 weeks and 5 weeks to intact rats caused reduction in their body weight of group III A,B and IVA,B while there was increase in group II A and group II B.

Organ weight (Table 1)

Testis

Oral administration of chlordane to rat caused a significant reduction ($P \leq 0.01$ and $P \leq 0.001$) in the weight of testis in comparison to control animals. The percent reduction was 12.54%, 13.64%, 12.94%, 17.25%, 20.39% and 28.62% respectively for IIA,B.,IIIA,B and IVA,B (Fig.1.1) .

Epididymides

A significant decrease ($P \leq 0.05$ and $P \leq 0.01$) in the weight of epididymides was observed after chlordane treatment at higher dose levels when compared with control rats.

Seminal vesicle

A significant decrease ($P \leq 0.05$ and $P \leq 0.01$) in seminal vesicle weight was observed after administration of chlordane at various dose levels for 2 weeks and 5 weeks, respectively (Fig. 1.2).

Prostate gland

A significant increase ($P \leq 0.05$) in the prostate gland weight was observed when chlordane administered at 600 mg/kg body weight dose level and duration as compared to control rats (Fig.1.3).

Liver

A significant increase ($P \leq 0.01$ & $P \leq 0.001$) in the weight of liver was brought about by chlordane treatment in different groups when compared with control group (Fig 1.4).

Kidney

Statistically non significant change was observed in the weight of kidney in the rats treated with chlordane in comparison to control rats.

Adrenal gland

Significant increase ($P \leq 0.05$) of the weight of adrenal gland was observed in the groups of rats exposed to higher dose levels of chlordane when compared with control rats.

4.1.2 SPERM DYNAMICS (Table 2)

Sperm motility (Table 2.1)

Cauda epididymides

The motility of spermatozoa in cauda epididymides decreased in a dose dependent manner i.e. 18.84%, 36.23%, 24.63%, 44.92% 75.36% and 76.81% in the rats treated with chlordane at 200, 400 and 600 mg/kg b.wt./day for 2 Weeks and 5 Weeks, respectively (Fig.2.1).

Sperm density (Table 2.2)

Testis

The chlordane exposed rats showed a significant (P ≤0.01 & P≤ 0.001) reduction testicular sperm density. The reduction was found in a dose dependent manner 35.33%, 80.28%, 78.84%, 83.17%, 68.75% and 79.56% in different groups i.e. IIA, B., IIIA, B. and IVA, B, respectively in comparison to control rats. (Fig.2.2)

Cauda epididymides

A significant decrease (P ≤0.01 & P≤ 0.001) 2.11%, 41.64%, 32.14%, 45.78%, 65.92% and 69.64% in the sperm concentration of chlordane treated rat was observed in different experimental groups i.e. IIA, B., IIIA, B. and IVA, B respectively in comparison to control rats (Fig.2.3).

Fertility test (Table 2.3)

Male rats and Female rats were kept for mating in the ratio of (1:2). Mating exposure test revealed that the control rats showed 100% positive fertility whereas 200 mg dose level showed 20% negative fertility, 400 mg dose level showed 40% and 60% negative fertility and 600 mg dose level showed 80% and 100% negative fertility (Fig.2.4). Numbers of pups were counted as indicator of fertility for all groups.

4.1.3 HEMATOLOGY (Table 3)

Total erythrocyte count (TEC)

A significant reduction in the erythrocyte count (P≤0.05 and ≤0.01) in chlordane treated rat at different dose levels was observed in comparison to control rats. (Fig.3.1)

Total Leukocyte count (TLC)

A significant increase ($P \leq 0.05$ and $P \leq 0.01$) in the leukocyte count of chlordane exposed rats 24.90, 4.94, 21.03, 33.94, 27.14 and 36.71% for IIA, B., IIIA, B. and IVA, B groups respectively was recorded in comparison to control rats.

Hemoglobin

Hemoglobin concentrations of different chlordane treated groups were found to be decreased significantly ($P \leq 0.001$) (Fig. 3.2).

Haematocrit percent

A significant decrease ($P \leq 0.05$, 0.01 and 0.01) in the percentage of haematocrit valuc was observed in chlordane exposed rats exclusively at higher dose level as compared to control group. However no significant effects were observed in low dose group (200 and 400mg/kg b.w.).

4.1.4 BIOCHEMICAL FINDINGS

Serum analysis (Table3, 4 and 5)

Blood Urea and Blood Sugar

A significant increase in blood sugar was observed in all the groups with exception of marginal increase in group like IIA and IIIA. Blood urea level in all the groups showed highly significant increase. Increase in blood urea was 70.89, 62.30, 53.08, 89.52, 79.47 and 87.64% respectively in group IIA, B, IIIA, B and IVA, B in comparison to control group.

Alanine amino transferase (ALT) and aspartate amino transferase (AST)

After exposure of chlordane to rats for 2 weeks and 5 weeks, it was observed that all the six groups of exposed rats showed highly significant ($P \leq 0.001$) increase in serum aspartate amino transferase. While alanine amino transferase was increased significantly

(P ≤0.01 and P≤ 0.001) in IIA,B and IVA,B all groups with no significant difference in group IIIA,B.. The respective increase in aspartate amino transferase was 58.77, 53.20, 55.43, 78.99, 62.53 and 73.53% and that of alanine amino transferase was 32.16, 22.71, 12.35, 23.93, 49.68 and 32.16% in IIA, B., IIIA, B and IVA, B in comparison to control animals. (Fig. 4.1 & 4.2)

Acid phosphates and Alkaline phosphates

A significant increase (P≤0.05) in the activity of acid phosphatase was observed after exposure to higher dose of chlordane to rats in comparison to control rats. Whereas significant (P≤0.01) decrease in the activity of alkaline phosphatase was observed in all the six groups of chlordane exposed rats in comparison to control rats. (Fig.4.3 & 4.4)

Bilirubin

Bilirubin concentration increased significantly (P≤ 0.01 and 0.001) after the oral administration of chlordane in group IIIA, B and IVA, B whereas no significant changes were observed in lower dose levels that are in group IIA, B as compared to control group.

Total protein (Table 5)

A significant (P≤ 0.001) increase in the amount of serum total protein was observed after 2 weeks and 5 weeks of chlordane exposed rats to all the six groups in comparison to control group.

Triglyceride

Triglyceride concentration in the serum of chlordane exposed rats showed significant increase (P≤ 0.01 and P≤ 0.001) at higher dose level (600mg/kg b.wt.) even after 2 weeks of treatment. Whereas at lower dose level the results did not show any significant changes (Fig.4.5).

Phospholipid

Treatment of chlordane resulted in almost a similar pattern of response as that of triglyceride. Here the insecticide resulted in about 43.41, 49.30 and 17.97, 17.04 % increase in group IIIA,B and IVA,B respectively as compared to control. However it did not show any significant changes in group IIA and B i.e. at low dose treatment (Fig.4.6).

Total Cholesterol

The total cholesterol was increased significantly ($P \leq 0.01$) in chlordane exposed rats which was much prominent in higher dose levels in comparison to control rats. Similarly the HDL, LDL and VLDL-Cholesterol contents were also found to be increased at higher dose levels of chlordane in all the six groups as compared to control group.

4.1.5 RADIO IMMUNO ASSAY (RIA):

Chlordane treatment resulted in a significant ($p \leq 0.05$) dose dependent decrease in the level of serum testosterone hormone, which was most prominent at highest concentration tested by us. At 600mg/kg b.wt. concentration, it showed about 2.5 folds of down regulation of testosterone level as compared to control (Fig. 4.7).
A significant decrease ($P \leq 0.05$) in serum testosterone level of chlordane treated rats at higher doses was observed as compared to control rats (Fig. 4.7).

ORGAN WEIGHT

Fig. (1.1) Testes

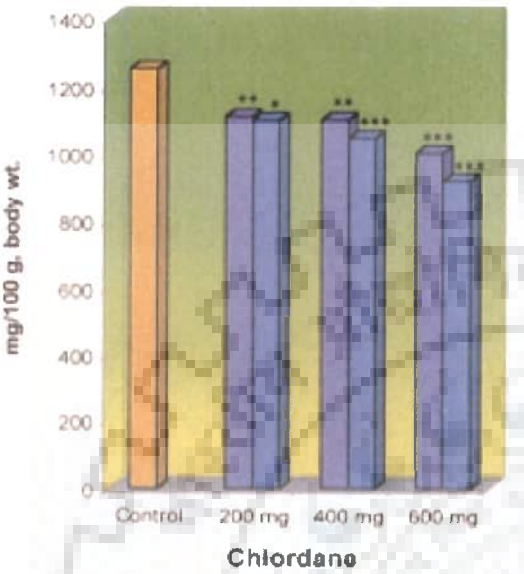


Fig. (1.2) Seminal vesicle

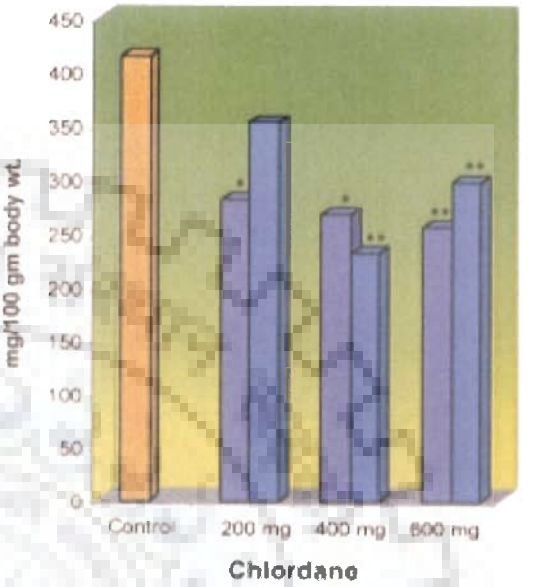


Fig. (1.3) Prostate gland

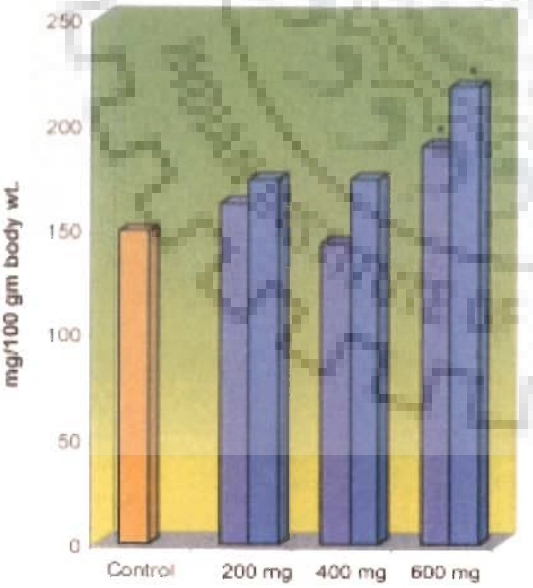
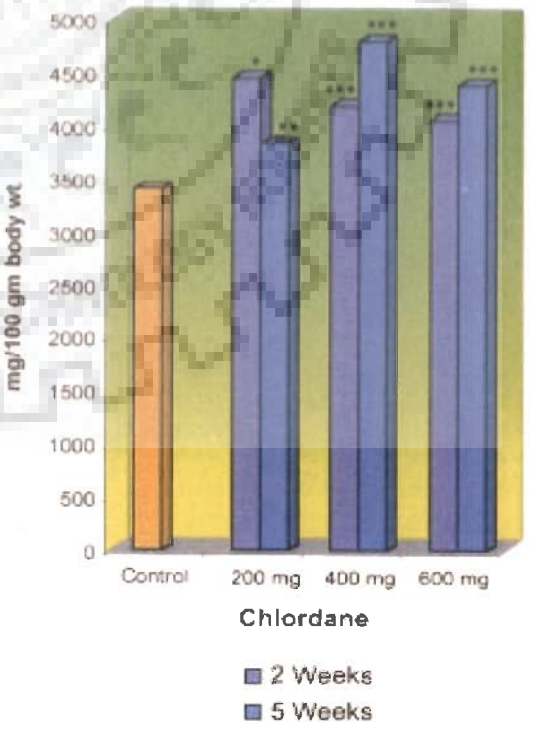


Fig. (1.4) Liver



* – P ≤ 0.05
** – P≤ 0.01
*** – P≤ 0.001

SPERM DYNAMICS AND FERTILITY

Fig. (2.1) Sperm Motility

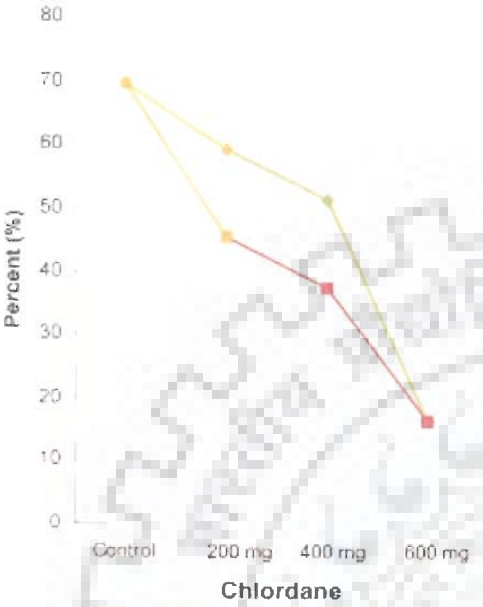


Fig. (2.2) Sperm Density (Testes)

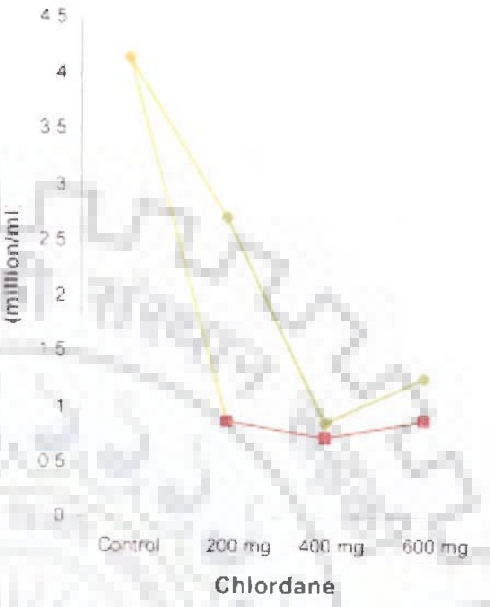
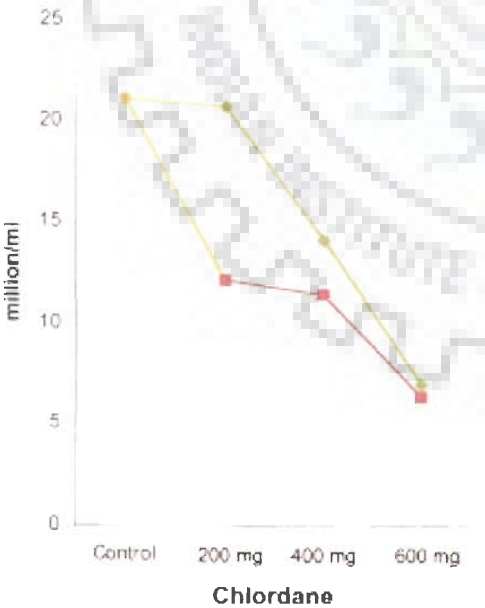
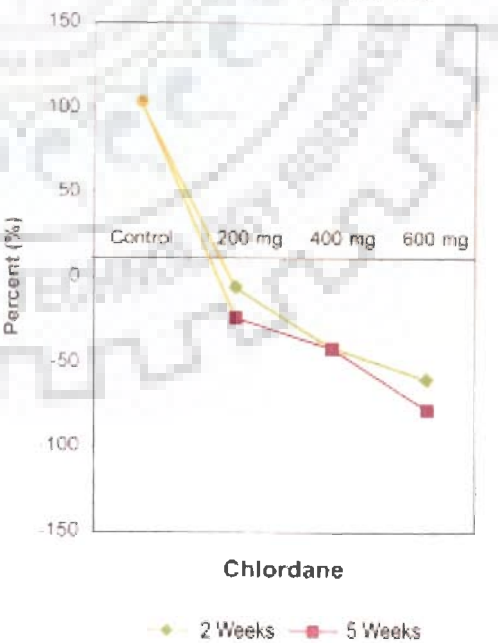


Fig. (2.3) Sperm Density (Cauda)



* = P ≤ 0.05
** = P≤ 0.01
*** = P≤ 0.001

Fig. (2.4) : Fertility (%)



BLOOD ANALYSIS

Fig. (3.1) : TEC

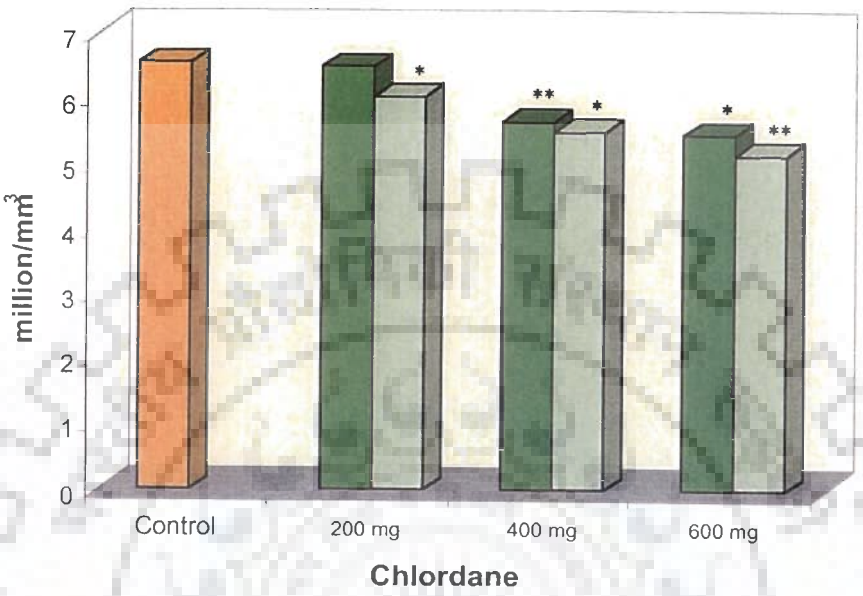
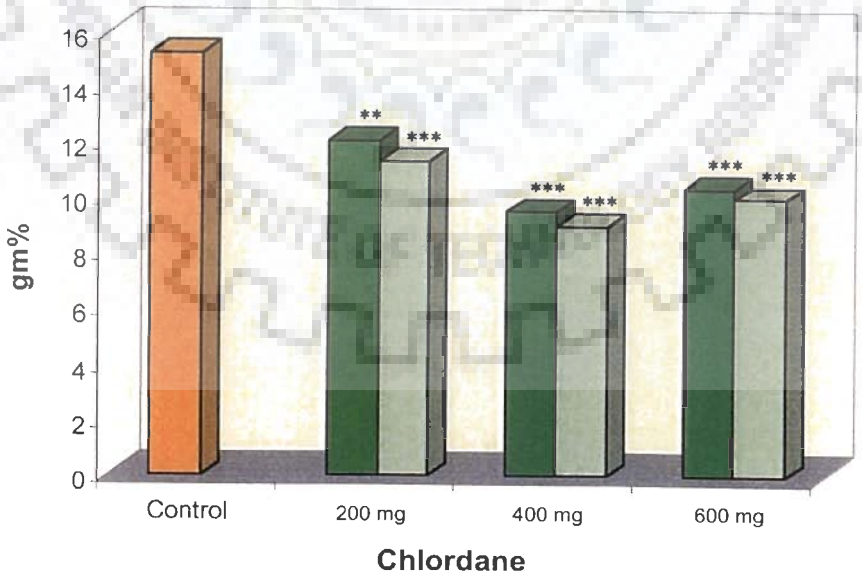


Fig. (3.2) Hemoglobin



* = P ≤ 0.05
* * = P≤ 0.01
* * * = P≤ 0.001

2 Weeks 5 Weeks

SERUM ANALYSIS

Fig. (4.1) ALT

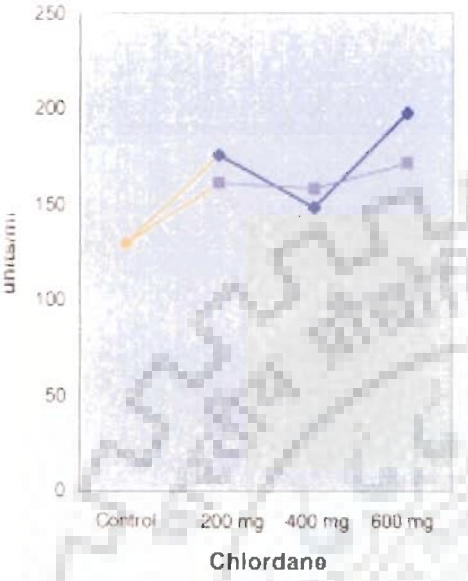


Fig. (4.2) AST

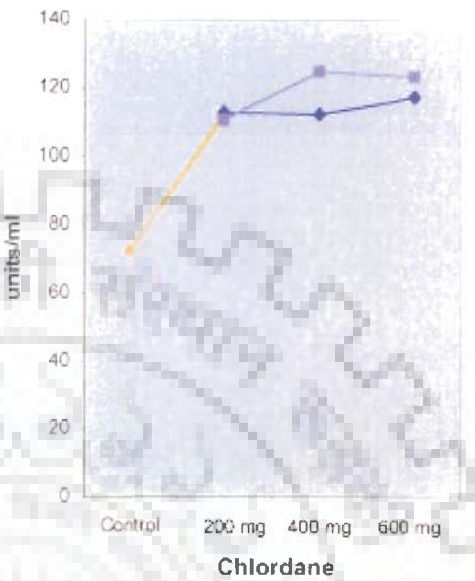


Fig. (4.3) Acid Phosphatase

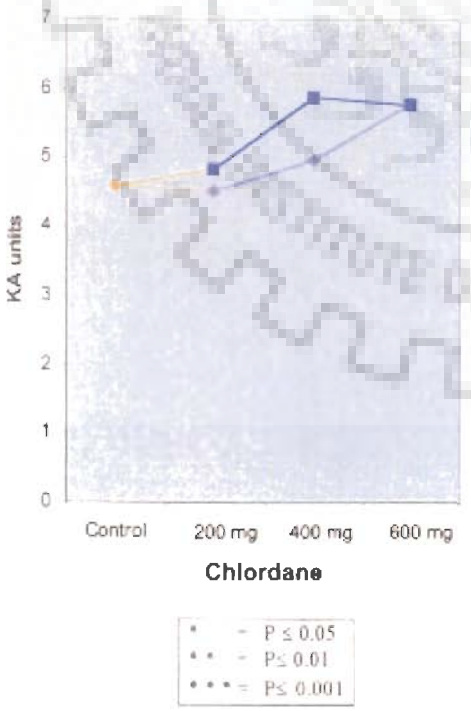
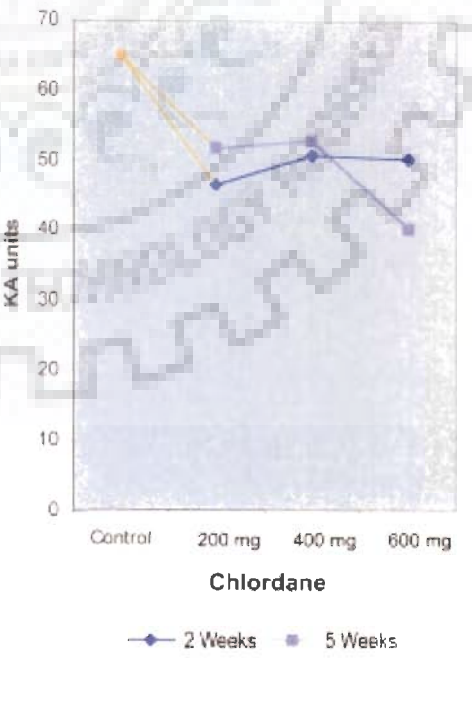


Fig. (4.4) Alkaline Phosphatase



SERUM ANALYSIS

Fig. (4.5) Triglyceride

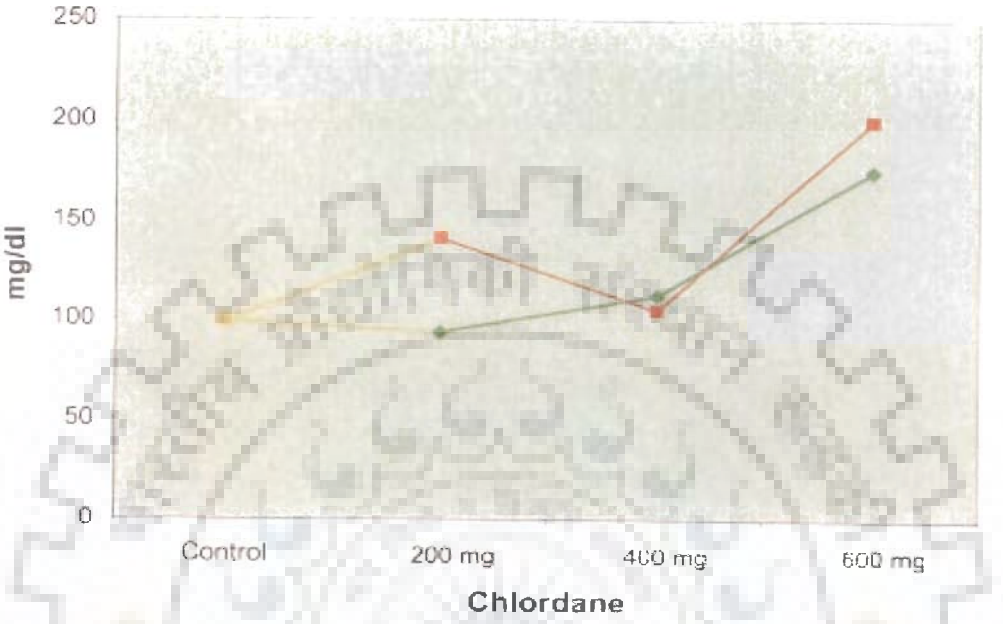
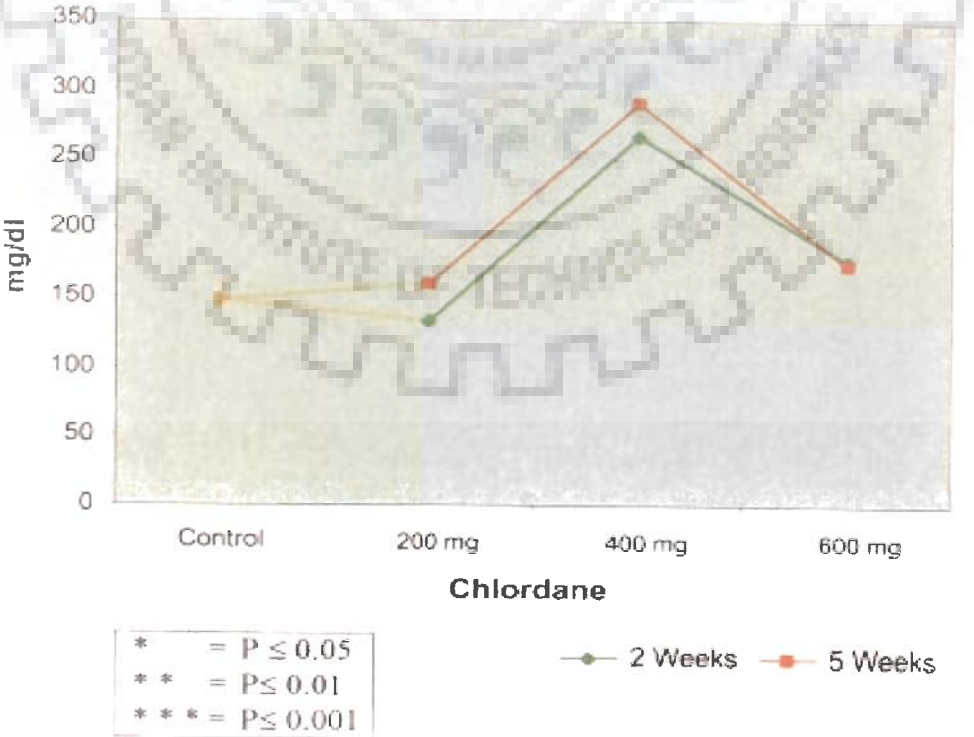
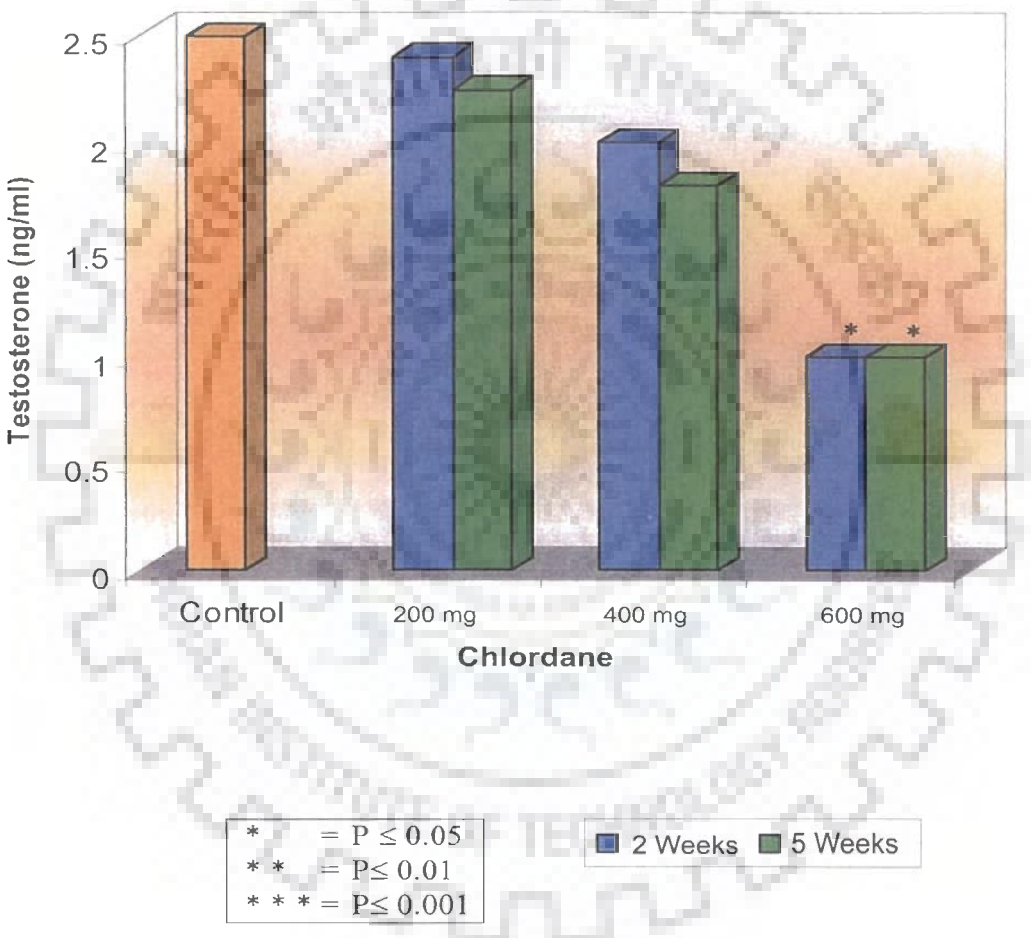


Fig. (4.6) Phospholipid



TESTOSTERONE LEVEL

Fig. (4.7) Serum Testosterone



HISTOLOGY OF CHORDANE TREATED RATS

PLATE-I

TESTIS

Fig.1 : Control

Microphotograph of control rat testis showing normal morphological architecture of seminiferous tubule, with all the successive stages of spermatogenesis. Lumen filled with spermatozoa. Leyding cells are present.

(H & E 200 X)

Fig.2 : Chlordane 200 mg/kg.b.wt./day for 2 weeks.

Microphotograph of seminiferous tubule exhibiting inhibition of spermatogenesis. Absence of sperm and irregular germinal epithelium could be seen.

(H & E 200 X)

Fig. 3 :Chlordane 400 mg/kg.b.wt./day for 2 weeks.

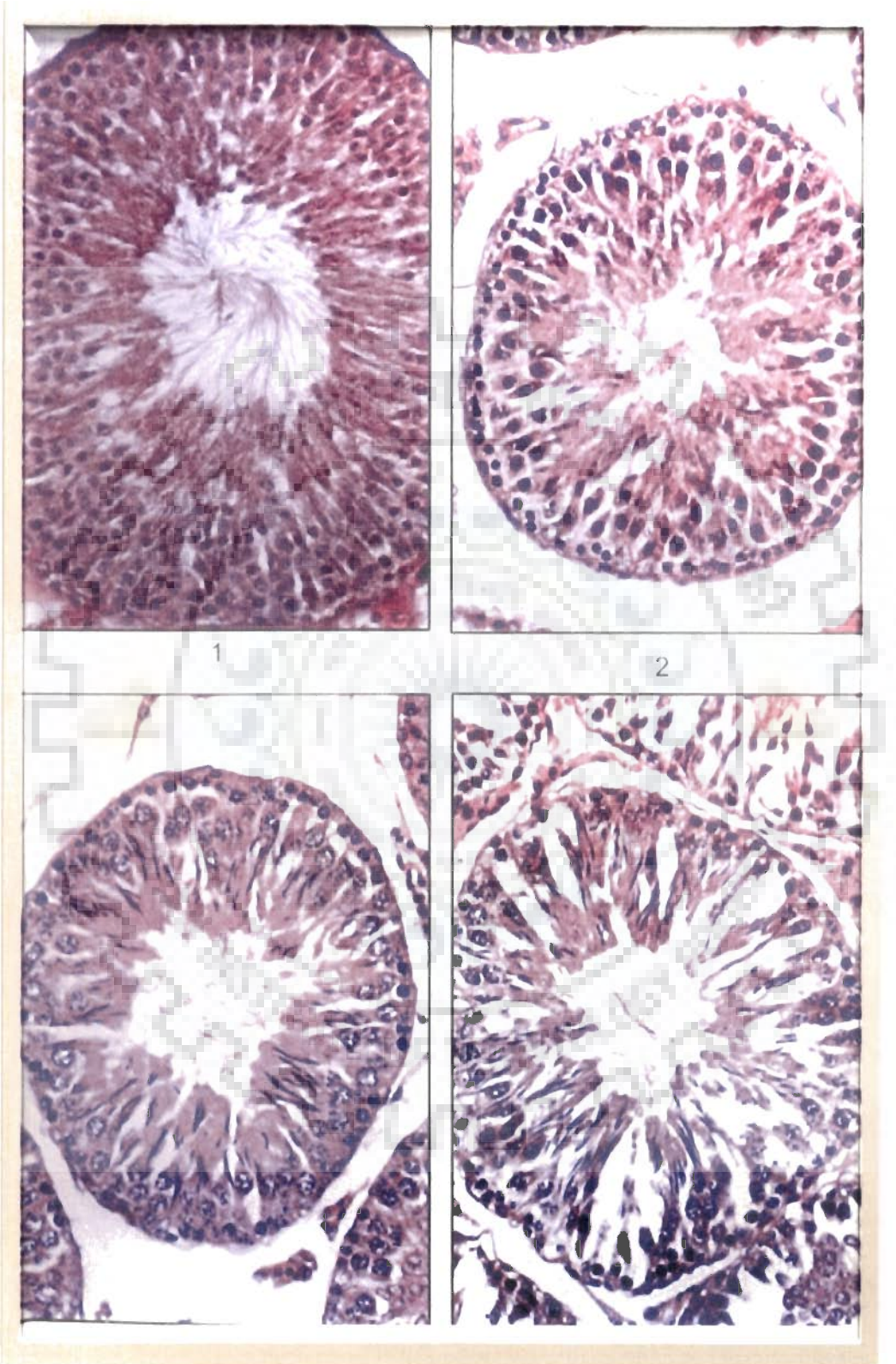
Microphotograph of testis showing reduced seminiferous tubule diameter and increase inter tubular space. Irregular and incomplete spermatogenesis.

(H & E 200 X)

Fig. 4: Chlordane 600 mg/kg.b.wt./day for 2 weeks.

Seminiferous tubule showing irregular epithelium loosened at several places. Spermatogenesis is highly inhibited. Decreased numbers of spermatogenic elements, few secondary spermatocytes and spermatids are present, lumen contain cellular debris.

(H & E 200 X)



1 2

3 4

PLATE-II

TESTIS

Fig.5 : Chlordane 200 mg/kg.b.wt./day for 5 weeks.

Microphotograph of testis showing degenerated seminiferous tubule and presence of vacuoles in the epithelium. Most of the germ cells have been shed, leaving only few spermatogenic elements. Lumen is filled with cellular debris.

(H & E 200 X)

Fig.6 : Chlordane 400 mg/kg.b.wt./day for 5 weeks.

Irregular tubule having vacuolated epithelium. Spermatogenic inhibition, vacuoles, cellular debris are present. Interstitial space showing degenerated leydig cells.

(H & E 200 X)

Fig. 7 :Chlordane 600 mg/kg.b.wt./day for 5 weeks.

Microphotograph showing degenerated seminiferous tubule, reduced in size and irregular in shape and showing inhibited spermatogenesis and lumen filled with debris. Leyding cells are scanty.

(H & E 200 X)

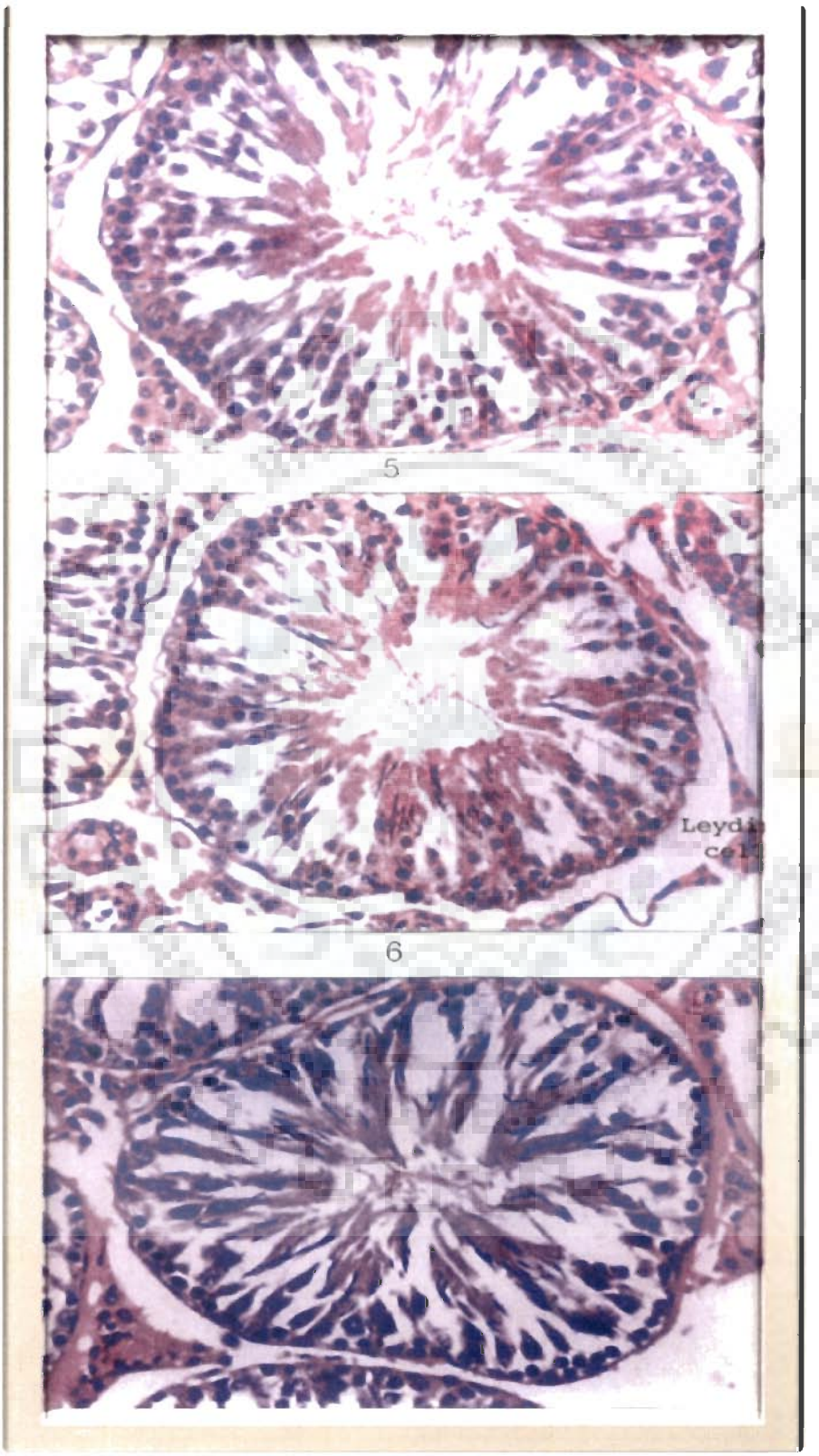

5
Leydi
ce
6
7

PLATE-III

VAS DEFERENS

Fig. 8 :Control

Vas deferens of control rat showing thick muscular layer of outer longitudinal and inner circular muscle fiber. Central lumen is lined with columnar epithelium containing long stereocilia. Lumen contains spermatozoa.

(H & E 100 X)

Fig. 9 :Chlordane 200 mg/kg.b.wt./day for 2 weeks.

Microphotograph showing thickened epithelium with vacuoles at some places. Fused stereocilia are present. Circular muscular coat is detached at few places, lumen contains cellular debris.

(H & E 100 X)

Fig. 10 : Chlordane 400 mg/kg.b.wt./day for 2 weeks.

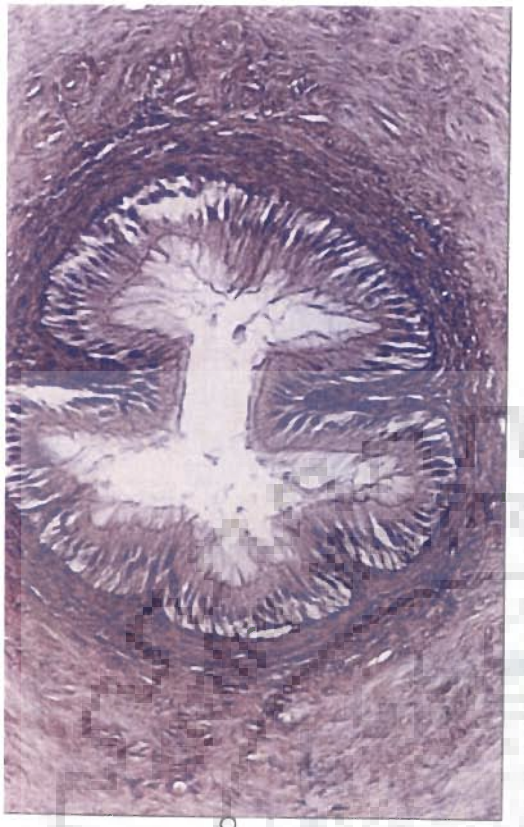
Vas deferens showing degenerated epithelium with wide lumen containing sperm debris. Muscular coat is detached at few places.

(H & E 100 X)

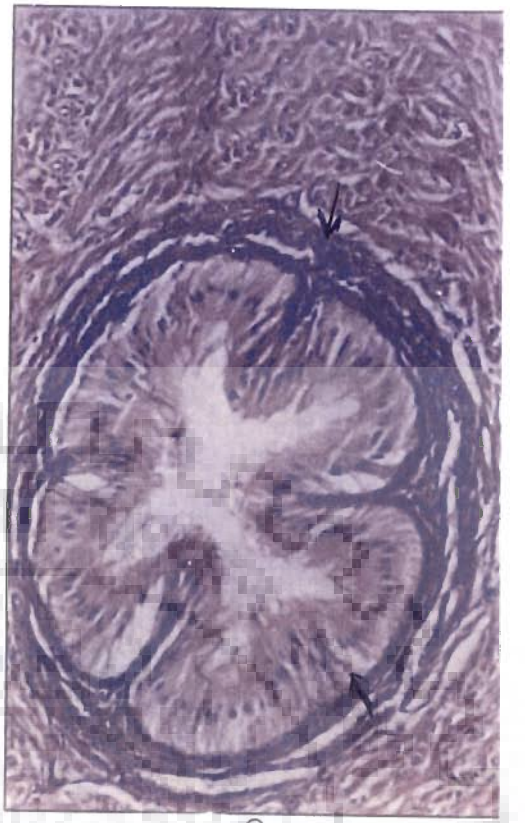
Fig. 11 : Chlordane 600 mg/kg.b.wt./day for 2 weeks.

Microphotograph of vas deferens showing increased epithelial height with vacuoles at some places. Folds of epithelial lining are reduced with fused stereocilia. Circular muscle layer is degenerated. Lumen is filled with debris.

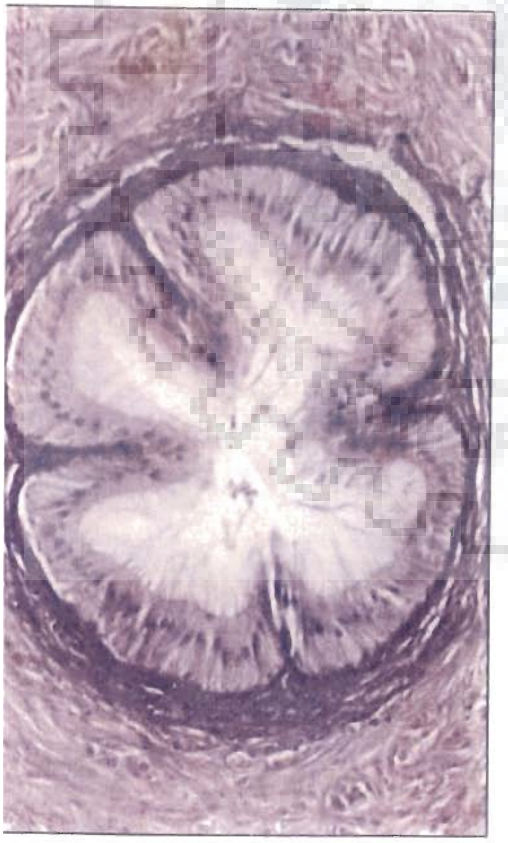
(H & E 100 X)



8



9



10

11

PLATE –IV

VAS DEFERENS

Fig. 12 : Chlordane 200 mg/kg.b.wt./day for 5 weeks.

Microphotograph showing degenerated epithelium with fused stereocilia. Muscular layer is disrupted and detached at several places. Lumen is without spermatozoa.

(H & E 100 X)

Fig.13 : Chlordane 400 mg/kg.b.wt./day for 5 weeks.

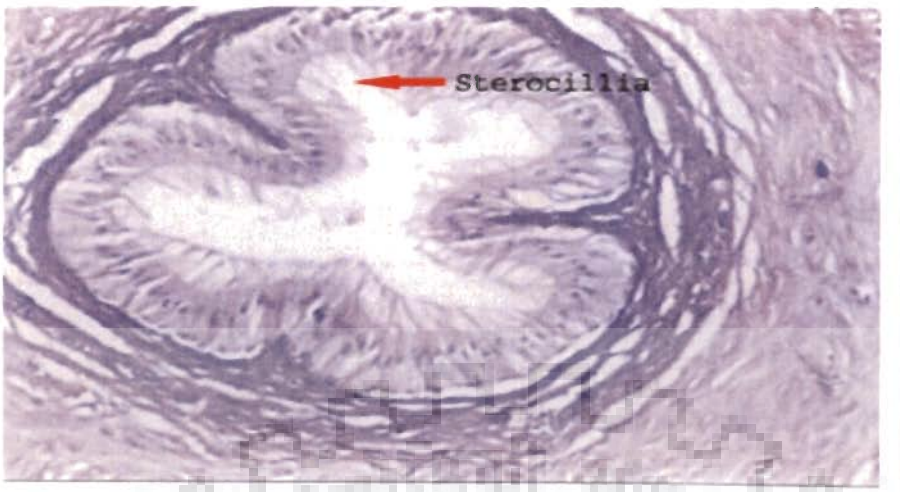
Vas deferens showing degenerated muscular coat with reduced folds of epithelium. Fused stereocilia and debris could be seen.

(H & E 100 X)

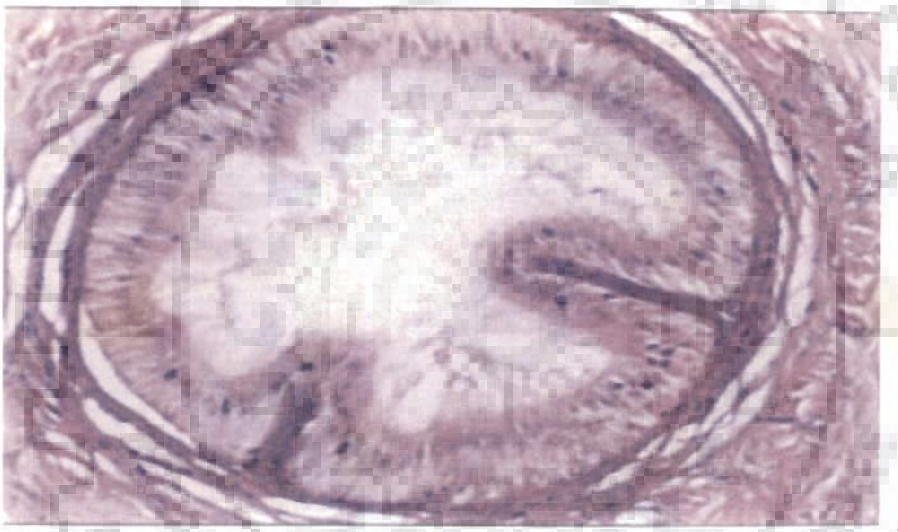
Fig. 14 : Chlordane 600 mg/kg.b.wt./day for 5 weeks.

Microphotograph showing degenerated vas deferens, degenerated muscular coat detached at several places and epithelium with no folds. Lumen is devoid of spermetoza but filled with cellular debris.

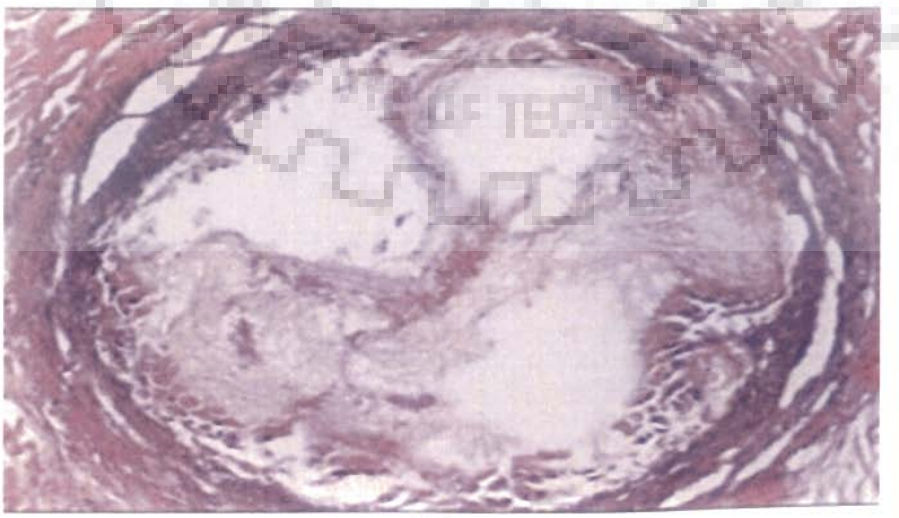
(H & E 100 X)



12



13



14

PLATE –V

SEMINAL VESICLE

Fig.15 : Control

Microphotograph of seminal vesicle showing normal muscular layer consisting of inner circular and outer longitudinal muscle fibers with mucosal folds of columnar epithelial cells extended deep into the lumen. Lumen is filled with eosinophilic secretion. (Shown by arrow).

(H & E 100 X)

Fig. 16 : Chlordane 200 mg/kg.b.wt./day for 2 weeks.

Microphotograph showing degenerative changes in epithelium and muscular layer. Little secretion is present in lumen.

(H & E 100 X)

Fig. 17 Chlordane 400 mg/kg.b.wt.day for 2 weeks.

Seminal vesicle showing degeneration in secretory epithelial cells. Muscular layer detached from epithelium. Lumen with less secretion.

(H & E 100 X)

Fig. 18 : Chlordane 600 mg/kg.b.wt./day for 2 weeks.

Microphotograph showing disorganized columnar epithelial cells. Muscular layer is disrupted. Diminished secretion in the lumen could be seen.

(H & E 100 X)

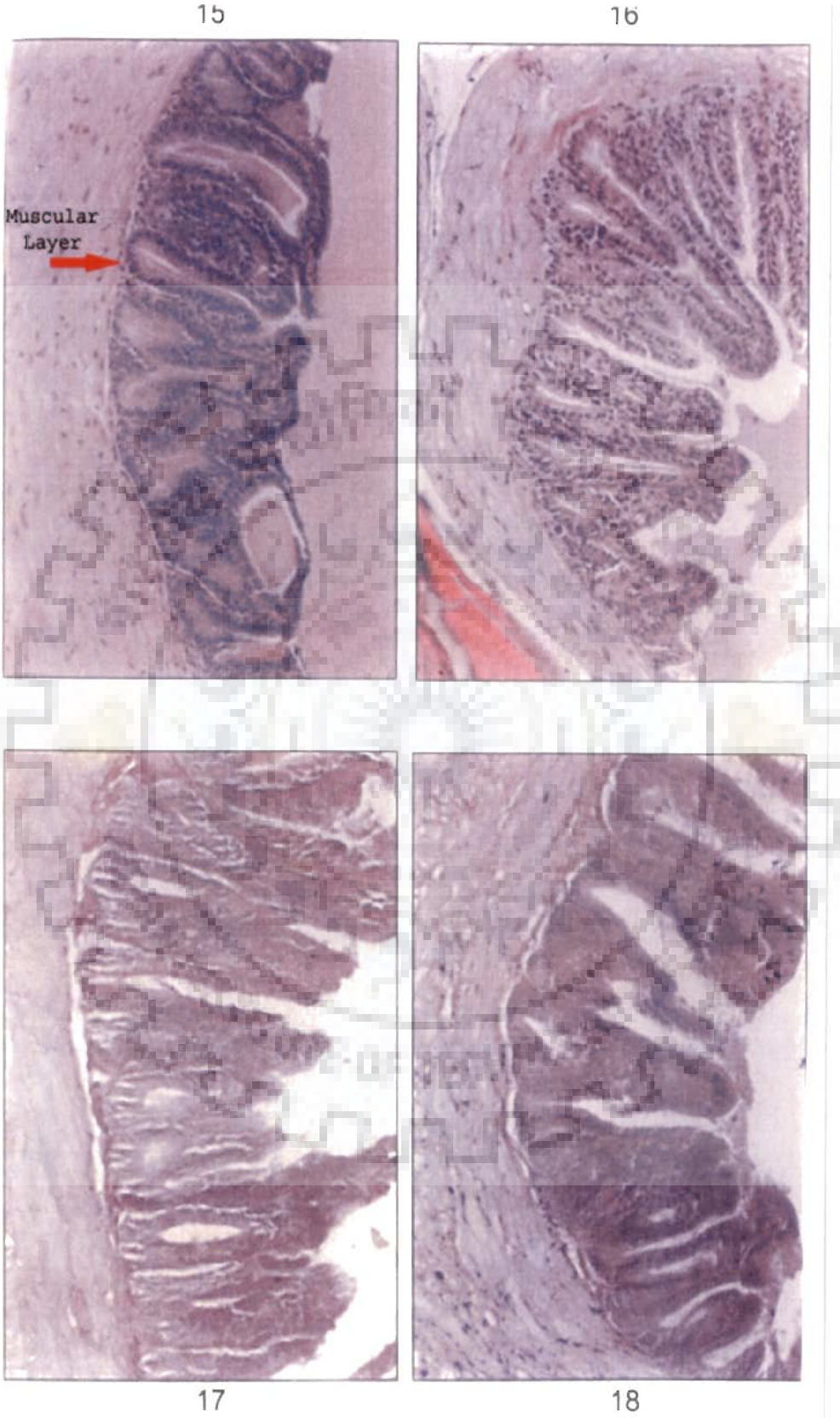

15
16
Muscular
Layer
17
18

PLATE-VI

SEMINAL VESICLE

Fig. 19 : Chlordane 200 mg/kg.b.wt./day for 5 weeks.

Microphotograph of seminal vesicle showing atrophy in epithelium. Lumen contains little secretion.

(H & E 100 X)

Fig. 20 : Chlordane 400 mg/kg.b.wt./day for 5 weeks.

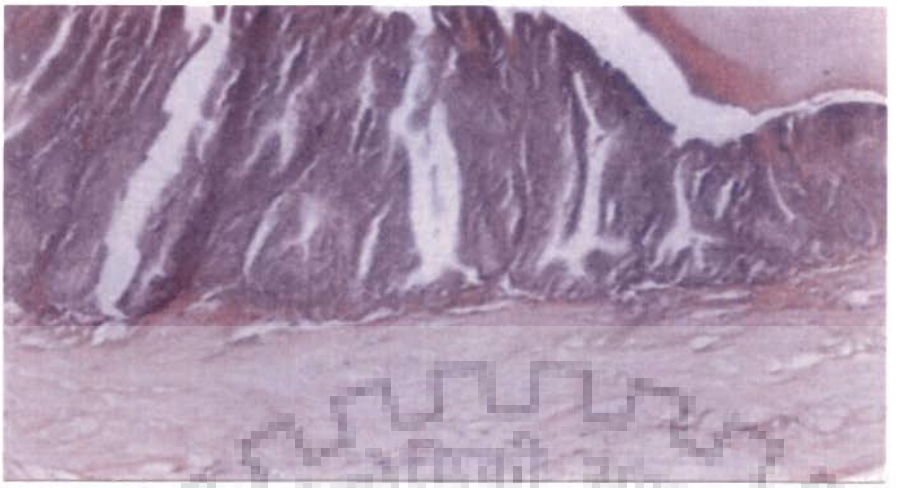
Seminal vesicle showing damaged epithelium with increased cell height. Muscular layer is disorganized. Lumen without secretion.

(H & E 100 X)

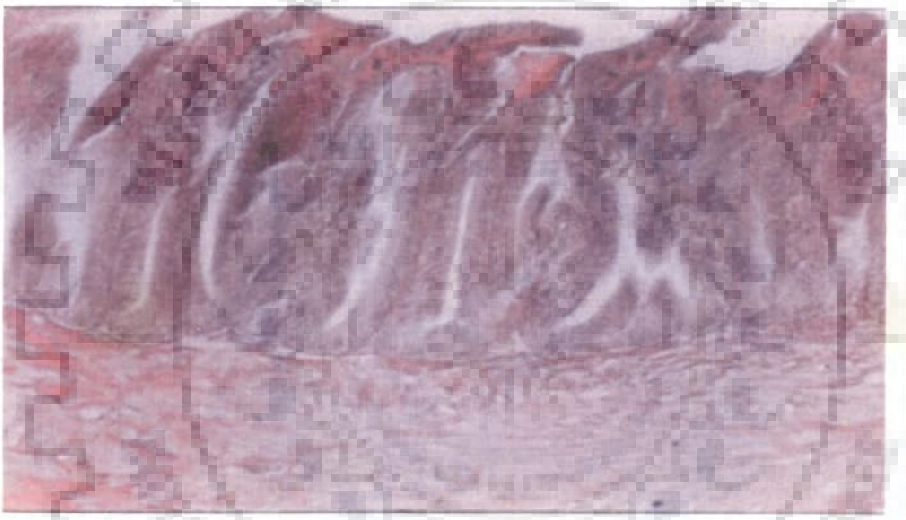
Fig. 21 : Chlordane 600 mg/kg.b.wt./day for 5 weeks.

Microphotograph showing degenerated secretory epithelial cells ruptured at some places. Disrupted muscular layer and scanty secretion could be seen.

(H & E 100 X)



19



20

21

PLATE-VII

CAUDA EPIDIDYMIS

Fig. 22 : Control

Cauda epididymis of control rat having large and compact tubules, lined with pseudostratified epithelial cells with stereocilia. Inter tubular stroma contains connective tissues and blood vessels. The lumen is packed with mature spermatozoa.

(H & E 100 X)

Fig. 23 : Chlordane 200 mg/kg.b.wt./day for 2 weeks.

Microphotograph showing degenerated epithelium with short stereocilia. Lumen with less spermatozoa.

(H & E 100 X)

Fig. 24 : Chlordane 400 mg/kg.b.wt./day for 2 weeks.

Microphotograph of cauda epididymis showing decreased epithelial cell height, cells without stereocilia. Lumen contains few spermatozoa.

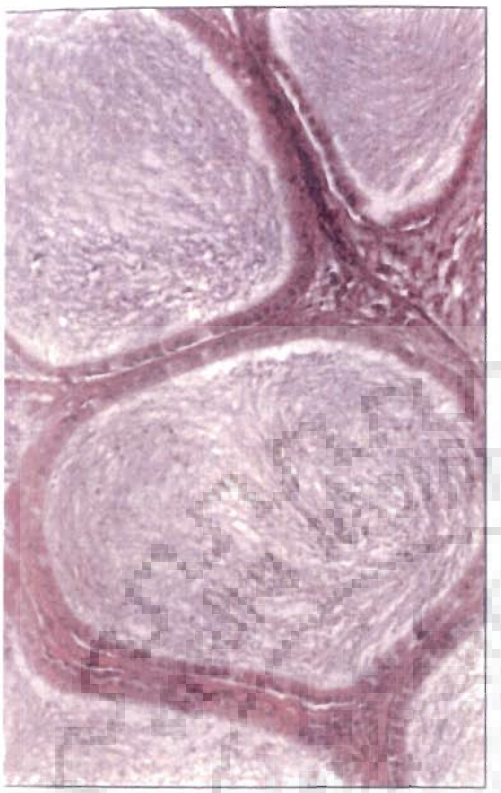
(H & E 100 X)

Fig. 25 : Chlordane 600 mg/kg.b.wt./day for 2 weeks.

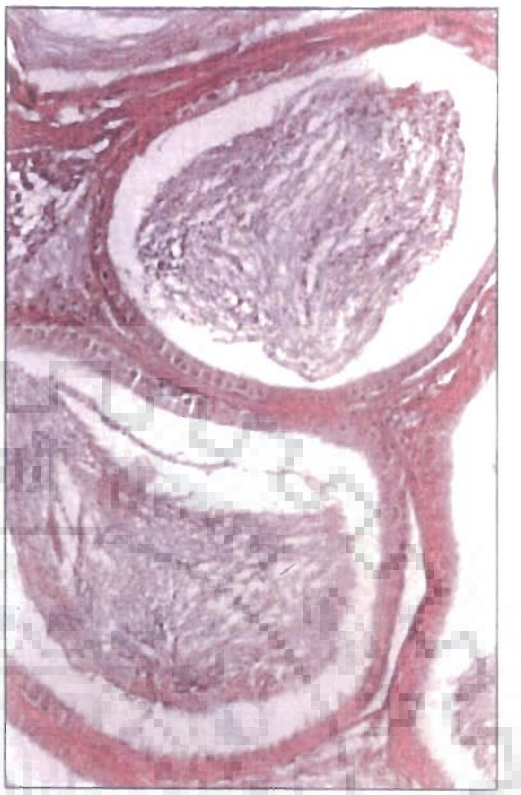
Cauda epididymis showing tubules with thin epithelium. Stereocilia are absent. Few spermatozoa are present in lumen. Increase in interstitial stroma.

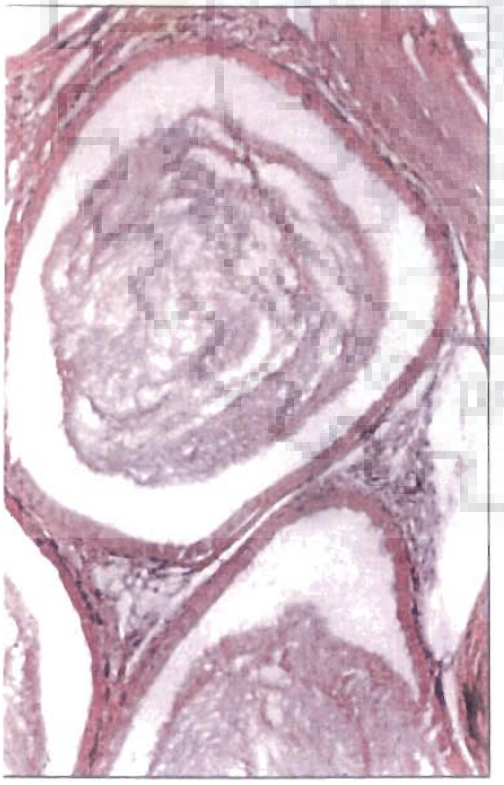
(H & E 100 X)

22

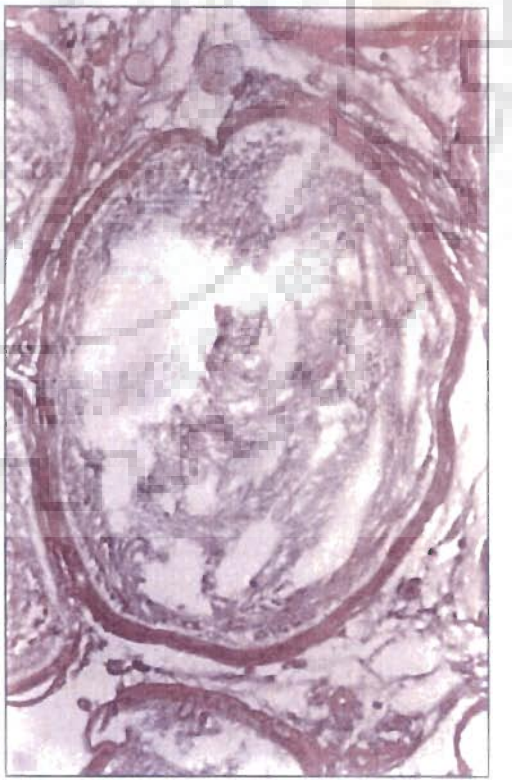


23





24



25

PLATE-VIII

CAUDA EPIDIDYMIS

Fig. 26 : Chlordane 200 mg/kg.b.wt./day for 5 weeks.

Microphotograph of cauda epididymis showing degenerated epithelium with decreased cell height, short stereocilia and lumen with less spermatozoa.

(H & E 100 X)

Fig. 27 : Chlordane 400 mg/kg.b.wt./day for 5 weeks.

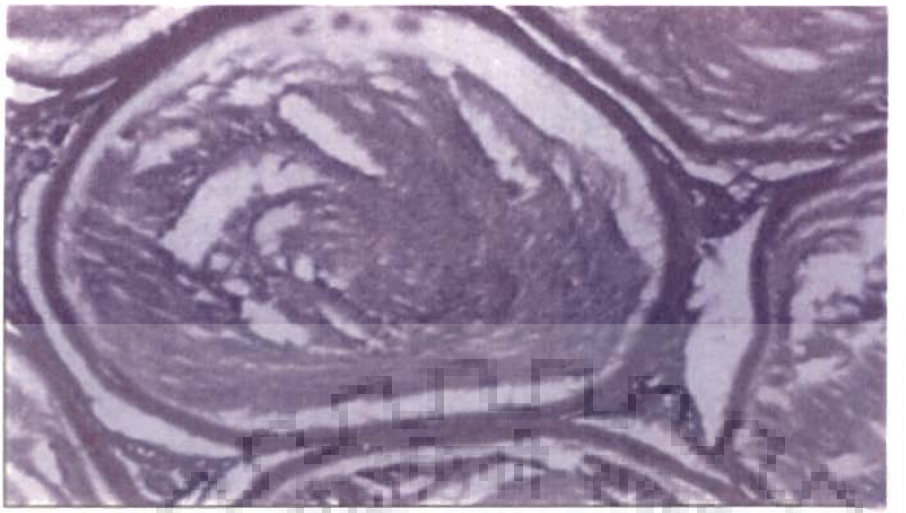
Microphotograph of cauda epididymis showing degenerated epithelium with fusion at certain points. Thin epithelium without stereocilia, lumen contains few spermatozoa.

(H & E 100 X)

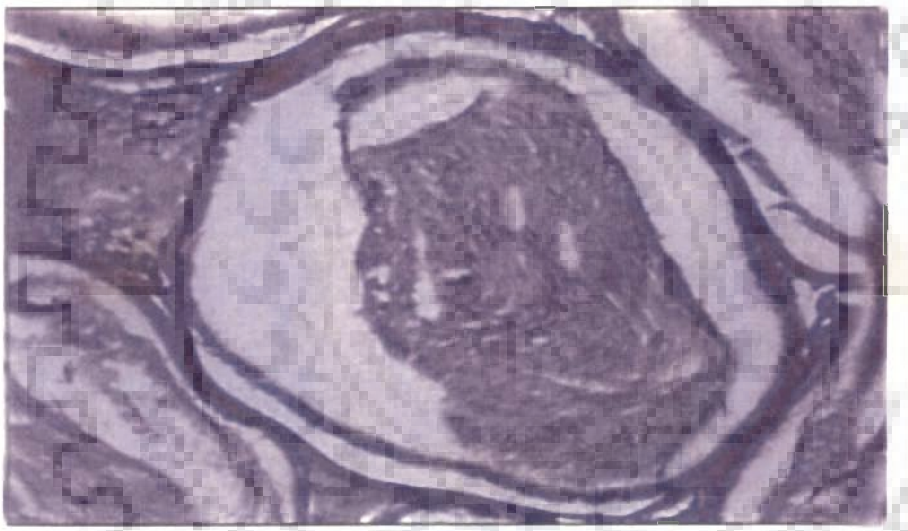
Fig. 28 : Chlordane 600 mg/kg.b.wt./day for 5 weeks.

Microphotograph of showing massive degenerative changes, the inter tubular stroma is increased. Very few spermatozoa are present in lumen. Stereocilia could not be seen.

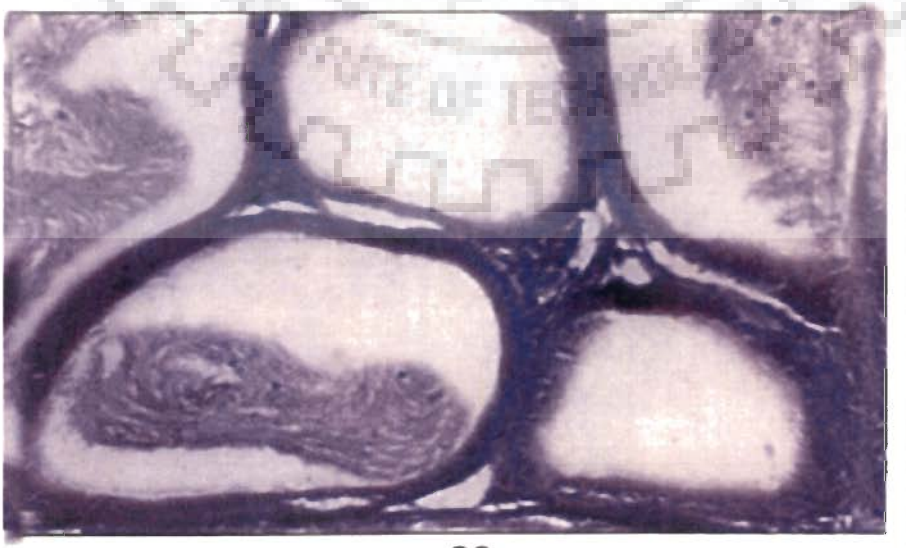
(H & E 100 X)



26

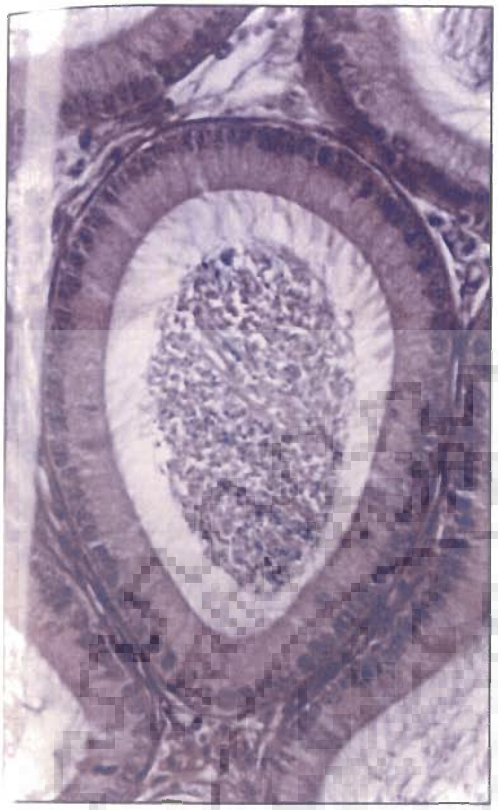


27

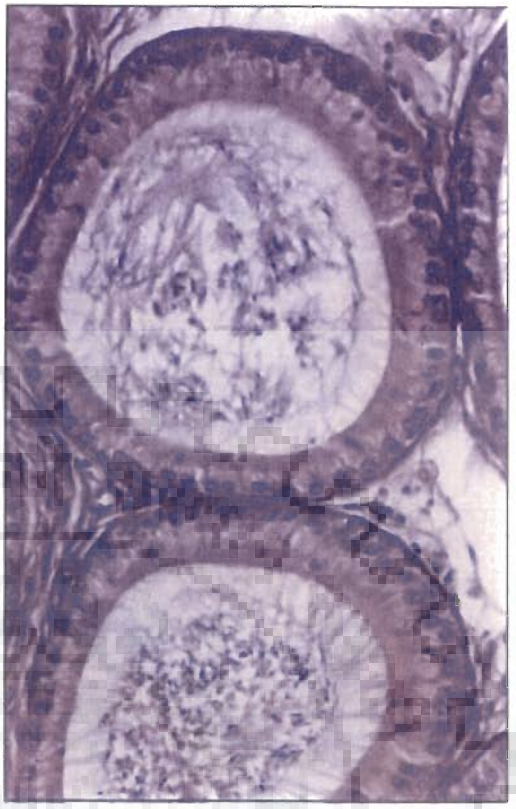


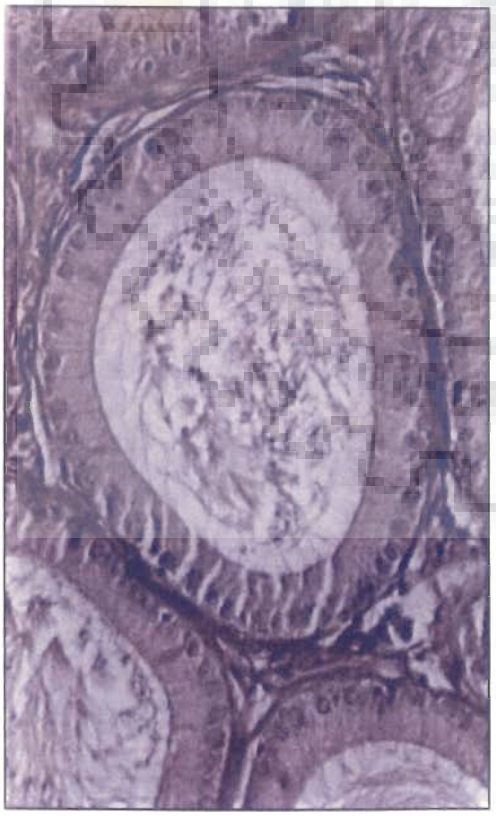
28

29

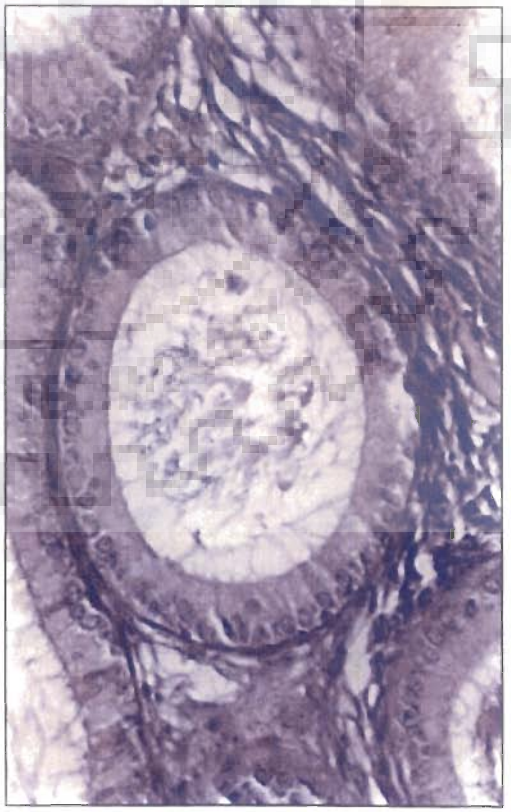


30





31



32

PLATE-IX

CAPUT EPIDIDYMIS

Fig. 29 : Control

The tubule of control caput epididymis are lined with pseudostratified columnar epithelium with long prominent stereocilia. Lumen containing high concentration of spermatozoa. The inter tubular spaces are filled with connective tissue and blood vessels.

(H & E 200 X)

Fig. 30 Chlordane 200 mg/kg.b.wt./day for 2 weeks.

Microphotograph of caput epididymis showing slightly thin epithelium. Lumen contains less spermatozoa.

(H & E 200 X)

Fig. 31 : Chlordane 400 mg/kg.b.wt./day for 2 weeks.

Microphotograph showing degenerated epithelium with fused stereocilia. Lumen contain few spermatozoa along with debris.

(H & E 200 X)

Fig. 32 : Chlordane 600 mg/kg.b.wt./day for 2 weeks.

Caput epididymis showing reduced tubular size and regressed epithelium. Lumen size is greatly reduced with very few spermatozoa. Increased interstitial stroma is also seen.

(H & E 200 X)

PLATE –X

CAPUT EPIDIDYMIS

Fig. 33 : Chlordane 200 mg/kg.b.wt./day for 5 weeks.

Microphotograph of caput epididymis showing reduction in the size of tubule. Lumen contains cellular debris.

(H & E 200 X)

Fig. 34 : Chlordane 400 mg/kg.b.wt./day for 5 weeks.

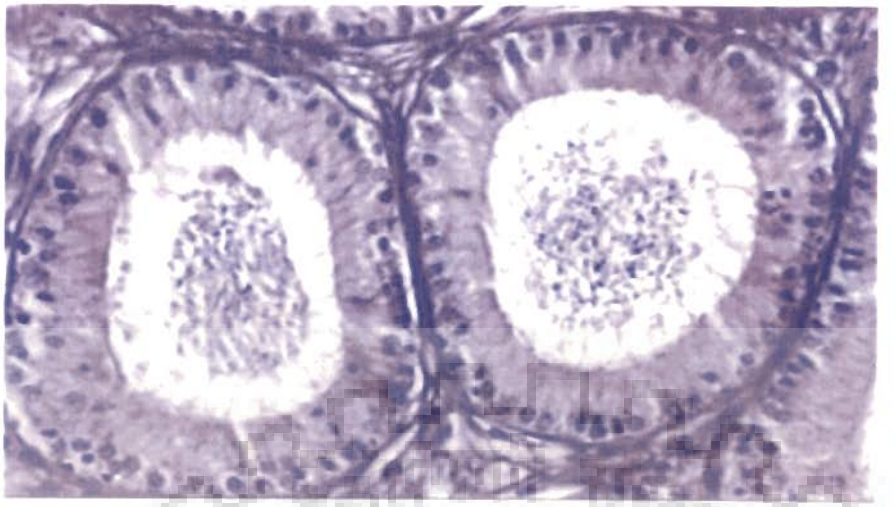
Caput epididymis showing increased interstitial stroma. Degenerated epithelium loosened at several places with fused stereocilia.

(H & E 200 X)

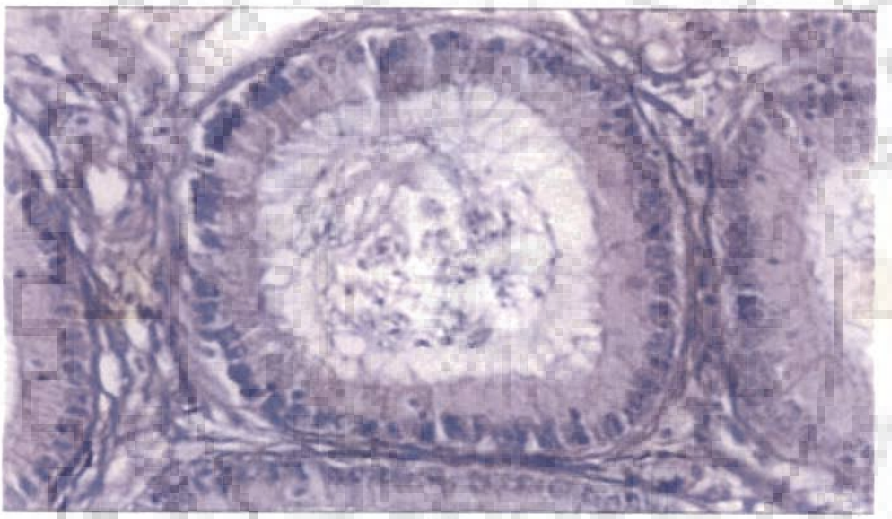
Fig. 35 : Chlordane 600 mg/kg.b.wt./day for 5 weeks.

Microphotograph showing damaged epithelium with few stereocilia. Interstitial stroma is degenerated and scanty sperm are present along with cellular debris.

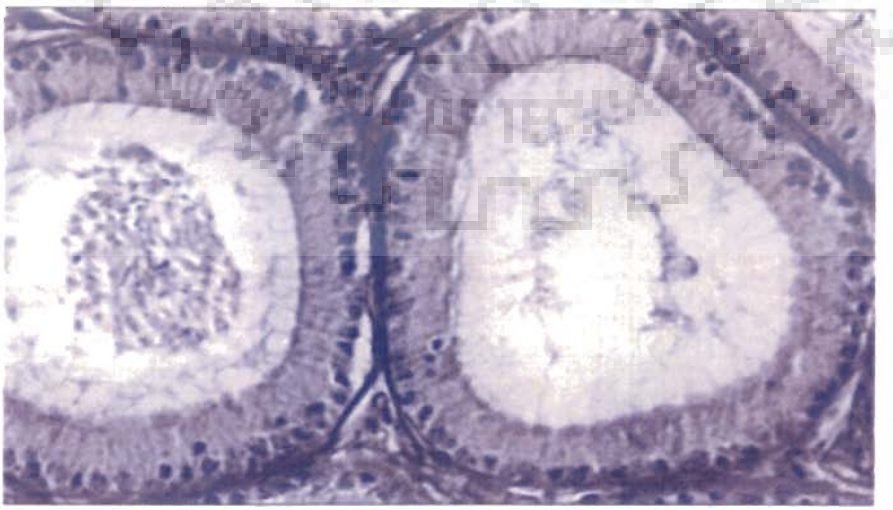
(H & E 200 X)



33



34



35

PLATE-XI

VENTRAL PROSTATE

Fig. 36 : Control

Microphotograph of prostate showing large alveoli with well developed cuboidal epithelium. The inter tubular spaces are filled with connective tissue and blood vessels. Lumen is full of eosinophilic secretion.

(H & E 100 X)

Fig. 37 : Chlordane 200 mg/kg.b.wt./day for 2 weeks.

Microphotograph showing alveoli with epithelium of varying height. Lumen with diminished secretion.

(H & E 100 X)

Fig. 38 : Chlordane 400 mg/kg.b.wt./day for 2 weeks.

Photograph of prostate showing alveoli with degenerated epithelium. Lumen contains less secretion. Increased interstitial space.

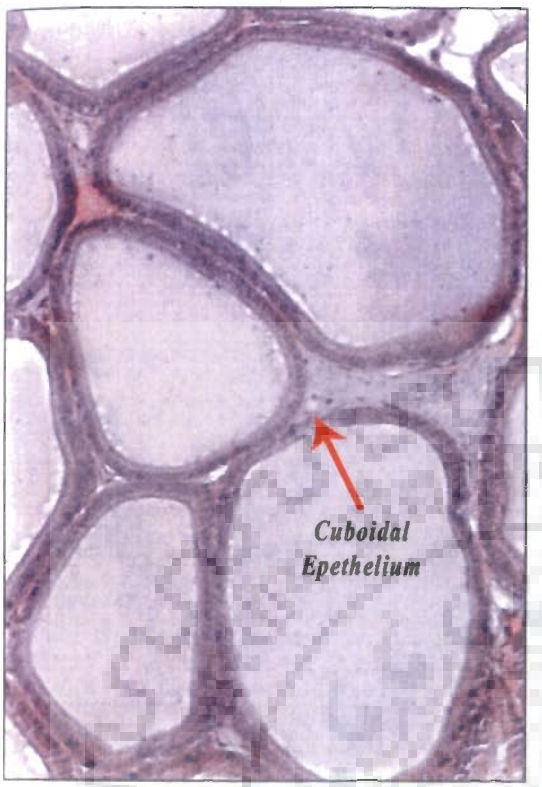
(H & E 100 X)

Fig. 39 : Chlordane 600 mg/kg.b.wt./day for 2 weeks.

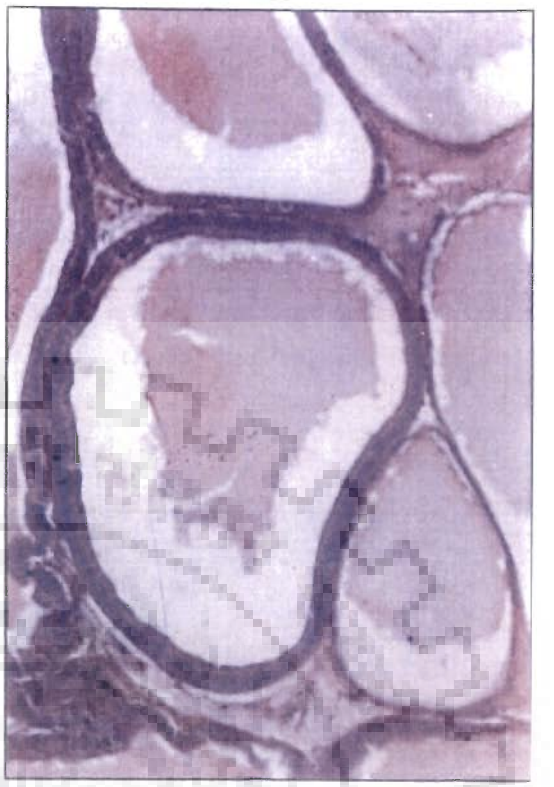
Microphotograph showing reduced alveolar size with degenerated and folded epithelium. Lumen with less secretion.

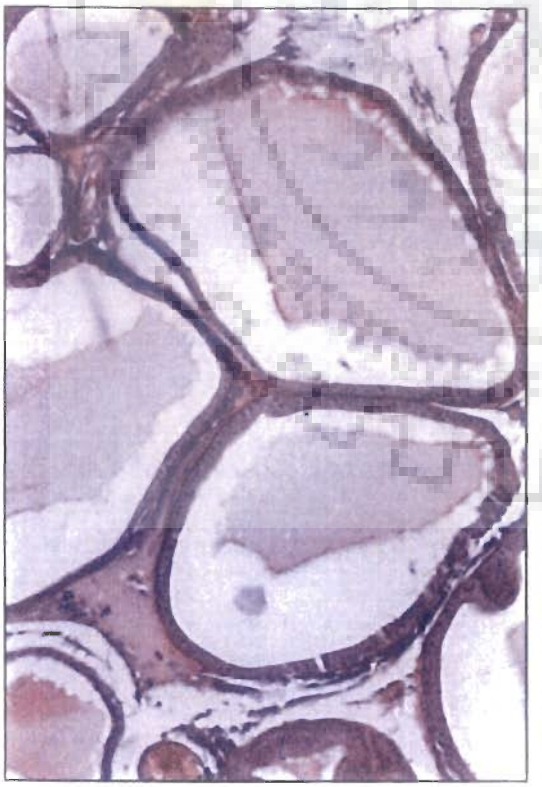
(H & E 100 X)

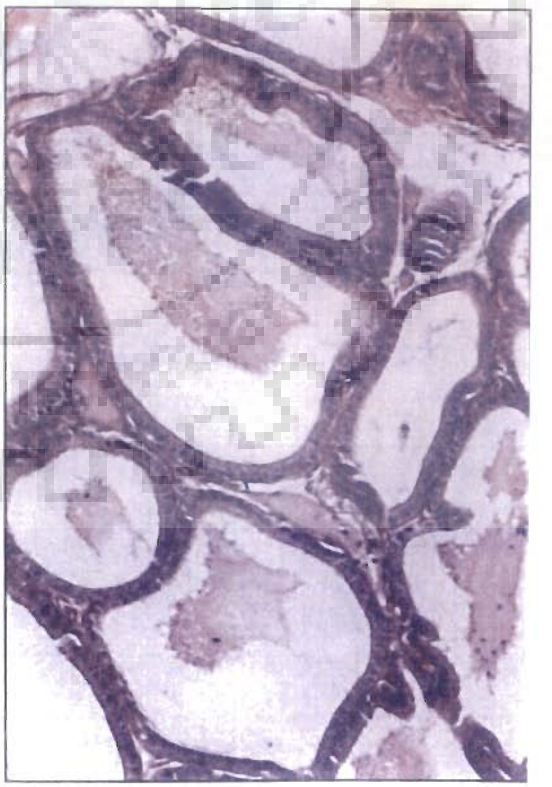
36



37







38

39

PLATE-XII

VENTRAL PROSTATE

Fig. 40 : Chlordane 200 mg/kg.b.wt./day for 5 weeks.

Microphotograph of ventral prostate showing degenerated and folded epithelium fused at some places. Lumen with diminished secretion.

(H & E 100 X)

Fig. 41 : Chlordane 400 mg/kg.b.wt./day for 5 weeks.

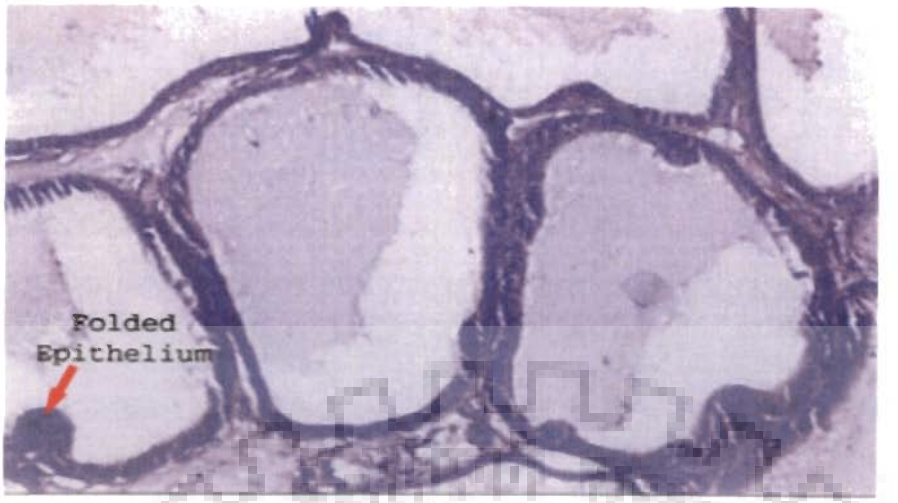
The picture of prostate shows fused and degenerated epithelial lining of alveoli. Interstitial stroma is disrupted. Lumen contains very less secretion.

(H & E 100 X)

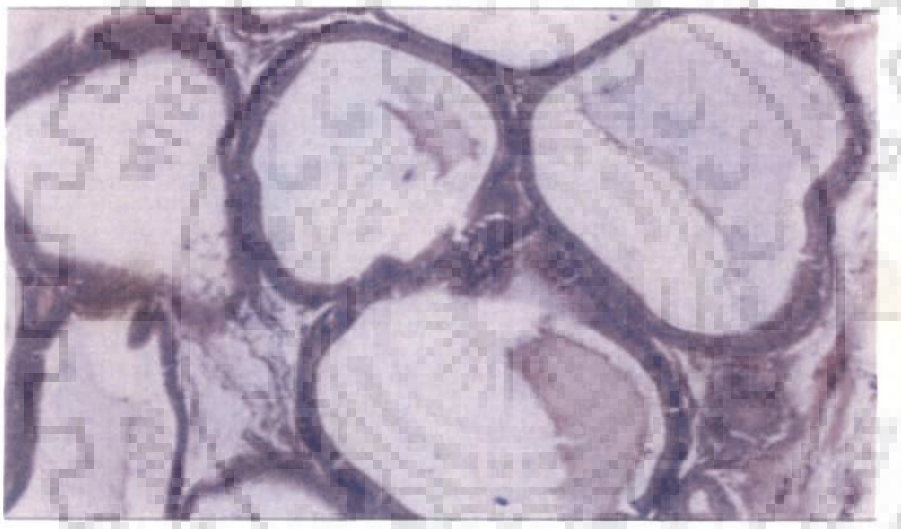
Fig. 42 : Chlordane 600 mg/kg.b.wt./day for 5 weeks.

Photograph showing alveoli with fused and folded epithelium of varying height. Very less secretion is present in lumen.

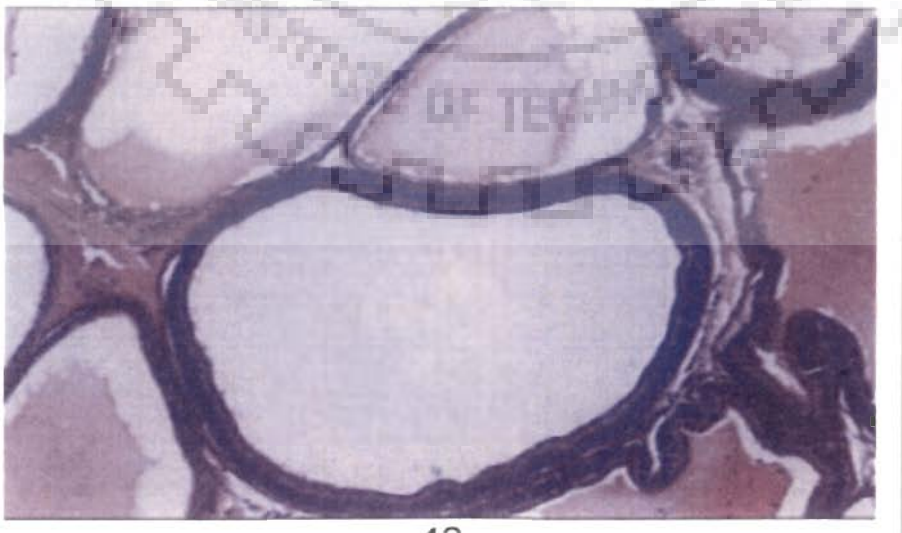
(H & E 100 X)



40



41



42

PLATE-XIII

LIVER

Fig.43 : Control

Photomicrography of control rat liver showing central vein, in the centre of the hepatic lobule. The hepatic sinusoids are seen between the plates of hepatic cells. Endothelial cells are also visible in sinusoids with small nuclei.

(H & E 100X)

Fig.44 : Chlordane 200 mg/kg. b. wt./day for 2 weeks.

Microphotograph of liver showing hepatocytic degeneration. Various nuclear aberrations are also present.

(H & E 100X)

Fig.45 : Chlordane 400 mg/kg. b. wt./day for 2 weeks.

Microphotograph showing extensive degeneration of hepatocytes.

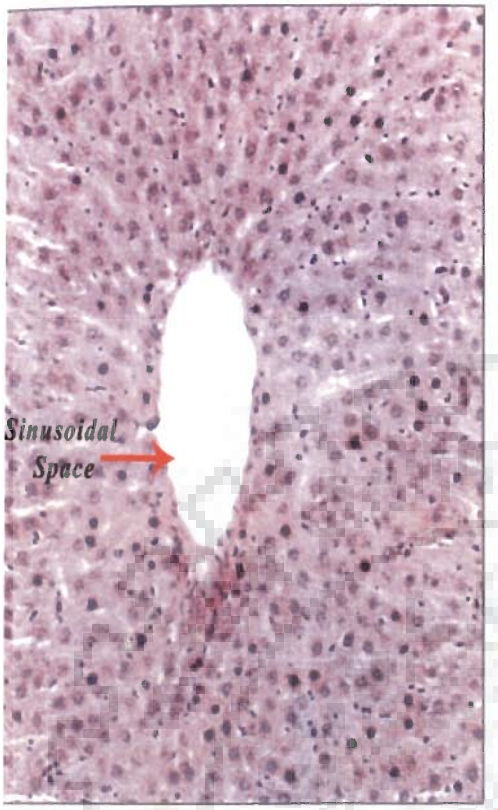
(H & E 100X)

Fig.46 : Chlordane 600 mg/kg. b. wt./day for 2 weeks.

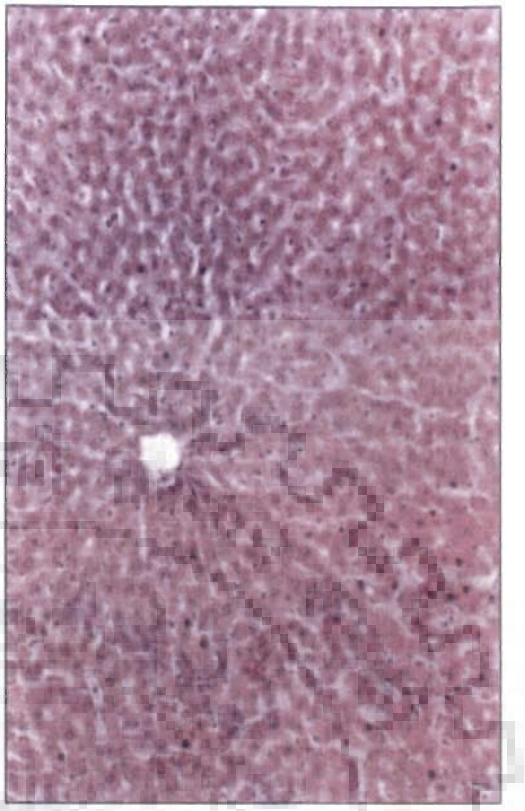
Microphotograph showing many degenerative changes marked by binucleated cells, hypertrophy in hepatocytes, vacuolization of hepatocytes. Dilation of sinusoidal spaces could be seen.

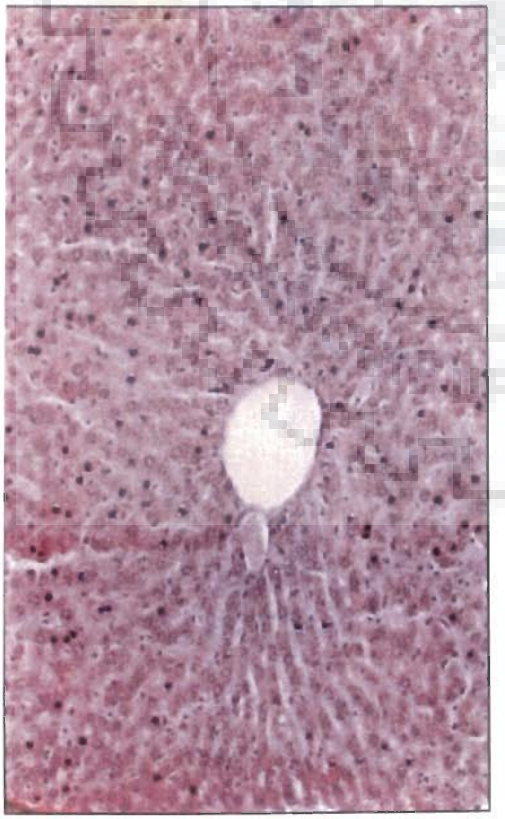
(H & E 100X)

43

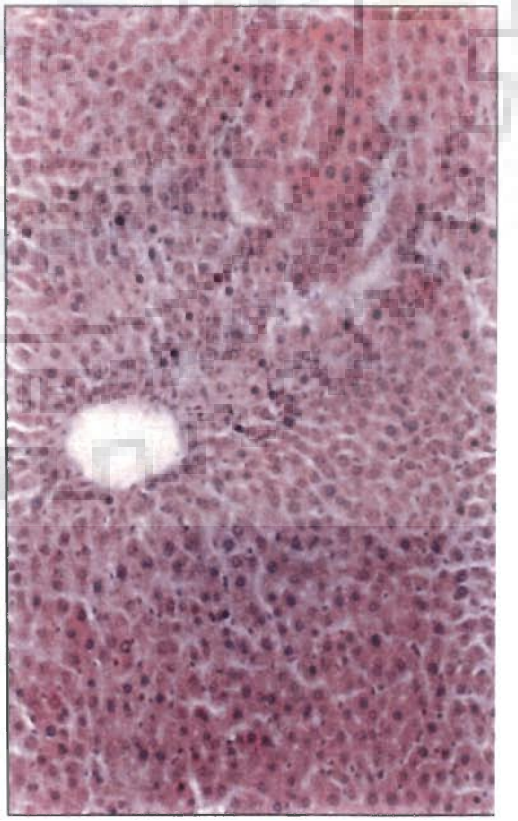


44





45



46

PLATE-XIV

LIVER

Fig.47 : Chlordane 200 mg/kg. b. wt./day for 5 weeks.

Microphotograph of liver showing hepatocytic degeneration forming cytoplasmic vacuolation and widening of sinusoidal space.

(H & E 100X)

Fig.48 : Chlordane 400 mg/kg. b. wt./day for 5 weeks.

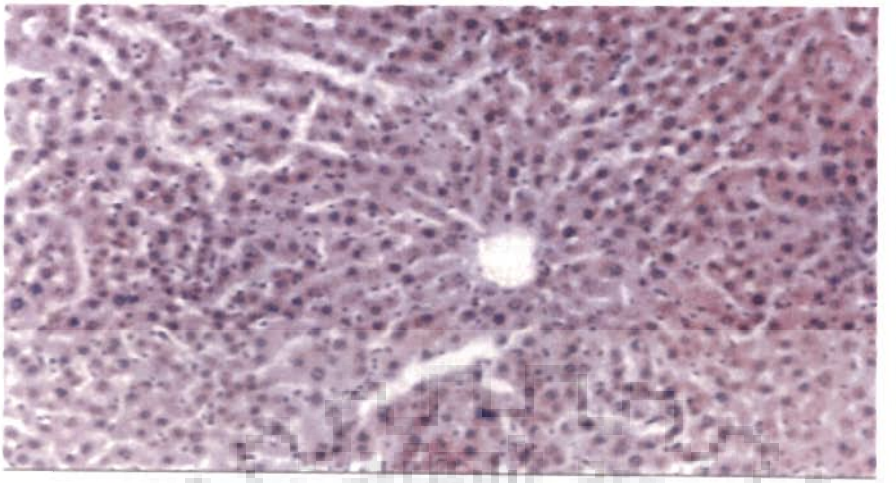
Microphotograph showing marked degenerative changes which includes disorganized hepatocytes, vacuolization, dilated sinusoidal spaces and lymphatic infiltration around the central vein.

(H & E 100X)

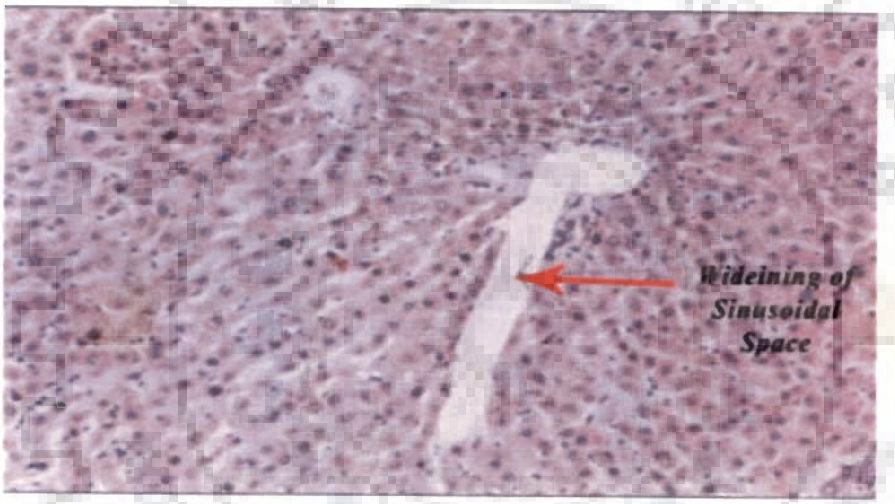
Fig.49 : Chlordane 600 mg/kg. b. wt./day for 5 weeks.

Photograph showing many degenerative changes marked by binucleated cells, hypertrophy in hepatocytes and infiltration around the central vein. Dilation of sinusoidal spaces.

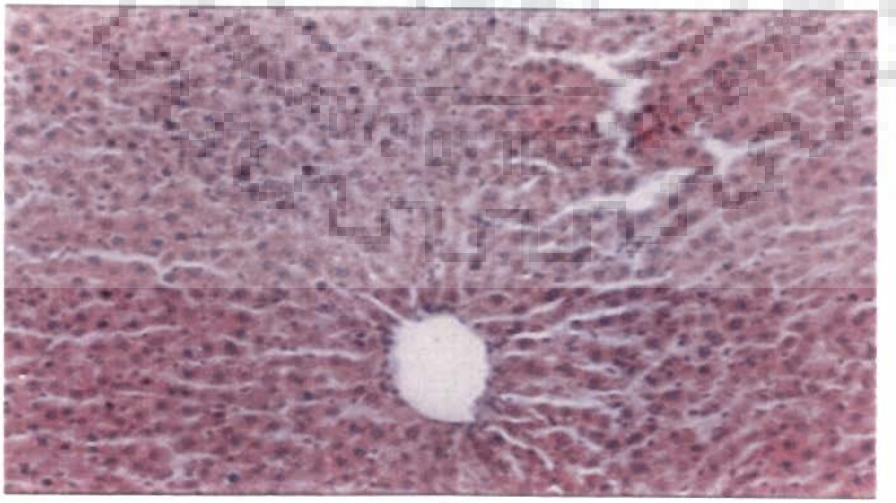
(H & E 100X)



47



48



49

PLATE-XV

KIDNEY

Fig.50 : Control

Microphotograph of control rat kidney exhibits the glomerular capsule with parietal and visceral layer separated by capsular space. Distal and proximal convoluted tubule alongwith collecting tubule is seen.

(H & E 100X)

Fig.51 : Chlordane 200 mg/kg. b. wt./day for 2 weeks.

Microphotograph showing hypercellularity with reduced glomerular size and accentuation of lobular architecture. Glomerular capillaries have diffused.

(H & E 100X)

Fig.52 : Chlordane 400 mg/kg. b. wt./day for 2 weeks.

Kidney photograph showing vacuolization of collecting tubule and degenerative changes in distal and proximal convoluted tubules with increased capsular space.

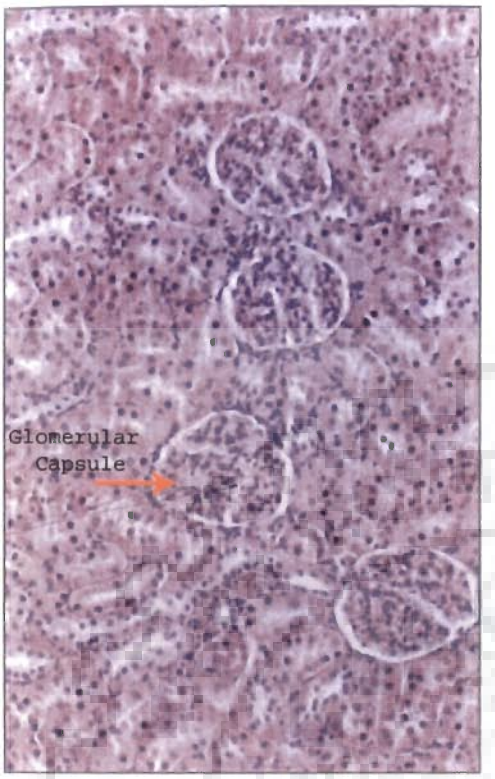
(H & E 100X)

Fig.53 : Chlordane 600 mg/kg. b. wt./day for 2 weeks.

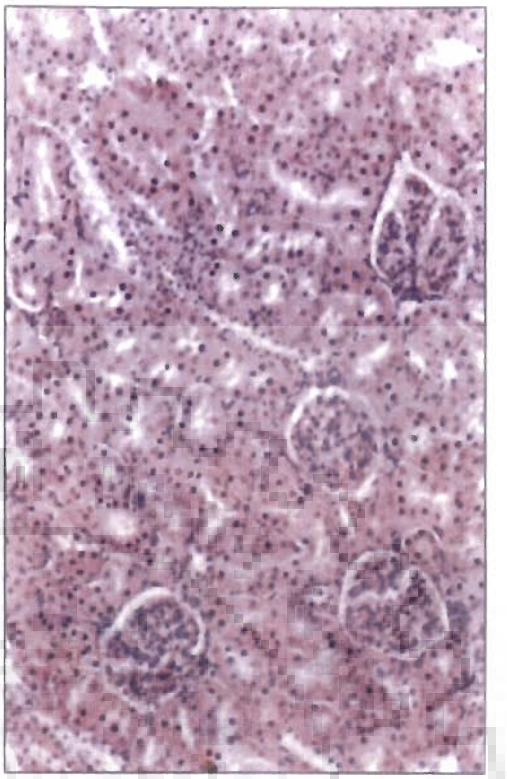
Microphotograph of treated rats showing reduced size of glomerulus. Sclerosed glomerulus with reduced cellularity and is adherent to Bowman's capsule.

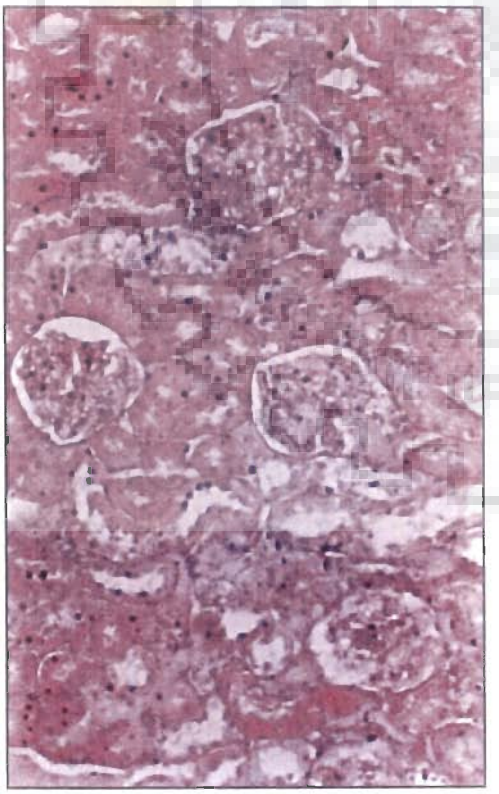
(H & E 100X)

50

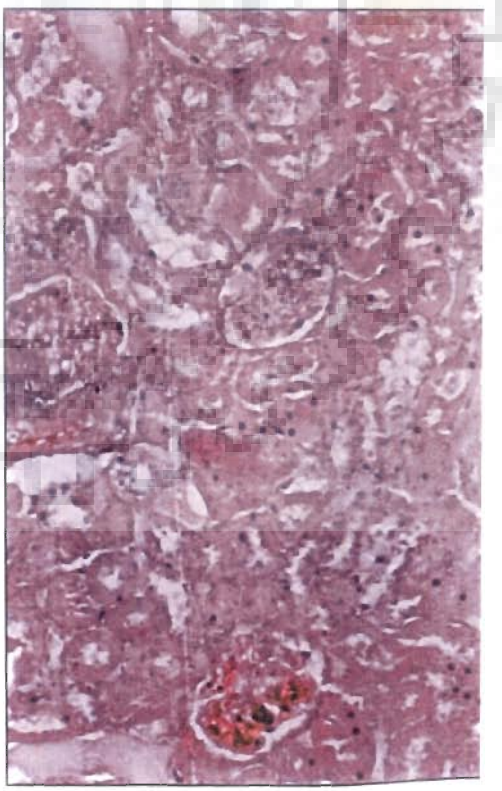


51





52



53

PLATE-XVI

KIDNEY

Fig.54 : Chlordane 200 mg/kg. b. wt./day for 5 weeks.

Microphotograph showing degenerative changes in the tubule and proliferation of intrinsic glomerular cells.

(H & E 100X)

Fig.55 : Chlordane 400 mg/kg. b. wt./day for 5 weeks.

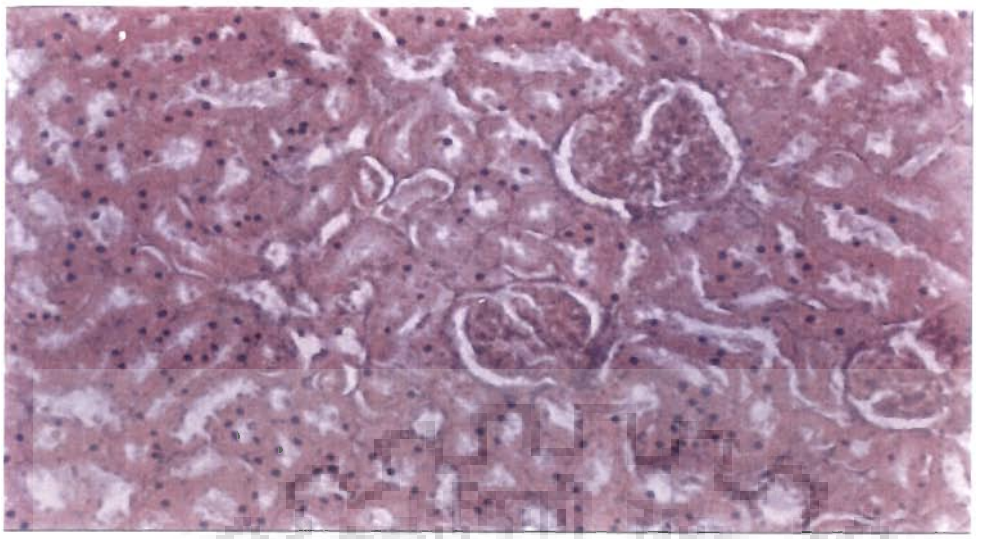
Microphotograph of kidney showing atropication of glomerulus. Reduction in the size of distal and proximal convoluted tubules is also seen with increased capsular space.

(H & E 100X)

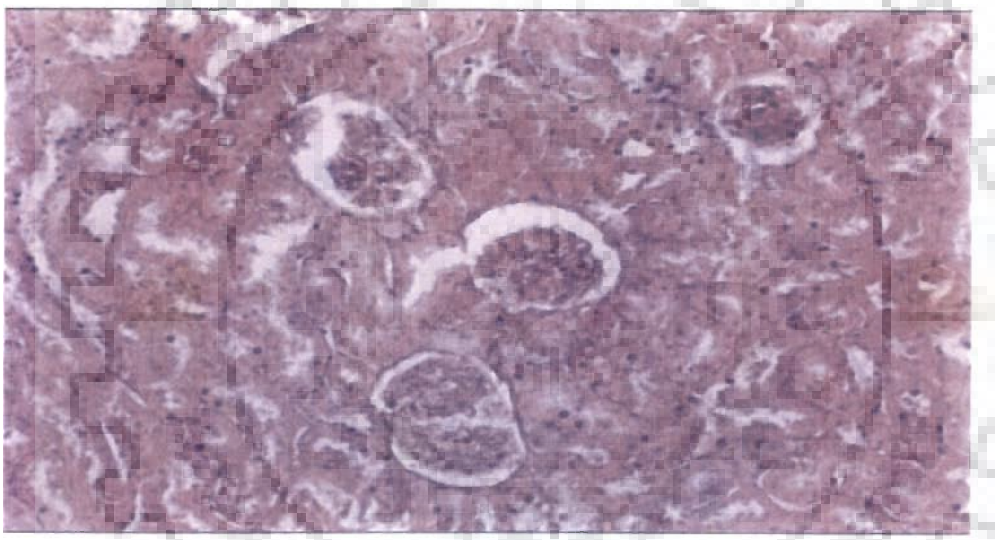
Fig.56 : Chlordane 600 mg/kg. b. wt./day for 5 weeks.

Kidney photograph showing many degenerative changes including glomerulonephritis, glomeruloscleroses and renal allogaft.

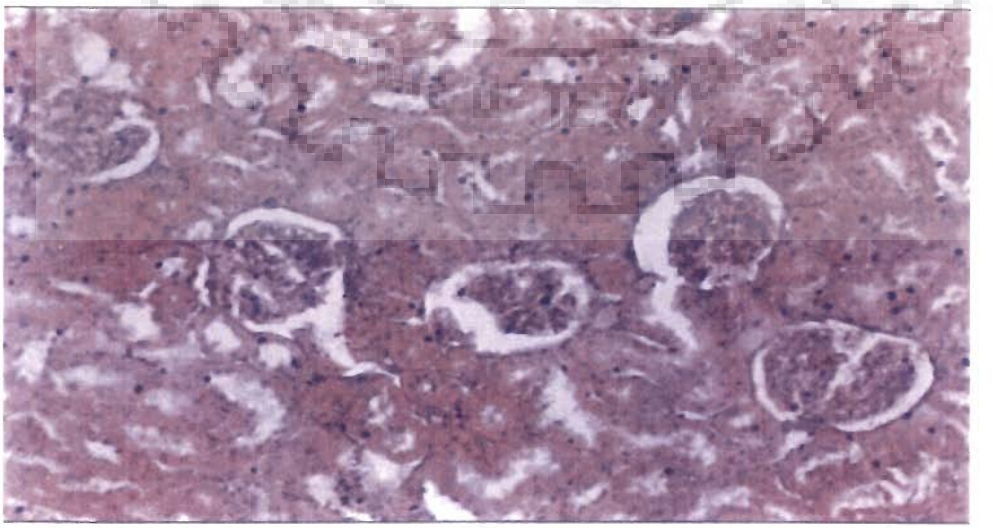
(H & E 100X)



54



55



56

[b] Monocrotophos

Table 6 : Body and Organ Weight

Treatment	Body weight		Testis	Epididymides	Seminal Vesicle	Prostate Gland	Liver	kidney	Adrenal Gland
	Initial	Final							
	gm		mg/100 gm body wt						
Group I Control (Vehicle treated)	195.67 ±9.10	196.75 ±7.60	1275.25 ±22.79	431.35 ±20.67	405.30 ±26.17	148.53 ±16.25	3420.16 ±84.18	695.12 ±31.16	19.12 ±0.96
6 mg/kg.b.wt./day of Monocrotophos									
Group IIA (2 Week)	170.10 ±7.2	161.20 ±3.10	1230.10 ±22.10	398.20 ±28.10	401.80 ±51.70	205.50 ±16.32	3810.20*** ±51.90	712.60± 8.20	18.20± 1.85
Group IIB (5 Week)	163.60 ±6.20	153.85 ±4.80	1270.18 ±44.25	406.17 ±10.90	346.50 ±56.80	221.17* ±13.15	2930.10 ±110.71	702.05 ±23.50	20.12 ±2.17
14 mg/kg.b.wt./day of Monocrotophos									
Group IIIA (2 Week)	182.50 ±5.70	181.20 ±6.15	741.60* ±20.17	418.25 ±46.40	321.70 ±42.20	162.15 ±10.30	3115.10 ±206.12	610.18 ±23.80	16.52 ±1.20
Group IIIB (5 Week)	178.00 ±6.00	172.00 ±7.50	632.80*** ±38.15	310.16* ±30.10	98.52*** ±6.92	180.30* ±13.20	3090.40 ±192.10	642.86 ±24.80	24.80* ±1.48
22 mg/kg.b.wt./day of Monocrotophos									
Group IVA (2 Week)	230.00 ±8.31	205.10 ±6.21	938.10*** ±38.40	403.30 ±19.27	336.20 ±26.27	162.65 ±9.2	3615.10* ±210.12	840.71* ±26.50	32.27** ±1.70
Group IVB (5 Week)	182.10 ±9.00	160.00 ±7.20	832.18*** ±56.10	408.10 ±27.95	199.75** ±30.20	215.60* ±13.78	3210.65* ±39.90	730.14 ±15.20	39.10** ±3.92

Group IIA, B, IIIA, B; IVA, B; compared with Group I

(Mean ± SEM of 5 animals)

* = $p \leq 0.05$

** = $p \leq 0.01$

*** = $p \leq 0.001$

Table 7: Sperm Dynamics and Fertility

Treatment	Sperm Motility (%) (Table 7.1)	Sperm Density (Million/ ml) (Table 7.2)		Fertility (%) (Table 7.3)
		Testis	Cauda Epididymides	
Group I Control (Vehicle treated)	69.19 ±3.75	4.16 ±0.07	21.25 ±0.42	100% (+)tve
6 mg/kg.b.wt./day of Monocrotophos				
Group IIA (2 Week)	49.20** ±2.31	2.95*** ±0.18	17.10* ±1.57	20%(-)ve
Group IIB (5 Week)	49.91** ±3.10	0.98*** ±0.05	15.35** ±1.01	40%(-)ve
14 mg/kg.b.wt./day of Monocrotophos				
Group IIIA (2 Week)	18.70*** ±2.21	3.10*** ±0.62	11.15*** ±1.21	60%(-)ve
Group IIIB (5 Week)	14.28*** ±2.16	0.72*** ±0.25	8.61*** ±1.08	60%(-)ve
22 mg/kg.b.wt./day of Monocrotophos				
Group IVA (2 Week)	19.58*** ±1.70	1.87*** ±0.22	8.08* ±0.80	80%(-)ve
Group IVB (5 Week)	9.87*** ±1.10	0.42*** ±0.06	3.81*** ±1.29	100%(-)ve

Group IIA, B,; IIIA, B; IVA, B; compared with Group I

(Mean ± SEM of 5 animals)

* = $p \leq 0.05$

** = $p \leq 0.01$

*** = $p \leq 0.001$

Table 8 : Blood Analysis (Hematology)

Treatment	Total erythrocyte Count (TEC)	Total leukocyte Count (TLC)	Hemoglobin	Haematocrit	Blood Sugar	Blood Urea
	------ million /mm^3 ----------		gm %	%	----- mg/dl------	
Group I Control (Vehicle treated)	6.64 ±0.24	5420 ±240	15.60 ±0.30	49.45 ±1.49	85.15 ±2.10	47.75 ±3.50
6 mg/kg.b.wt./day of Monocrotophos						
Group IIA (2 Week)	6.21 ±0.31	8010** ±419.10	15.25 ±0.21	54.15 ±1.16	89.2 ±1.8	53.10 ±5.9
Group IIB (5 Week)	5.80 ±0.38	7827.30* ±811.10	13.12** ±0.36	51.2 ±0.9	92.7 ±2.1	76.45*** ±2.12
14 mg/kg.b.wt./day of Monocrotophos						
Group IIIA (2 Week)	6.57 ±0.19	7010.50** ±4600.25	12.58*** ±0.31	41.6** ±1.28	92.1 ±3.9	96.25** ±8.18
Group IIIB (5 Week)	5.69** ±0.18	9425.10** ±810.2	11.20*** ±0.33	38.2* ±2.8	95.6 ±3.61	97.10*** ±7.80
22 mg/kg.b.wt./day of Monocrotophos						
Group IVA (2 Week)	5.60* ±0.22	8110** ±778.1	11.30** ±0.55	41.50** ±1.32	121.10** ±8.2	81.10** ±4.2
Group IVB (5 Week)	4.78*** ±0.17	8422.5** ±541.1	10.02*** ±0.18	38.50** ±1.38	112.6** ±6.15	78.14** ±5.10

Group IIA, B,; IIIA, B; IVA, B; compared with Group I

(Mean ± SEM of 5 animals)

* = $p \leq 0.05$

** = $p \leq 0.01$

*** = $p \leq 0.001$

Table 9 : Serum Analysis

Treatment	Alanine amino transferase (ALT)	Aspartate amino transferase (AST)	Acid Phosphatase	Alkaline Phosphatase	Bilirubin
	--------- units/ml ----------		----------KA units----------		Mg %
Group I Control (Vehicle treated)	131.28 ±7.12	71.80 ±1.80	4.51 ±.07	65.70 ±2.20	0.22 ±0.02
6 mg/kg.b.wt./day of Monocrotophos					
Group IIA (2 Week)	146.50 ±2.90	83.20** ±2.90	10.27** ±1.62	47.75*** ±0.90	0.31 ±0.08
Group IIB (5 Week)	147.20 ±6.85	87.75** ±3.98	11.65** ±1.78	42.15*** ±0.38	0.68 ±0.08
14 mg/kg.b.wt./day of Monocrotophos					
Group IIIA (2 Week)	178.60** ±4.12	97.50*** ±14.10	11.41** ±0.92	44.65** ±2.88	0.90** ±0.69
Group IIIB (5 Week)	168.40** ±4.81	92.6** ±3.18	11.86** ±1.29	42.12*** ±1.40	0.85** ±0.71
22 mg/kg.b.wt./day of Monocrotophos					
Group IVA (2 Week)	161.50** ±3.10	88.63** ±4.71	13.81** ±2.18	40.00*** ±0.81	2.25** ±1.20
Group IVB (5 Week)	177.20** ±3.72	105.10*** ±4.97	12.16** ±2.76	41.95*** ±1.72	2.72** ±0.95

Group IIA, B,; IIIA, B; IVA, B; compared with Group I

(Mean ± SEM of 5 animals)

* = $p \leq 0.05$

** = $p \leq 0.01$

*** = $p \leq 0.001$

Table 10 : Serum Analysis

Treatment	Total protein	Phospholipid	Triglyceride	Total – Chol.	HDL- Col.	LDL – Chol.	VLDL- Chol.
	mg/dl						
Group I Control (Vehicle treated)	16950.15 ±621.19	146.12 ±4.10	99.16 ±1.21	96.72 ±4.9	41.10 ±5.70	37.50 ±6.20	18.80 ±2.60
6 mg/kg.b.wt./day of Monocrotophos							
Group IIA (2 Week)	15870.15 ±602.1	131.50 ±11.9	100.00 ±10.12	88.5 ±5.00	41.75 ±2.46	24.12 ±2.48	22.20 ±2.10
Group IIB (5 Week)	15340.41 ±642.3	163.10* ±2.42	163.25* ±13.75	118* ±5.21	56.71* ±2.39	26.00 ±2.92	32.50* ±2.35
14 mg/kg.b.wt./day of Monocrotophos							
Group IIIA (2 Week)	25260.28** ±2718.30	134.00 ±10.85	119.50 ±13.32	103.25 ±4.8	46.20 ±5.10	38.27 ±3.10	26.50 ±2.80
Group IIIB (5 Week)	29310.28** ±2218.1	173.50** ±5.52	141.65* ±12.25	148.00* ±11.10	71.10** ±5.69	49.50 ±7.51	28.25 ±2.40
22 mg/kg.b.wt./day of Monocrotophos							
Group IVA (2 Week)	28261.38** ±1152.51	198.50*** ±2.30	155.00* ±25.00	107.15 ±5.21	58.00 ±5.12	31.00 ±5.00	37.25* ±4.90
Group IVB (5 Week)	29172.15** ±2862.34	213.50*** ±9.10	198.00*** ±7.5	171.5*** ±10.75	62.00** ±10.10	51.20 ±8.10	41.00* ±9.00

Group IIA, B,; IIIA, B; IVA, B; compared with Group I

(Mean ± SEM of 5 animals)

* = $p \leq 0.05$

** = $p \leq 0.01$

*** = $p \leq 0.001$

INSECTICIDES

4.2 MONOCROTOPHOS

The exposure of rat with technical grade monocrotophos was carried out at three dosage levels 6, 14, and 22 mg/kg b.wt./day for 2 weeks and 5 weeks, respectively.

4.2.1 WEIGHT RESPONSE

Body weight (Table 6)

The treatment with monocrotophos at all the three dosage levels for 2 weeks and 5 weeks to intact rats caused reduction in their body weights.

Organ weight (Table 6)

Testis

Oral administration of monocrotophos to rat caused a significant reduction at higher dose levels ($P \leq 0.01$ and $P \leq 0.001$) in the weight of testis in comparison to control animals. The percent reduction was 3.54, 0.40, 41.85, 50.37, 26.43 and 34.76% in group IIA, IIB, IIIA, IIIB, IVA and IVB respectively. (Fig.4.7)

Epididymides

No significant changes ($P \leq 0.05$ and $P \leq 0.01$) in the weight of epididymides were observed after monocrotophos treatment even at higher dose levels when compared with control rats.

Seminal vesicle

A significant decrease ($P \leq 0.01$ and $P \leq 0.001$) in seminal vesicle weight was observed at higher dose levels after administration of monocrotophos for 2 and 5 weeks, respectively. The change was more prominent when treated for 5 weeks. (Fig. 4.8)

Prostate gland

A significant increase (P≤0.05) in the weight of the prostate gland was observed when monocrotophos was administered for a longer duration of time (5 weeks) at all dosage level. (Fig.4.9).

Liver

A significant increase (P ≤0.05 & P≤ 0.001) in the weight of liver was brought about by monocrotophos treatment in group II A and IV A, B when compared with control group. On the contrary these were almost no significant change at 14mg/kg b.wt/day. (Fig 4.10).

Kidney

Statistically there was almost no significant change in the weight of kidney in the rats treated with monocrotophos in comparison to control rats.

Adrenal gland

Significant increase (≤0.01 & P≤ 0.01) in the weight of adrenal gland was observed in the group III B and IVA,B of rats exposed to higher dose levels of monocrotophos when compared with control rats.

4.2.2 SPERM DYNAMICS (Table 7)

Sperm motility (Table 7.1)

Cauda epididymides

The motility of spermatozoa in cauda epididymides decreased (P≤0.01 and P ≤0.001) in a dose dependent manner i.e. 28.89%, 27.87%, 72.97%, 79.36% 71.81% and 85.73% observed in the rats treated with monocrotophos at 6, 14 and 22 mg/kg b.wt./day for 2 Weeks and 5 Weeks respectively (Fig.4.11).

Sperm density (Table 7.2)

Testis

The monocrotophos exposed rats showed a significant ($P \leq 0.001$) reduction in testicular sperm density. The reduction was found in a dose dependent manner 29.08%, 76.44%, 25.48%, 82.69%, 55.04% and 89.90% in comparison to control rats. The effect was more prominent on long term treatment. (Fig.4.12).

Cauda epididymides

A significant decrease, dose and time dependent, ($P \leq 0.05$, $P \leq 0.01$ & $P \leq 0.001$) in the sperm concentration of monocrotophos treated rat was observed in different experimental groups as compared to control groups. The percent decrease was found to be 19.52%, 27.76%, 47.52%, 59.52%, 61.97% and 82.11% in groups IIA, IIB, IIIA, IIIB, IVA and IVB. respectively as compared to control (Fig.4.13).

Fertility test (Table 7.3)

On mating between male and female rats, the mating exposure test revealed that the control rats showed 100% positive fertility whereas 6 mg dose level showed 20% negative fertility, 14 mg dose level showed 40% and 60% negative fertility and 22 mg dose level showed 80% and 100% negative fertility for 2 and 5 weeks of treatment respectively. In this experiment, one male rat was placed with two female rats. Numbers of pups were counted as indicator of fertility for all groups. (Fig.4.14)

4.2.3 HEMATOLOGY (Blood Analysis) - (Table 8)

Total Erythrocyte Count (TEC)

A significant reduction in the erythrocyte count ($P \leq 0.05$ and ≤ 0.001) in monocrotophos treated rat at higher dose levels was observed in comparison to control rats. (Fig.4.15)

Total Leukocyte Count (TLC)

A significant increase (P ≤0.05 and P≤ 0.01) in the leukocyte count of monocrotophos exposed rats was found to be 47.78, 44.40, 29.33, 73.89, 49.63 and 55.38% in groups IIA, IIB, III A, IIIB, IV A, IVB respectively as compared to control rats.

Hemoglobin

Hemoglobin concentrations of different monocrotophos treated groups were found to be decreased (P≤0.01 and P ≤ 0.001) at almost all the tested dosage levels and for each durations. (Fig. 4.16)

Haematocrit percent

A significant decrease (P≤0.05 and 0.01) in the percentage of haematocrit treated groups value was observed in monocrotophos exposed rats at 14 and 22 mg/kg.b.wt./day as compared to control groups. The pesticide failed to induce any significant effect at 6 mg/kg.b.wt./day dosage levels.

4.2.4. BIOCHEMICAL ANALYSIS

Serum analysis (Table 8, 9 and 10)

Blood Urea and Blood Sugar

Blood sugar level although did not show any significant changes at 6 and 14 mg/kg. b.wt./dosage level, but it showed significant increase at 22 mg/kg.b.wt. dosage level for both 2 and 5 weeks time span. The increase was11.20%, 60.01%, 101.5%, 103.35%, 69.84% and 63.64% respectively over control. Significant increase in blood sugar was observed at higher dose level groups (P≤0.01).

On the contrary, blood urea level showed highly significant increase (P≤0.01 and P ≤ 0.001) almost in all the groups of treated animals. Increase in blood urea was 11.20%, 60.01%, 101.5%, 103.35%, 69.84% and 63.64% respectively in group IIA, B, IIIA, B and IVA, B in comparison to control group.

Alanine amino transferase (ALT) and aspartate amino transferase (AST)

After exposure of monocrotophos to rats for 2 weeks and 5 weeks, it was observed that group III A,B and IV A,B showed highly significant ($P \leq 0.01$) increase in alanine amino transferase. While aspartate amino transferase was increased significantly ($P \leq 0.01$ and $P \leq 0.001$) in all groups (including groups IIA and B). The respective increase in aspartate amino transferase was 15.87%, 22.21%, 35.79%, 28.96%, 23.44% and 47.55% and that of alanine amino transferase was 11.59%, 12.12%, 36.04%, 28.27%, 23.01% and 34.97% in groups IIA, B, IIIA, B, IV A, and IVB respectively in comparison to control animals. (Fig. 4.17 & 4.18)

Acid phosphates and Alkaline phosphates

A significant increase ($P \leq 0.01$ and $P \leq 0.05$) in the activity of acid phosphatase was observed after exposure to monocrotophos to rats in comparison to control rats. Whereas the level of alkaline phosphatase was found to the decreased in all the six groups of monocrotophos exposed rats in comparison to control rats (Fig.4.19 & 4.20).

Bilirubin

Bilirubin concentration increased significantly ($P \leq 0.01$) after the oral administration of monocrotophos in group IIIA, B, IVA and IV B, whereas no significant changes were observed in lower dose levels i.e. in group IIA, B as compared to control group.

Total protein (Table 10)

A significant increase ($P \leq 0.01$) in serum level of total protein was observed after 2 and 5 weeks of monocrotophos exposed at higher dose levels (14 and 22 mg./kg.b.wt./day) in comparison to control group. While no significant changes were observed at lower dosage.

Triglyceride

Triglyceride concentration in the serum of monocrotophos exposed rats showed significant increase ($P \leq 0.05$ and $P \leq 0.001$) in the group of IIB, IIIB and IVA, B (Fig.4.21).

Phospholipid

A similar pattern was observed in phospholipids in tissues where it showed a significant up regulation ($P \leq 0.001$) in serum phospholipids levels at highest dose concentration (22mg/kg b.wt) for 2 and 5 weeks time span. Although it failed to cause significant elevation at lower dose but it showed some significant elevation ($P \leq 0.05$) at long treatment group (5 weeks) of animals at lower dose in comparison to control group. (Fig.4.22).

Total Cholesterol

The total cholesterol was increased significantly in monocrotophos exposed rats at all the dose levels but the response was most significant on long treatment group of animals (5 Weeks) in comparison to control rats. The HDL, LDL and VLDL-Cholesterol contents were also found to be increased at higher dose levels of monocrotophos as compared to control group.

4.2.5 RADIO IMMUNO ASSAY-

Significant decrease ($P \leq 0.01$ and $P \leq 0.001$) in serum testosterone level of monocrotophos treated rats at higher doses was observed as compared to control rats (Fig.4.23). Serum level testosterone showed a dose dependent reduction ($P \leq 0.01$ and $P \leq 0.001$), which was most prominent at highest dose tested (22 mg/kg b.wt/day).Where it showed more than 100% reduction as compared to control group (Fig. 4.23)

ORGAN WEIGHT

Fig. (4.7) Testes

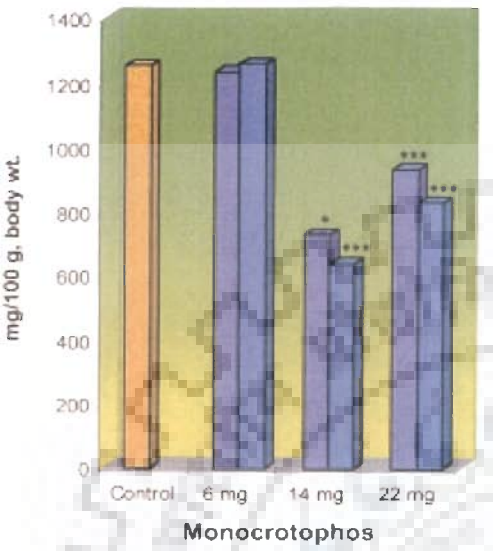


Fig. (4.8) Seminal vesicle

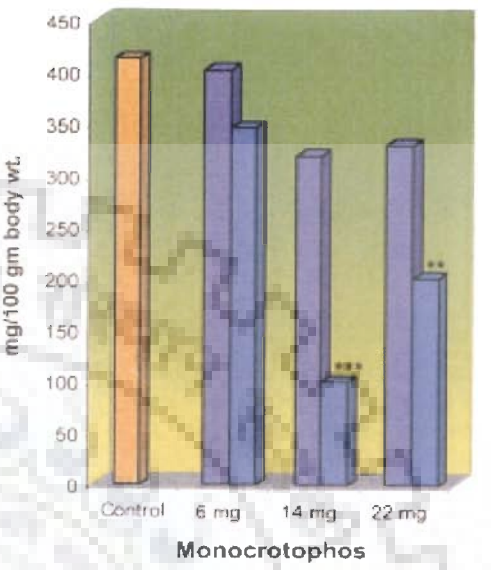


Fig. (4.9) Prostate gland

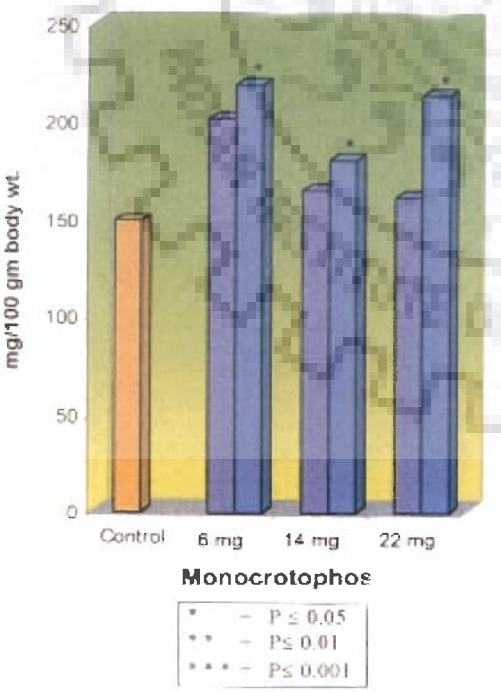
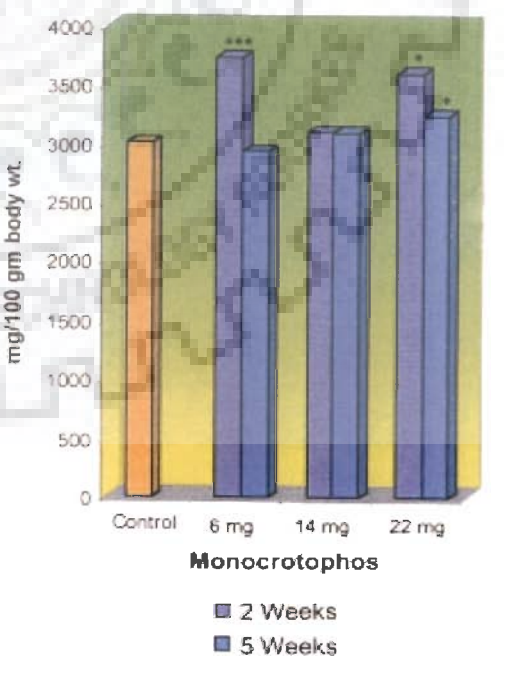


Fig. (4.10) Liver



SPERM DYNAMICS AND FERTILITY

Fig.(4.11) Sperm Motility

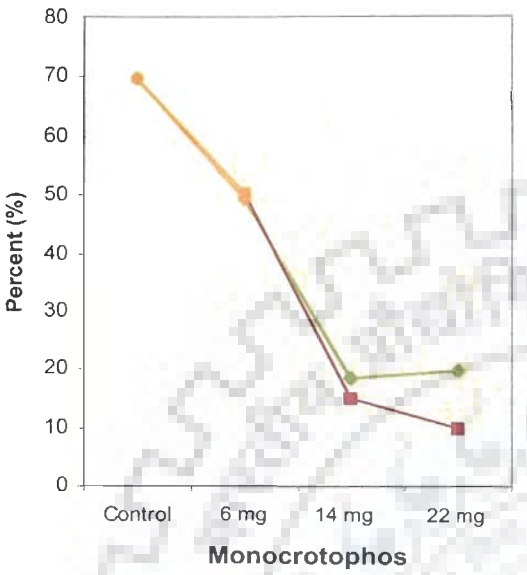


Fig.(4.12) Sperm Density (Testes)

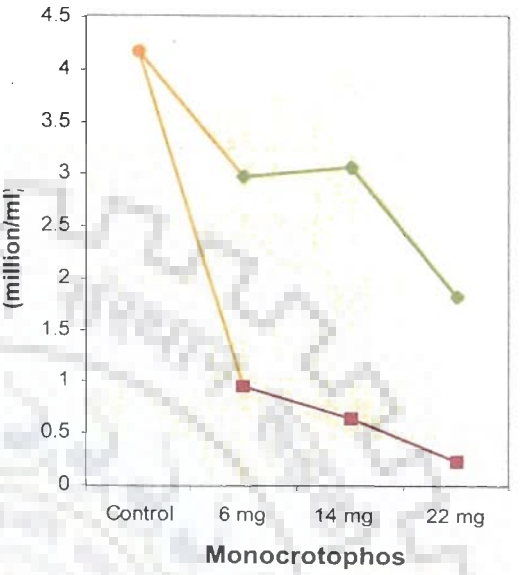
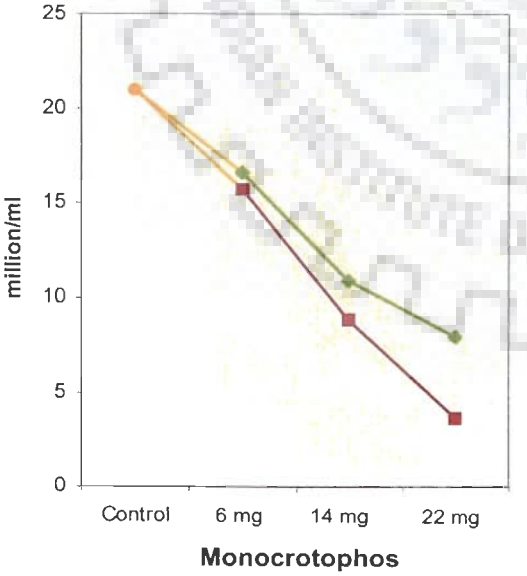
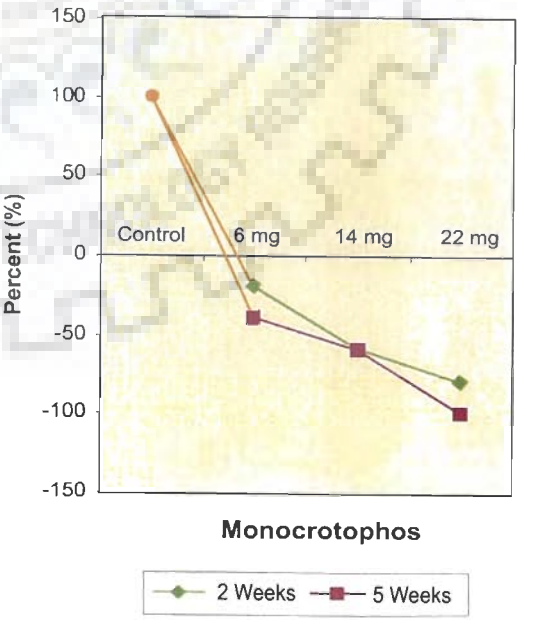


Fig.(4.13) Sperm Density (Cauda)



* = P ≤ 0.05
** = P≤ 0.01
*** = P≤ 0.001

Fig.(4.14) : Fertility (%)



BLOOD ANALYSIS

Fig. (4.15): TEC

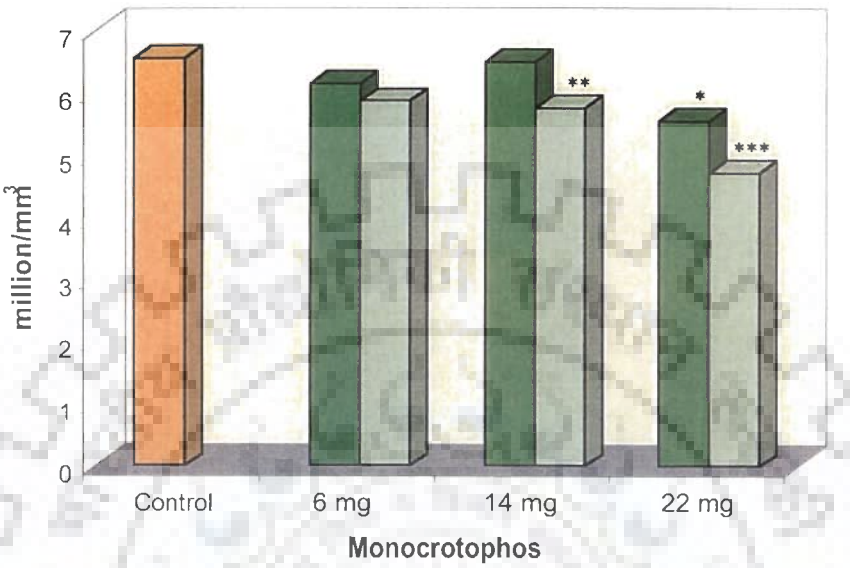
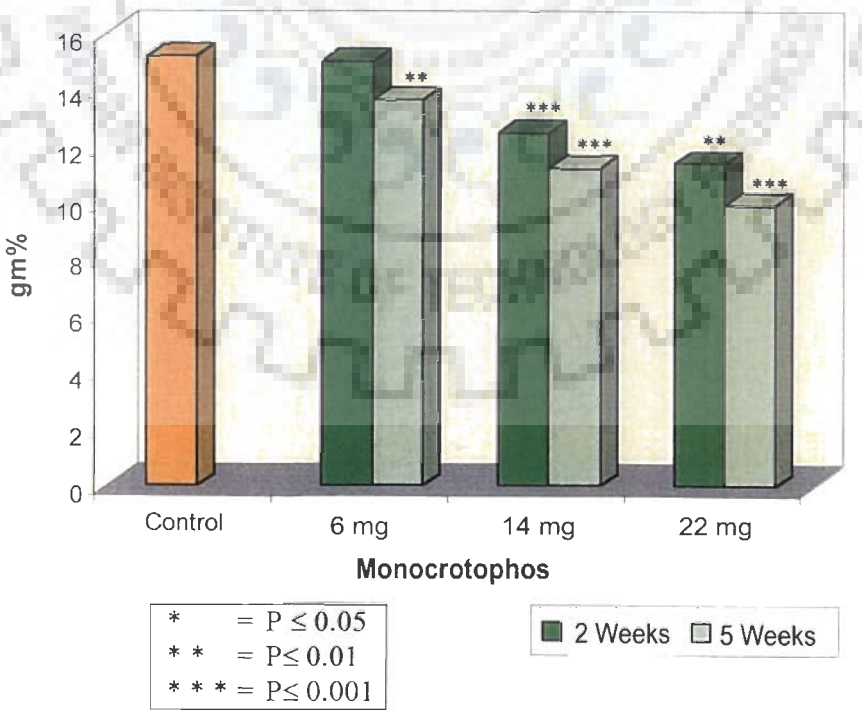


Fig. (4.16) Hemoglobin



SERUM ANALYSIS

Fig. (4.17) ALT

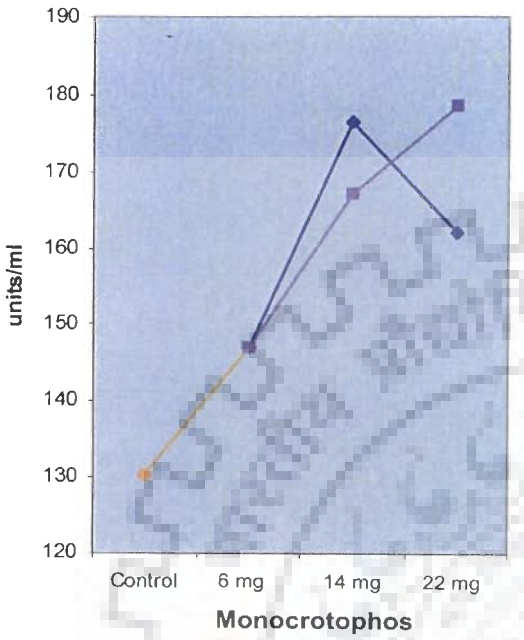


Fig. (4.18) AST

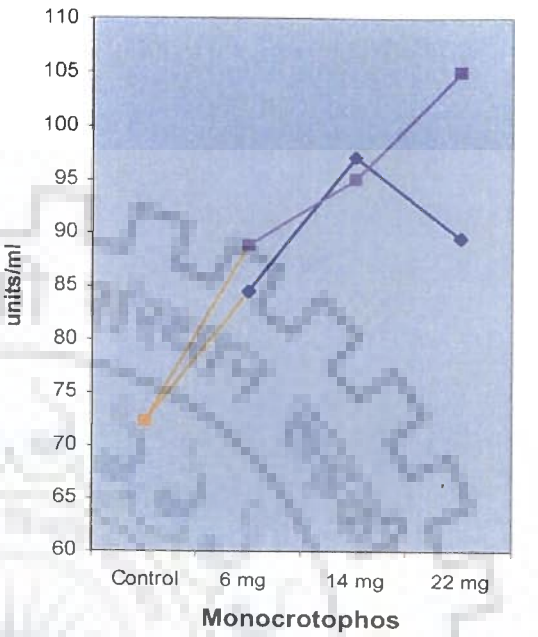


Fig. (4.19) Acid Phosphatase

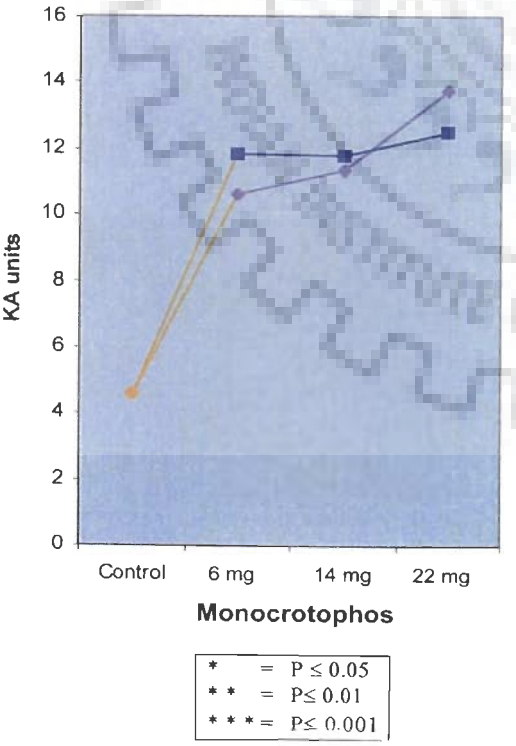
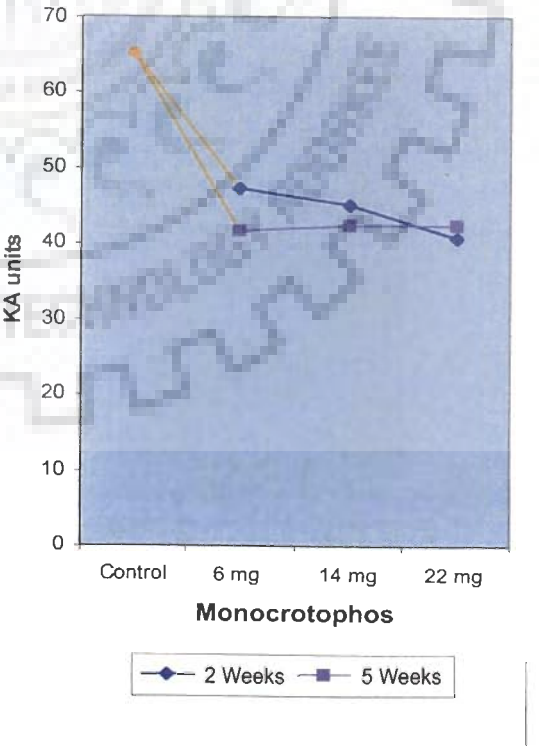


Fig. (4.20) Alkaline Phosphatase



* = P ≤ 0.05
** = P≤ 0.01
*** = P≤ 0.001

2 Weeks 5 Weeks

SERUM ANALYSIS

Fig. (4.21) Triglyceride

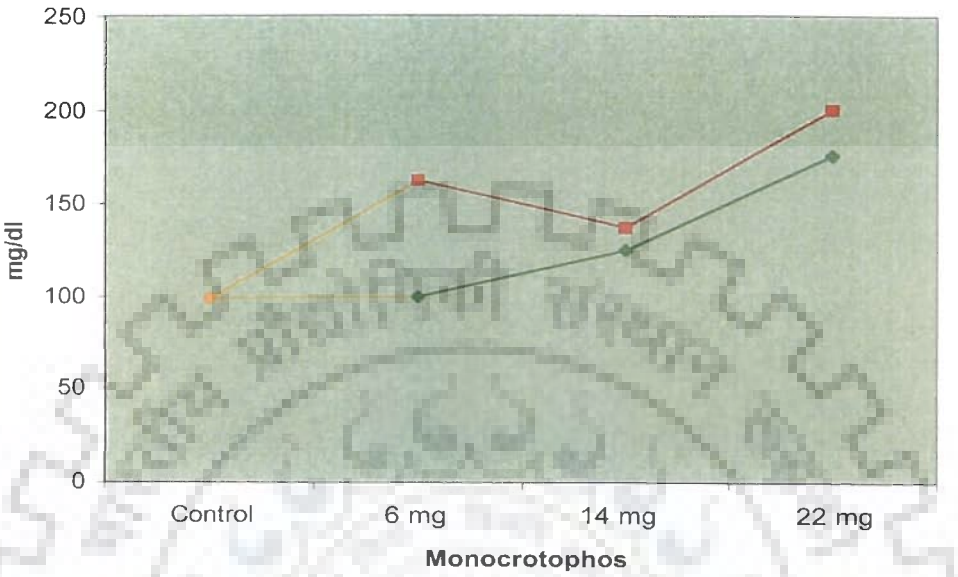
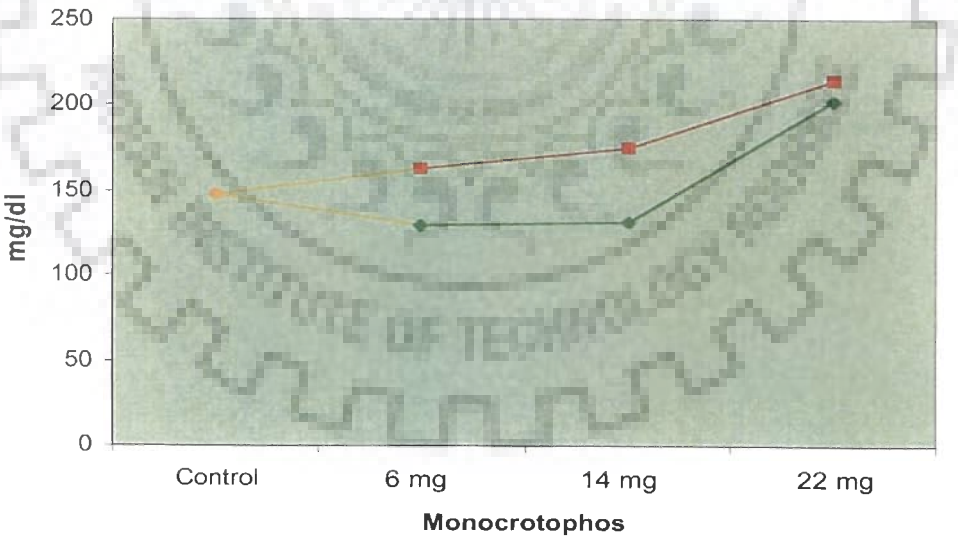


Fig. (4.22) Phospholipid

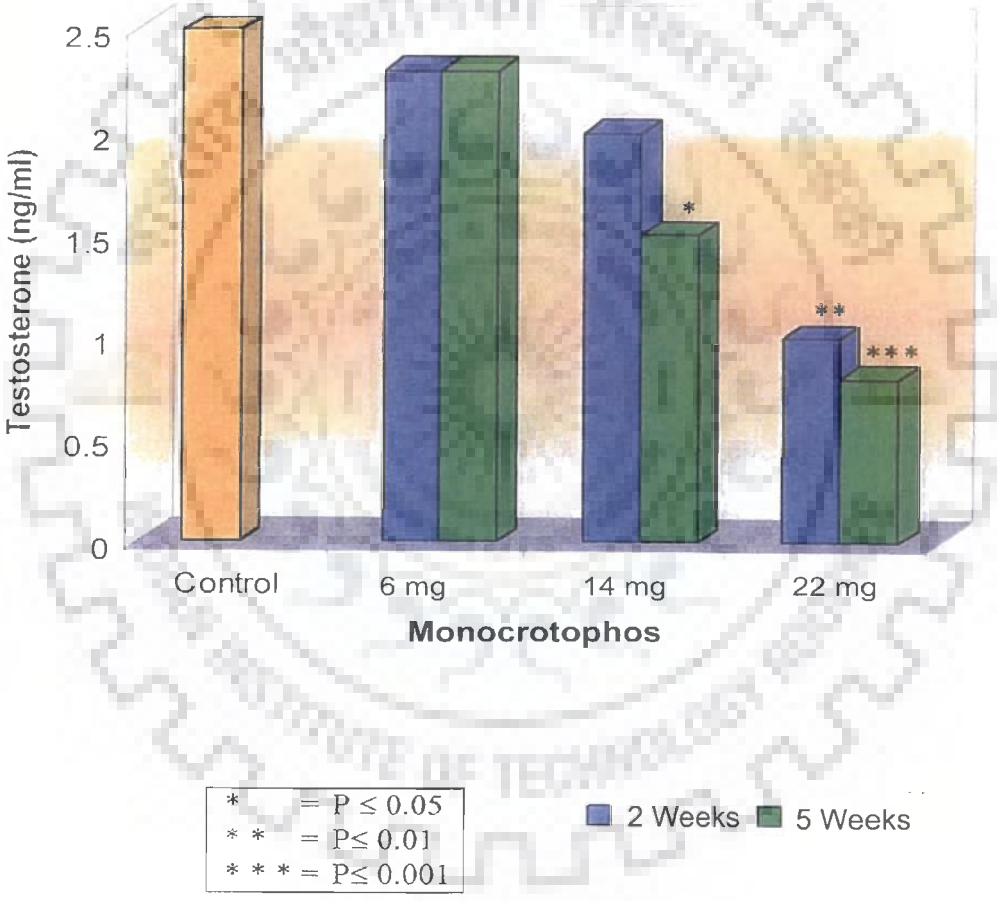


* = P ≤ 0.05
* * = P≤ 0.01
* * * = P≤ 0.001

2 Weeks 5 Weeks

TESTOSTERONE LEVEL

Fig. (4.23) Serum Testosterone



HISTOLOGY OF MONOCROTOPHOS TREATED RATS

PLATE- XVII

TESTIS

Fig.1 Control

Microphotograph of testis from vehicle treated control rat showing seminiferous tubules with all the successive stages of spermatogenesis. Sertoli cells are present. Lumen is filled with sperm. Leydig cells could be seen in the intertubular spaces.

(H & E 200 X)

Fig.2 Monocrotophos 6 mg./kg. b.wt./ day for 2 Weeks

Microphotograph showing reduced seminiferous tubule contained degenerated sertoli cells and few spermatogonia and are surrounded by a thickened basement membrane.

(H & E 200 X)

Fig.3 Monocrotophos 6 mg. /kg. b.wt./ day for 5 Weeks

Seminiferous tubule exhibiting inhibition of spermatogenesis. Absence of sperm and presence of germ cells in the tubular lumen could be seen. Interstitial cells are disrupted.

(H & E 200 X)

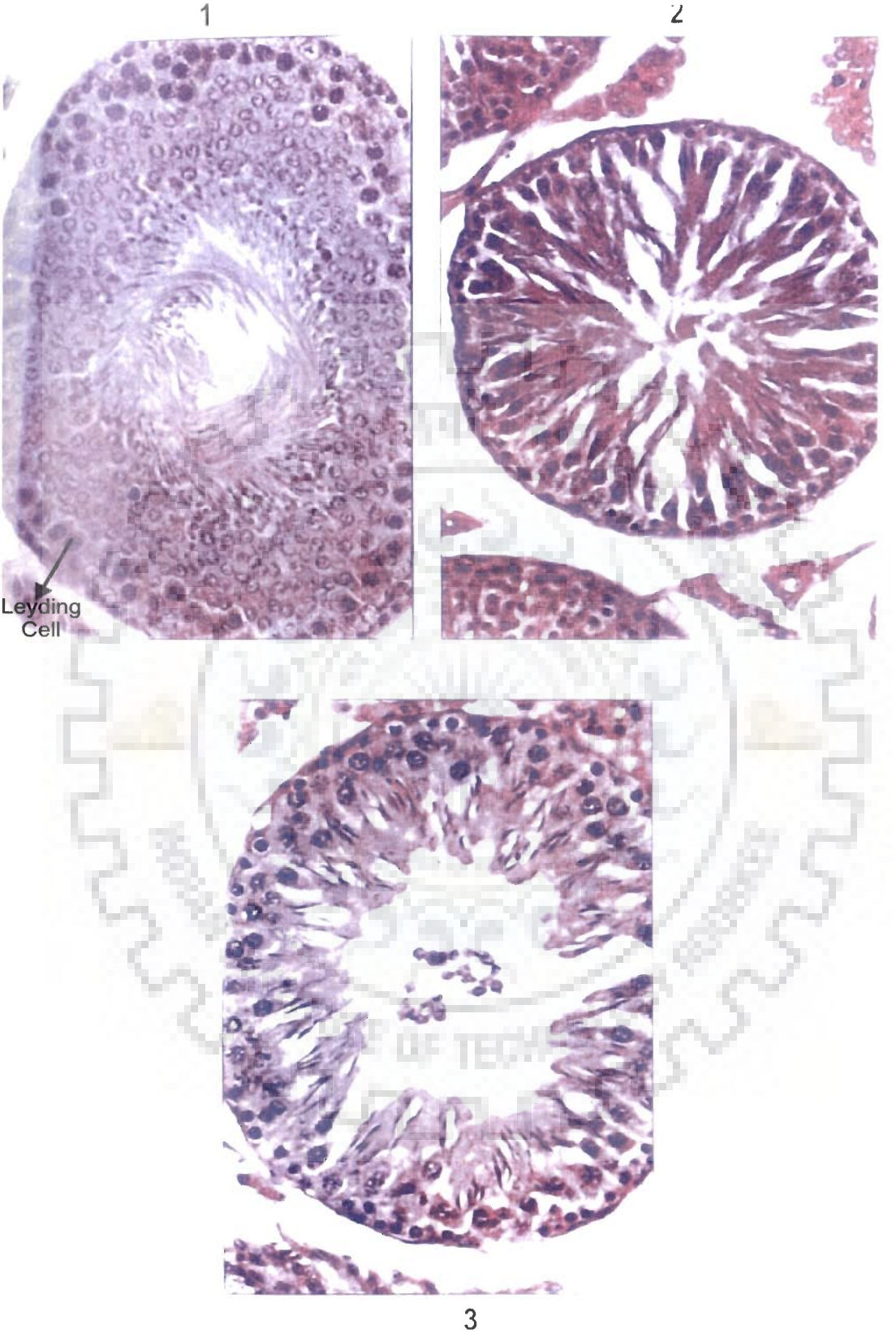

1
2
Leyding
Cell
3

PLATE- XVIII

TESTIS

Fig.4 Monocrotophos 14mg./kg. b.wt./ day for 2 Weeks

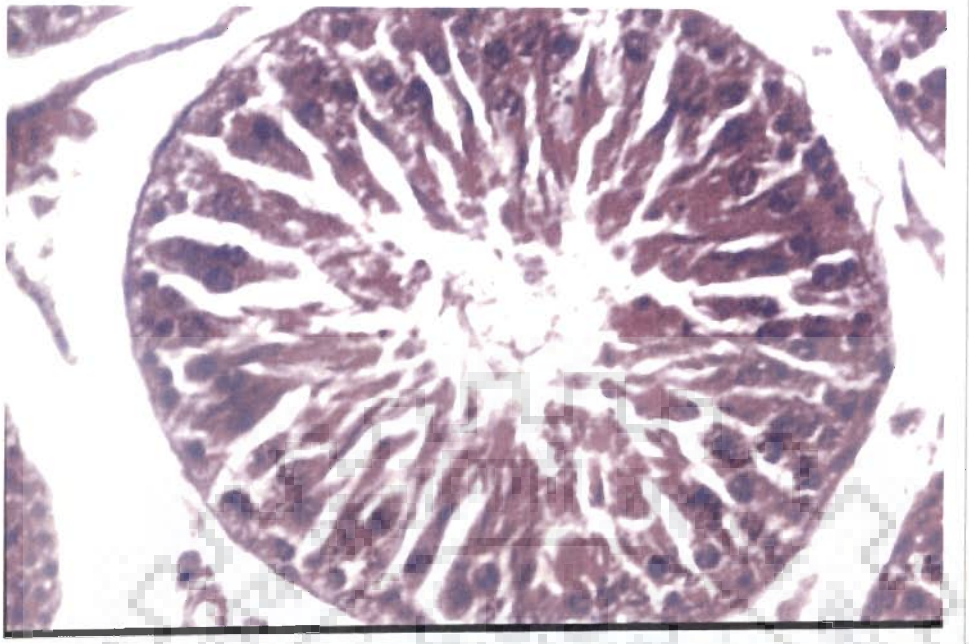
Microphotograph showing inhibition of spermatogenesis and presence of degenerated sertoli cells with few spermatogonia. Increased interstitial spaces and disrupted leydig cells are present. Lumen contained cellular debris.

(H & E 200 X)

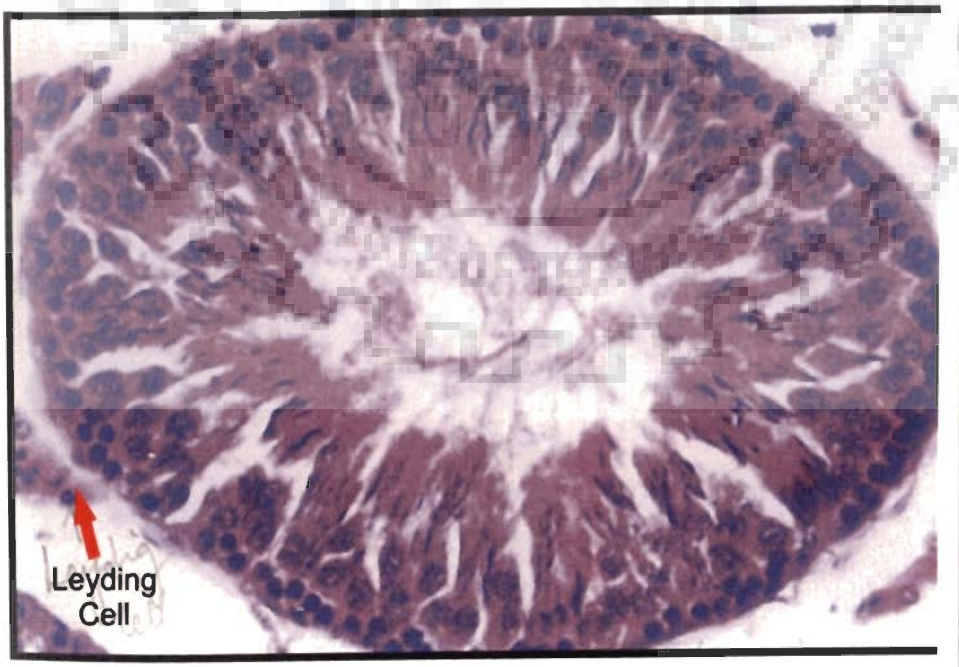
Fig.5 Monocrotophos 14 mg./kg. b.wt./ day for 5 Weeks

Seminiferous tubule showing decrease number of spermatogenic elements. Interstitial space showing degenerated leydig cells. Tunica propria is loosed at some places. Lumen is occupied by few sperm along with debris.

(H & E 200 X)



4



5

PLATE XIX

TESTIS

Fig.6 Monocrotophos 22 mg./kg. b.wt./ day for 2 Weeks

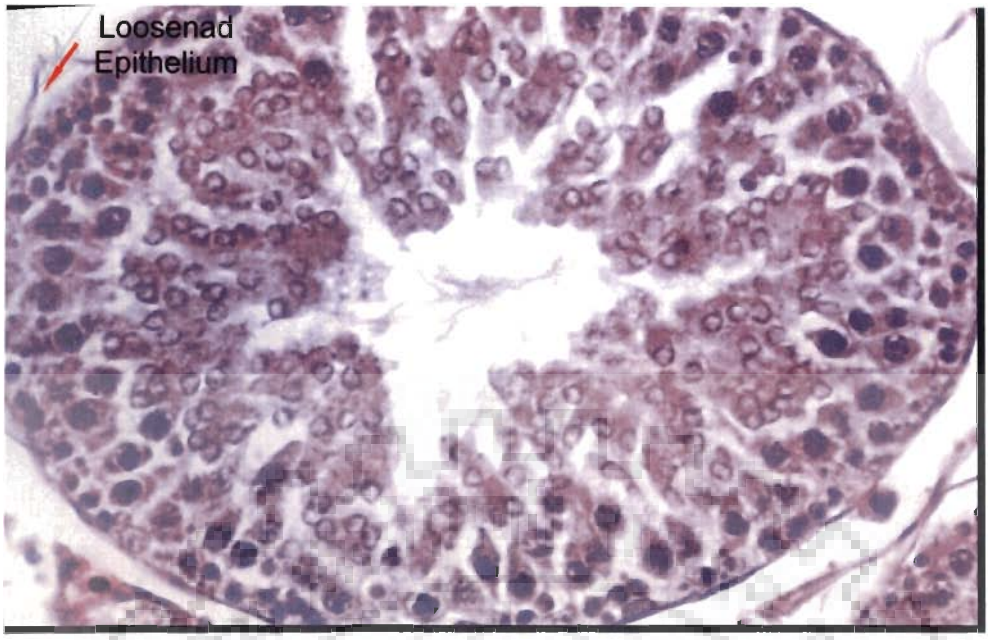
Microphotograph showing semniferous tubules with irregular epithelium loosened at some sites. Interstitial space showing degenerated leydig cells. Lumen contain few sperm.

(H & E 200 X)

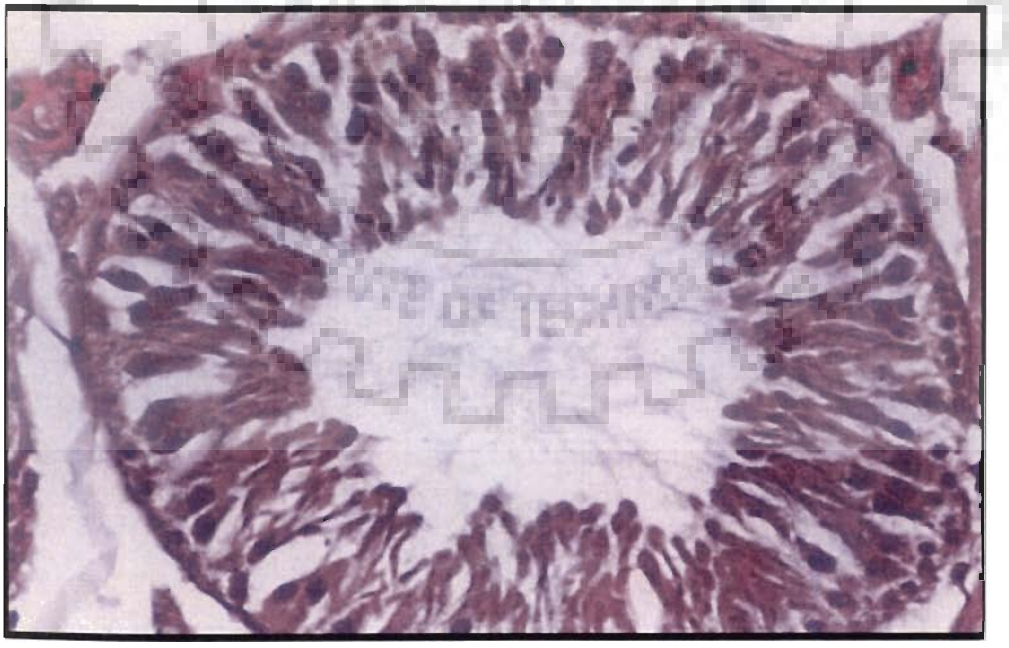
Fig.7 Monocrotophos 22 mg./kg. b.wt./ day for 5 Weeks

Microphotograph showing inhibited spermatogenesis in the seminiferous tubule and surrounded by thickened membrane. Only few spermatogenic elements are present and lumen contained lesser number of sperm. Disrupted leydig cells also seen.

(H & E 200 X)



6



7

PLATE XX

CAUDA EPIDIDYMIS

Fig.8 Control

Microphotograph of cauda epididymis from vehicle treated control rat showing compact tubules with columnar epithelial cell lining. Stereocilia projected towards lumen. Lumen is filled with spermatozoa. Connective tissue and blood vessels are seen in the intertubular stroma.

(H & E 100 X)

Fig.9 Monocrotophos 6 mg./kg. b.wt./ day for 2 Weeks

Cauda epididymis showing degenerated wall of the tubule and absence of stereocilia. Lumen contains reduced numbers of spermatozoa.

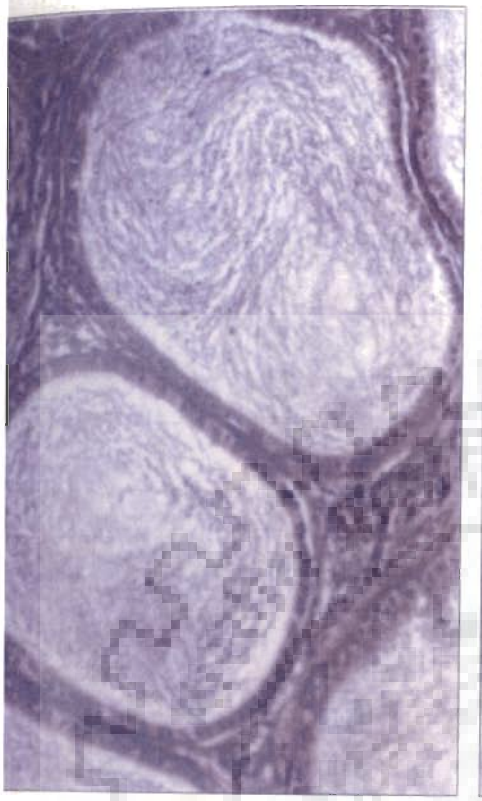
(H & E 100 X)

Fig.10 Monocrotophos 6 mg./kg. b.wt./ day for 5 Weeks

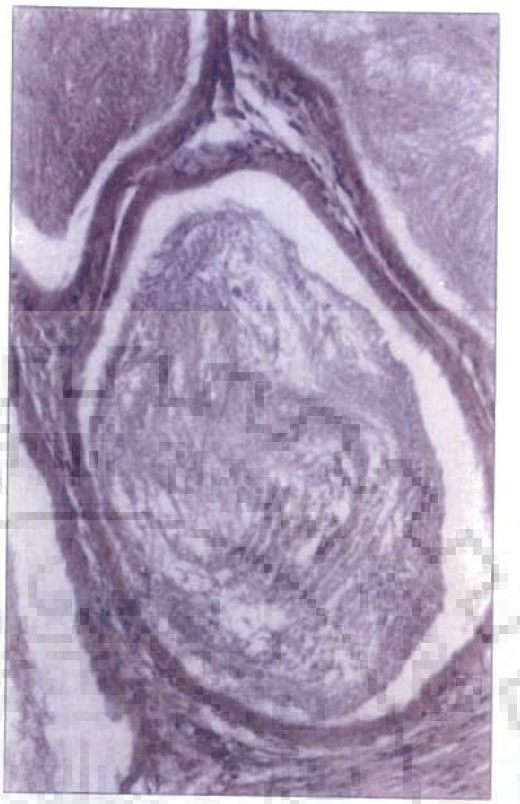
Microphotograph showing various degenerative changes in the epithelium. Lumen with less number of sperm.

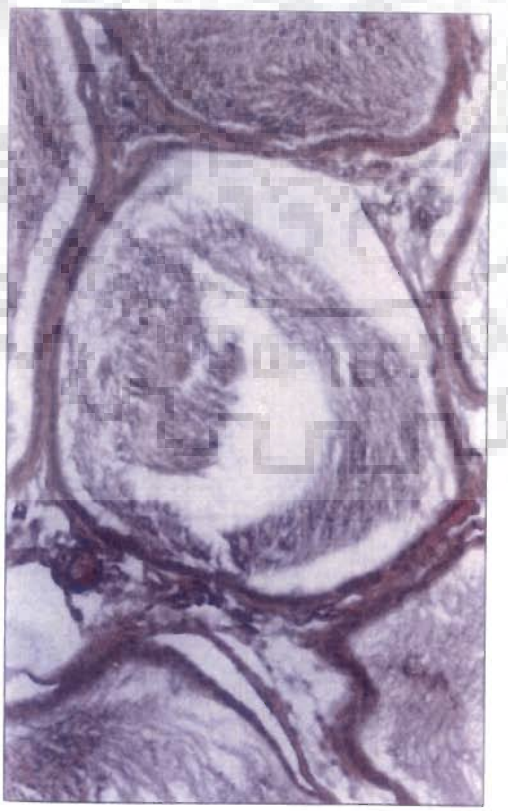
(H & E 100 X)

8



9





10

PLATE – XXI

CAUDA EPIDIDYMIS

Fig.11 Monocrotophos 14 mg./kg. b.wt./ day for 2 Weeks

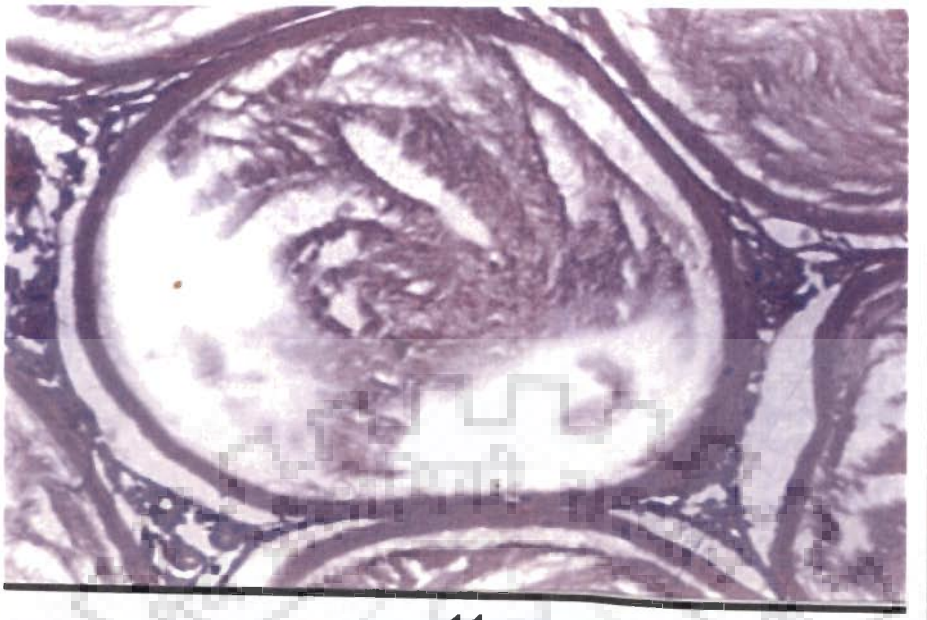
Microphotograph of cauda epididymis showing tubules with marginal changes in epithelium and short stereocilia. Intertubular stroma is disrupted. Lumen contains spermatozoa with some cellular debris.

(H & E 100 X)

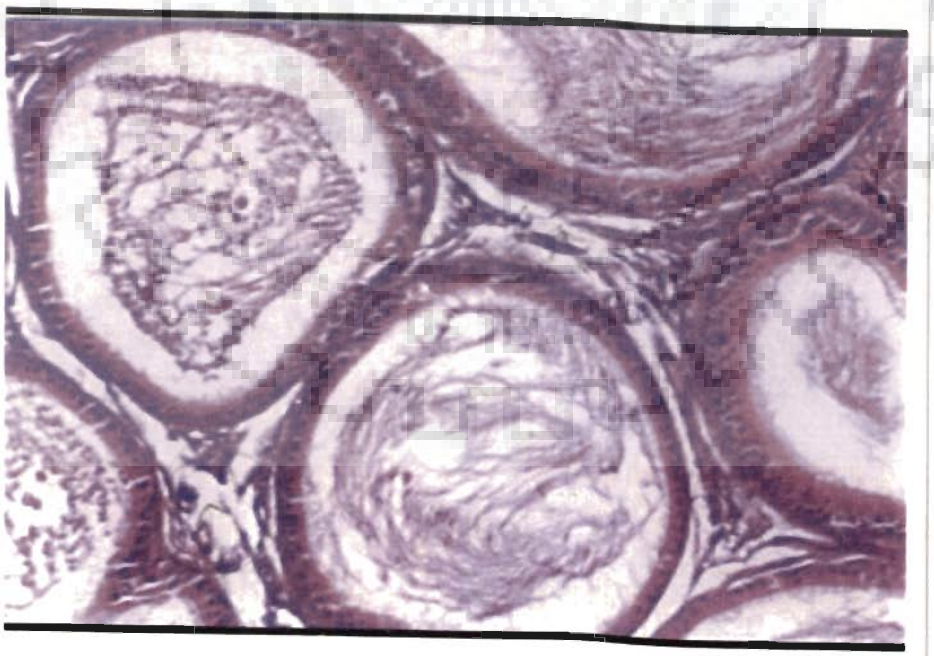
Fig.12 Monocrotophos 14 mg./kg. b.wt./ day for 5 Weeks

Microphotograph of cauda epididymis showing reduced tubule with highly damaged epithelium, disrupted interstitial stroma and lumen with less spermatozoa.

(H & E 100 X)



11



12

PLATE- XXII

CAUDA EPIDIDYMIS

Fig.13 Monocrotophos 22 mg./kg. b.wt./ day for 2 Weeks

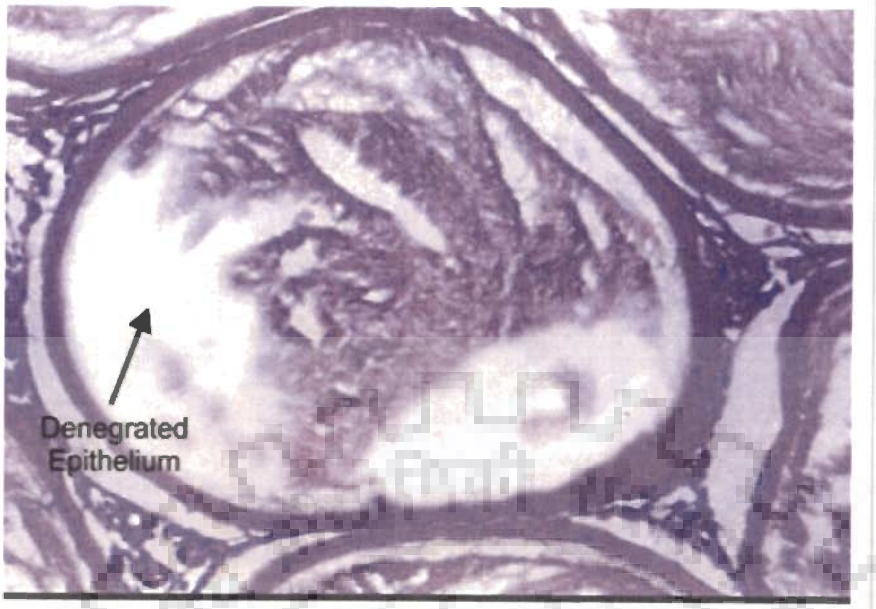
Microphotograph showing tubule with thin and degenerated epithelium. Diffused stereocilia are also seen. Increased intertubular space with disrupted stroma.

(H & E 100 X)

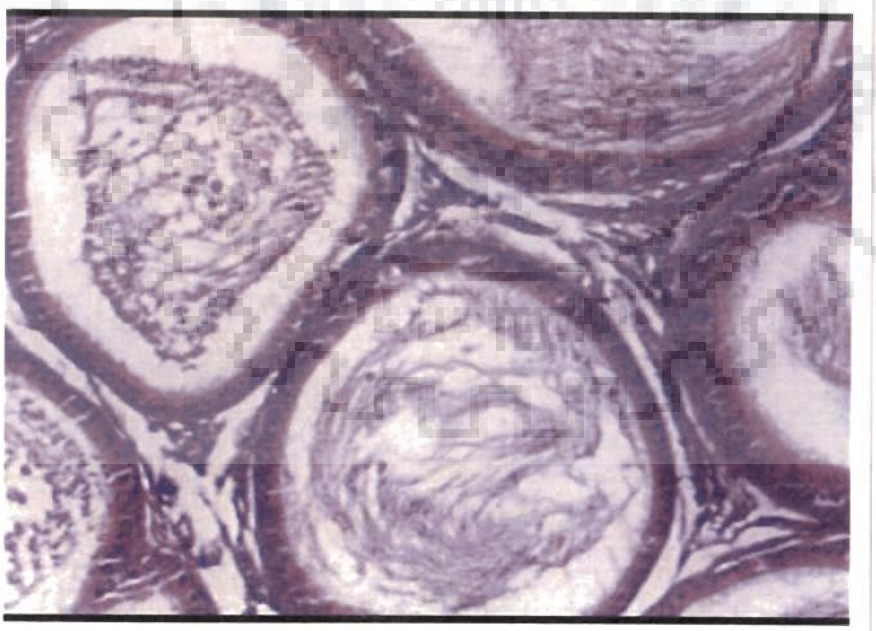
Fig.14 Monocrotophos 22 mg. /kg. b.wt./ day for 5 Weeks

Irregular and highly damaged tubules of cauda epididymis exhibits degererated epithelium without stereocilia. Cluster of spermatozoa are present mostly in the center of lumen. Intertubular stroma is disrupted.

(H & E 100 X)



13



14

PLATE XXIII

CAPUT EPIDIDYMIS

Fig.15 Control

Caput epididymis of control rat showing tubule with columnar epithelial cell lining and long prominent stereocilia projected towards lumen. Lumen is filled with spermatozoa, connective tissue and blood vessels could be seen in the intertubular spaces.

(H & E 200 X)

Fig.16 Monocrotophos 6 mg./kg. b.wt./ day for 2 Weeks

Microphotograph of caput epididymis showing damaged epithelium with fused stereocilia. Lumen contain sperm with debris.

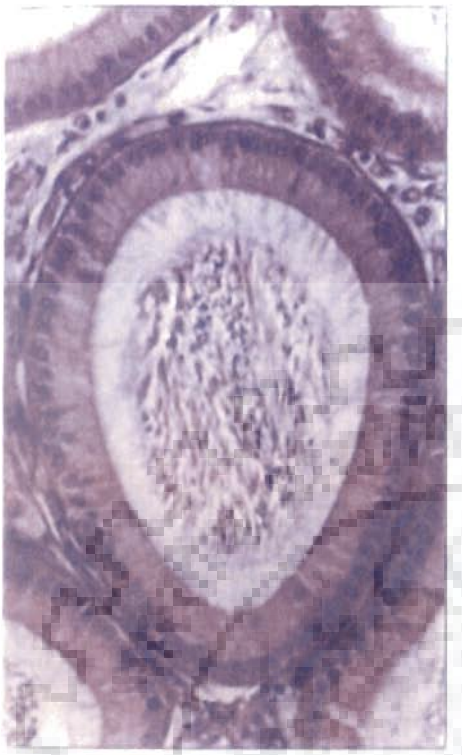
(H & E 200 X)

Fig.17 Monocrotophos 6 mg./kg. b.wt./ day for 5 Weeks

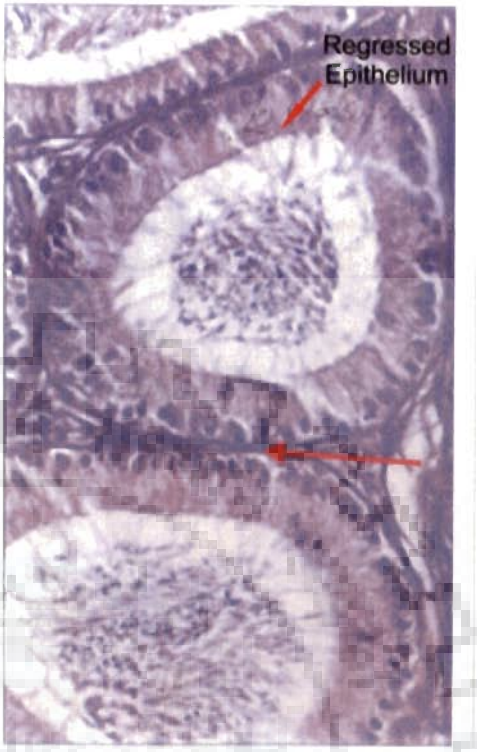
Microphotograph showing tubule with degenerated epithelium and short stereocilia, reduced spermatozoa and cellular debris are present in the Lumen.

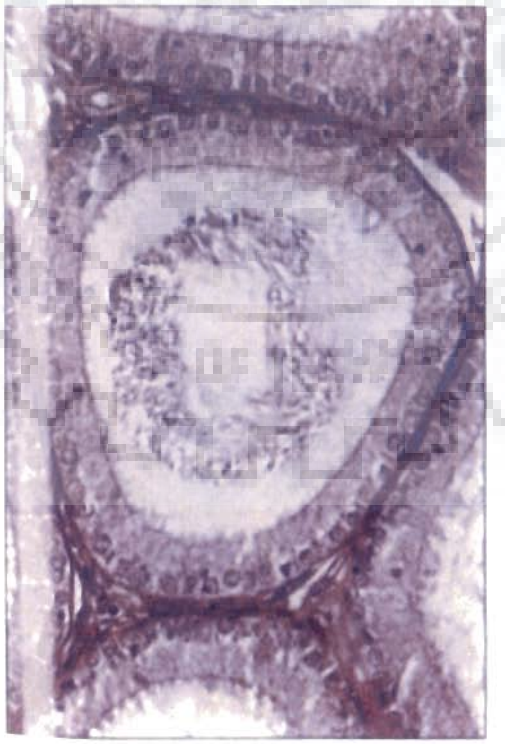
(H & E 200 X)

15



16





17

PLATE- XXIV

CAPUT EPIDIDYMIS

Fig. 18 Monocrotophos 14 mg/kg.b.wt./ day for 2 Weeks

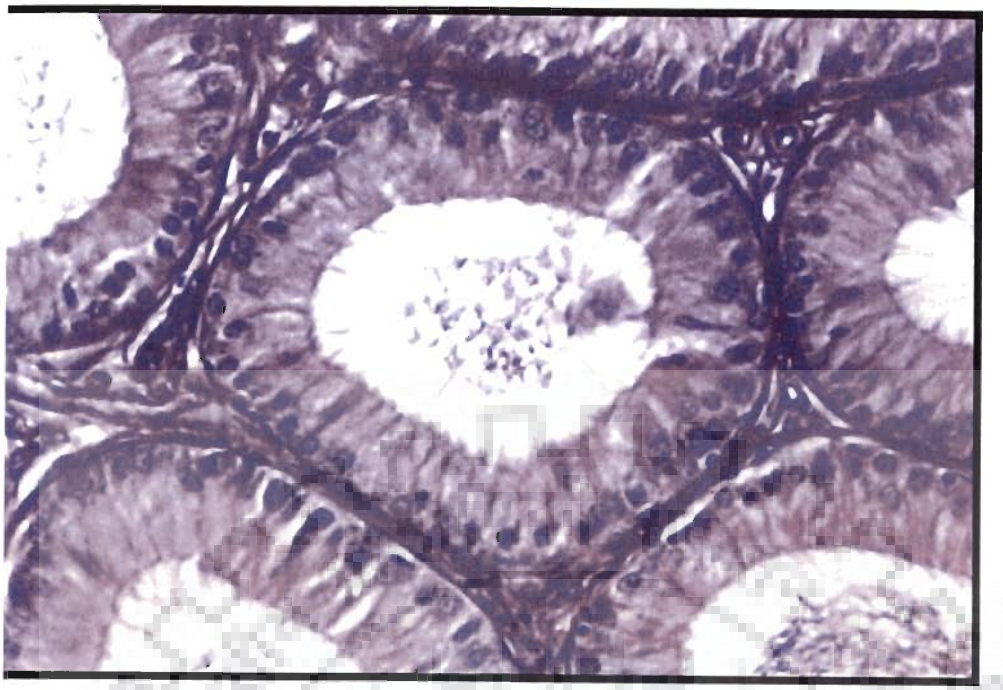
Microphotograph showing tubules of reduced size and thickening of epithelium. Fused stereocilia and cellular debris are also visible in lumen.

(H & E 200 X)

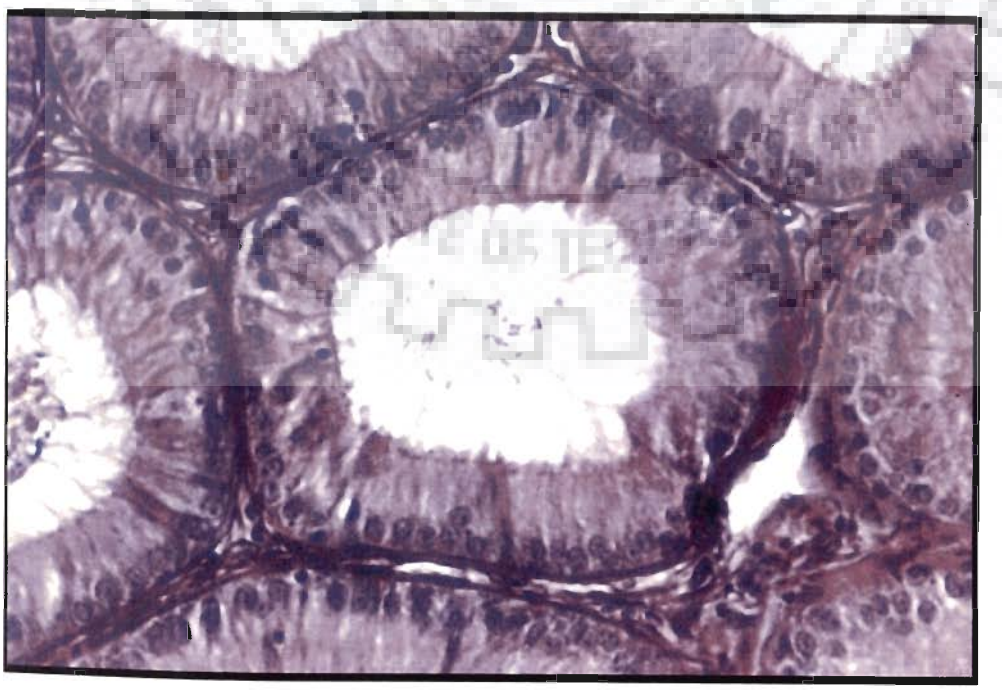
Fig. 19 Monocrotophos 14 mg/kg.b.wt./ day for 5 Weeks.

Photograph of caput epididymis showing reduction in lumen size and thickened epithelium with fused stereocilia. Lumen contained little debris.

(H & E 200 X)



18



19

PLATE- XXV

CAPUT EPIDIDYMIS

Fig.20 Monocrotophos 22 mg/kg.b.wt./ day for 2 Weeks.

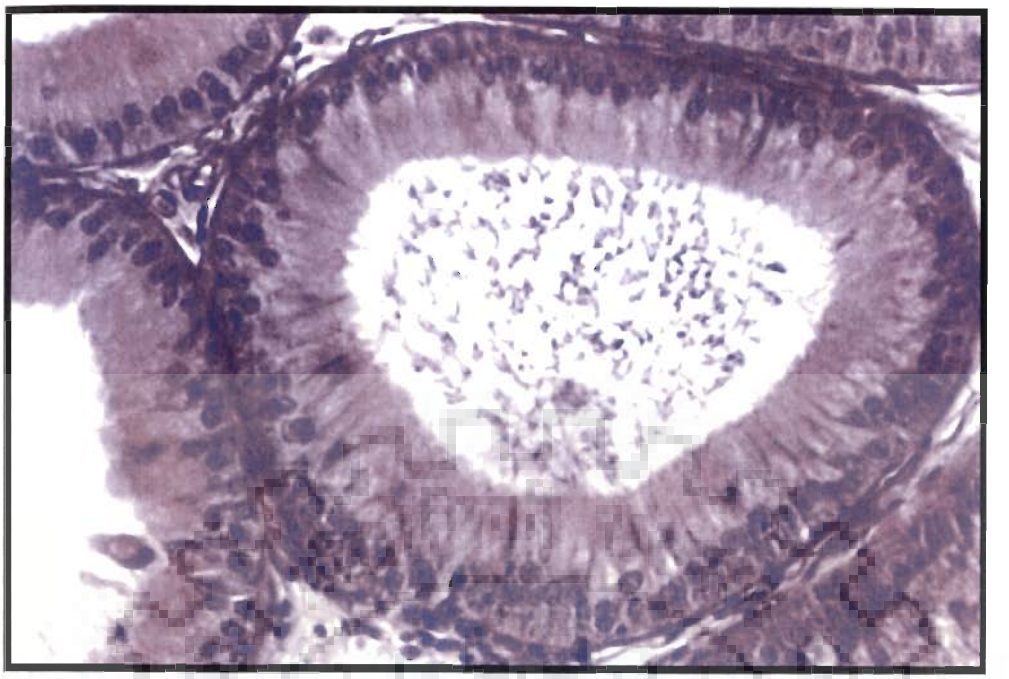
Microphotograph showing reduced lumen size of the tubule and thickened epithelium. Scanty sperm along with cellular debris are present in lumen.

(H & E 200 X)

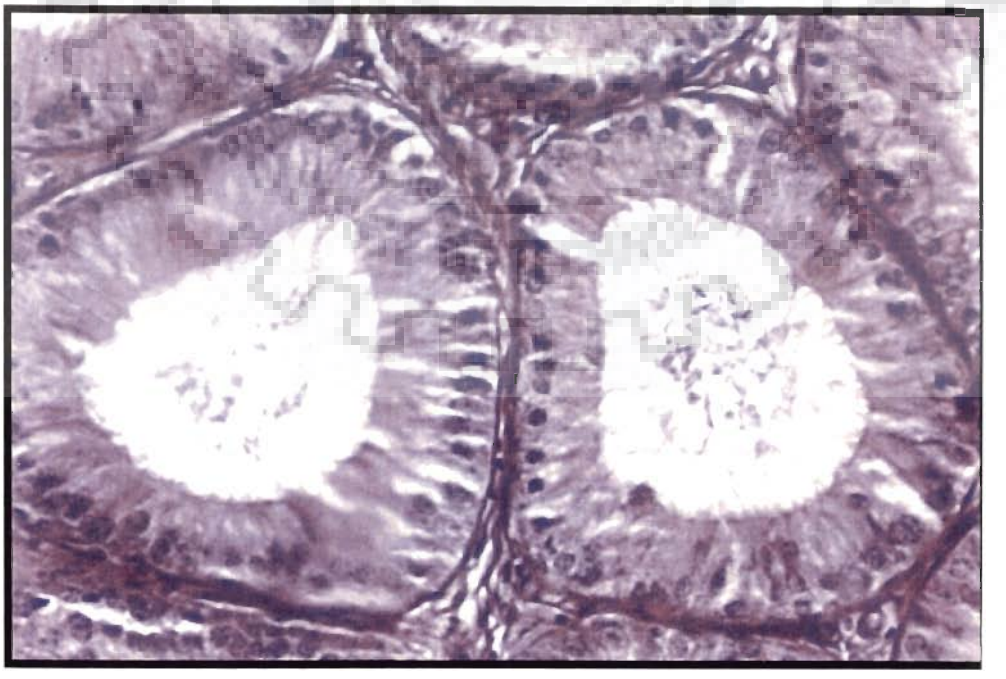
Fig.21 Monocrotophos 22 mg/kg.b.wt./ day for 5 Weeks.

Caput epididymis showing thick but highly damaged epithelial layer of tubules with fused stereocilila. Lumen contain only debris.

(H & E 200 X)



20



21

PALTE – XXVI

SEMINAL VESICLE

Fig.22 Control

Microphotograph of seminal vesicle from vehicle treated control rat showing basement membrane consisting of inner circular and outer longitudinal muscle fibres. Mucosal folds of columnar epithelial cells are extended in to the lumen.

(H & E 100 X)

Fig.23 Monocrotophos 6 mg/kg.b.wt./ day for 2 Weeks.

Seminal vesicle showing degenereated epithelial cells. Little secretion is present in the lumen. Muscular layer is intact.

(H & E 100 X)

Fig.24 Monocrotophos 6 mg/kg.b.wt./ day for 5 Weeks.

Microphotograph showing disorganized and deformed columnar epithelial cells. Muscular layer is vacuolated. Scanty secretion could be seen.

(H & E 100 X)

22

23

24

PLATE – XXVII

SEMINAL VESICLE

Fig. 25 Monocrotophos 14 mg/kg.b.wt./ day for 2 Weeks

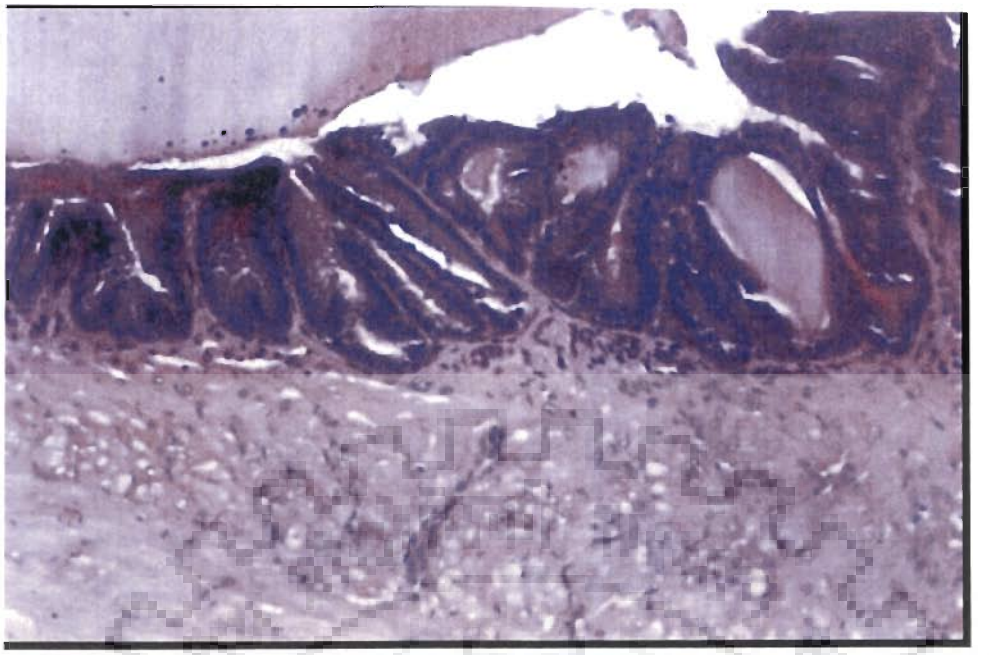
Microphotograph showing degenerated epithelium and vacuolated muscular layer. Scanty secretion could be seen.

(H & E 100 X)

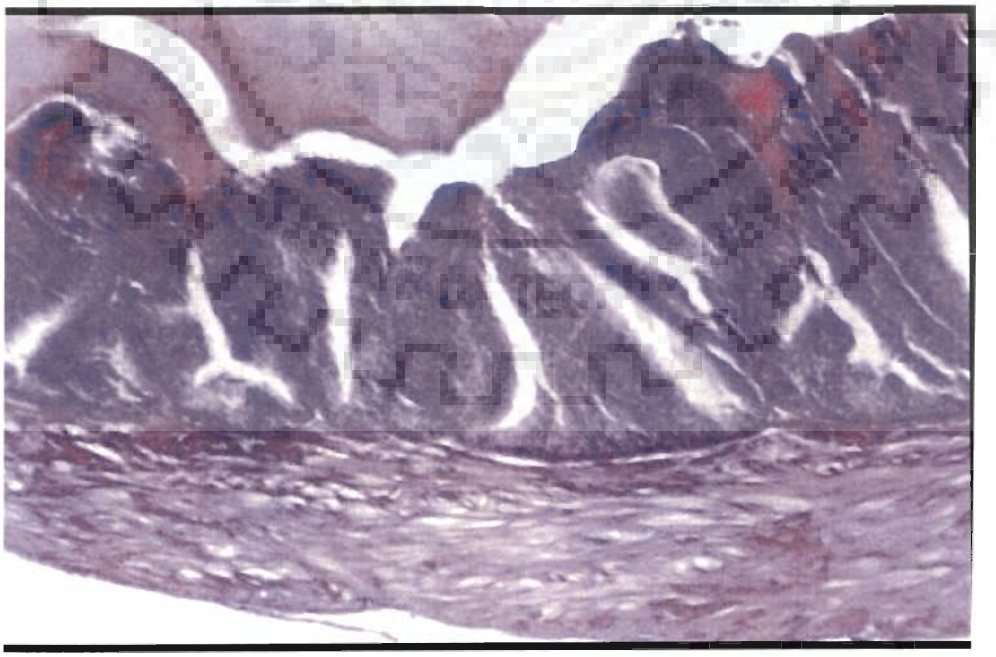
Fig. 26 Monocrotophos 14 mg/kg.b.wt./ day for 5 Weeks

Seminal vesicle showing disorganized secretory epithelium, degenerated muscular layer and lumen with less secretion.

(H & E 100 X)



25



26

PLATE XXVIII

SEMINAL VESICLE

Fig.27 Monocrotophos 22 mg/kg.b.wt./ day for 2 Weeks

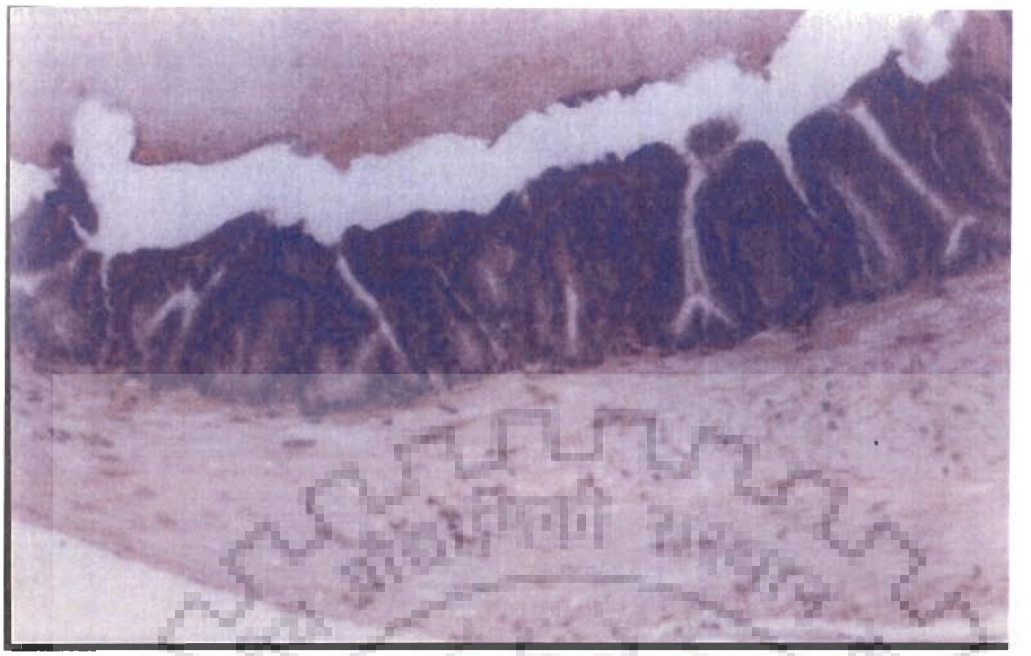
Microphotograph of seminal vesicle showing disorganized epithelial cell layer and muscle. Scanty secretion could be seen.

(H & E 100 X)

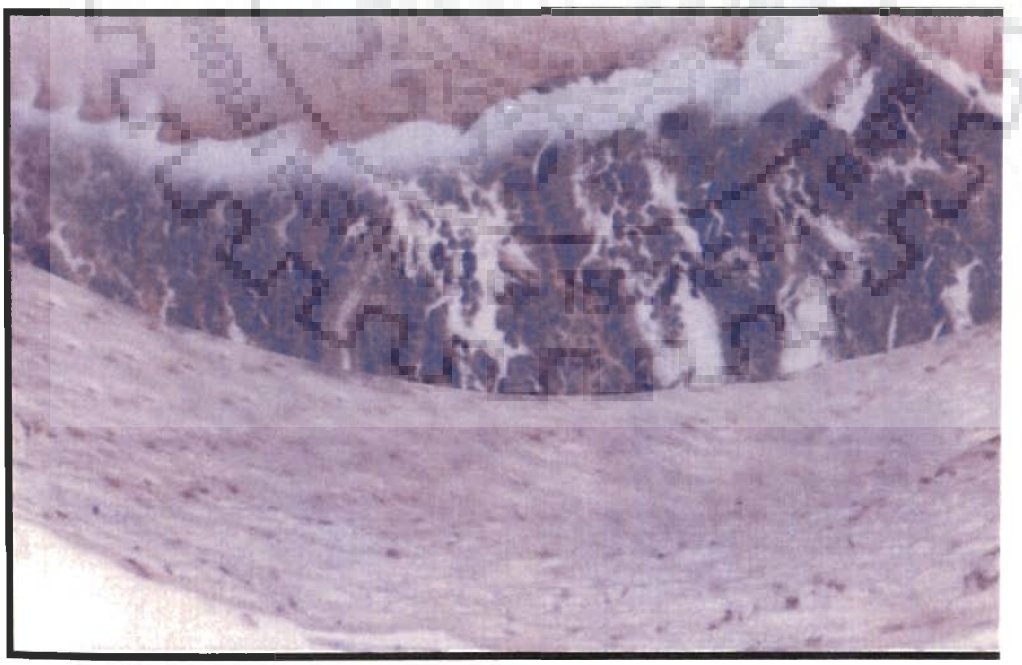
Fig. 28 Monocrotophos 22 mg/kg.b.wt./ day for 5 Weeks.

Microphotograph showing ruptured secretory epithelium started detaching from muscular layer. Scanty or almost no secretion in lumen.

(H & E 100 X)



27



28

PLATE – XXIX

VENTRAL PROSTATE

Fig. 29 Control

Microphotograph of control rat prostate showing alveoli with well developed epithelium. Lumen is full of eosinophilic secretion. Intertubular spaces are filled with connective tissue and blood vessels.

(H & E 100 X)

Fig.30 Monocrotophos 6 mg/kg.b.wt./ day for 2 Weeks.

Microphotograph showing epithelium of varying height fused at some places. Lumen contains less secretion.

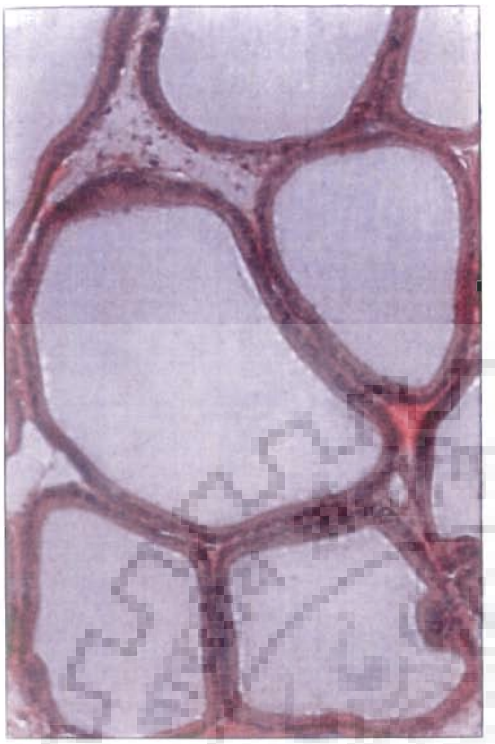
(H & E 100 X)

Fig.31 Monocrotophos 6 mg/kg.b.wt./ day for 5 Weeks.

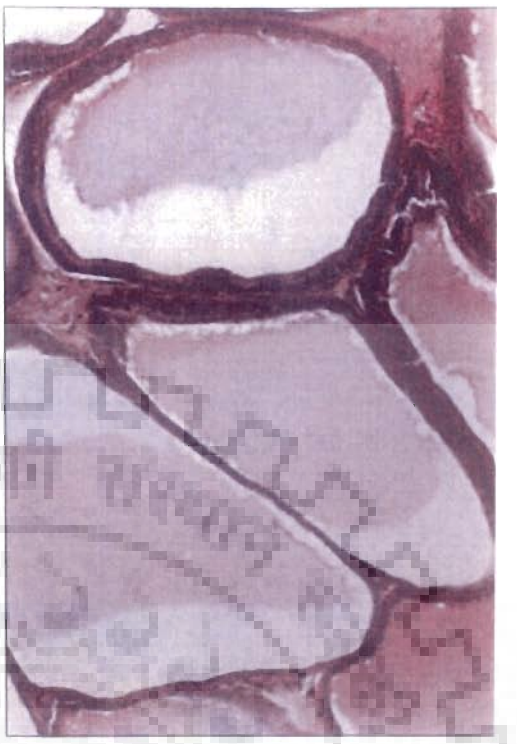
Photograph of prostate showing fused and folded epithelial lining of alveoli. Lumen with very little secretion.

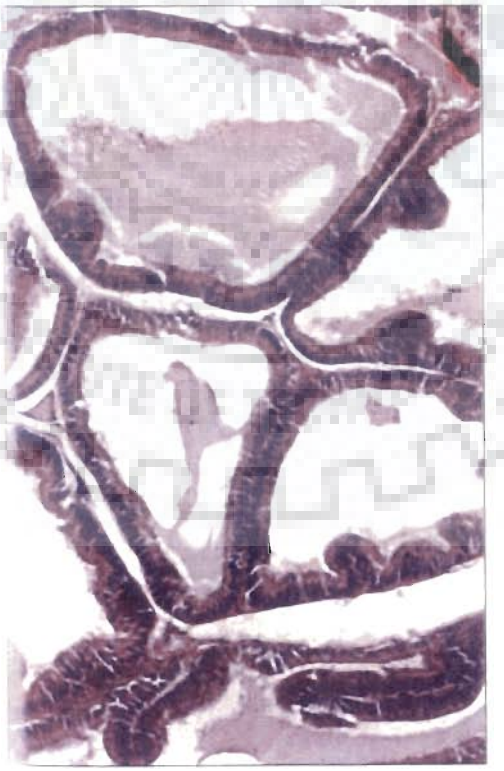
(H & E 100 X)

29



30





31

PALTE- XXX

VENTRAL PROSTAE

Fig. 32 Monocrotophos 14 mg/kg.b.wt./ day for 2 Weeks.

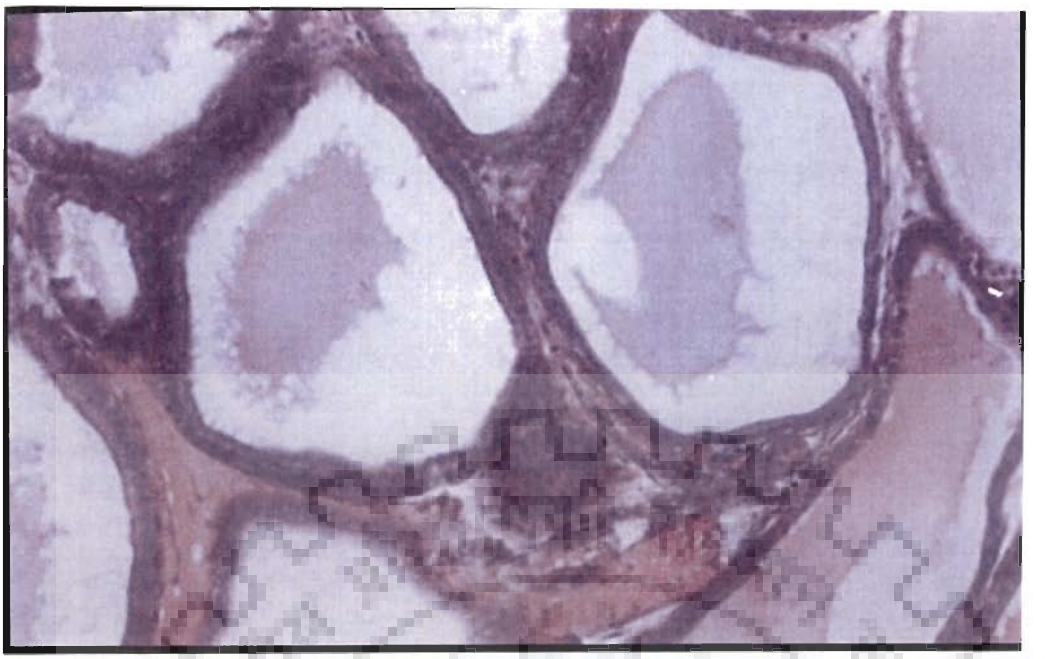
Microphotograph of prostate showing damaged and fused epithelial lining of alveoli with less secretion. Intertubular spaces are filled with degenerated stroma.

(H & E 100 X)

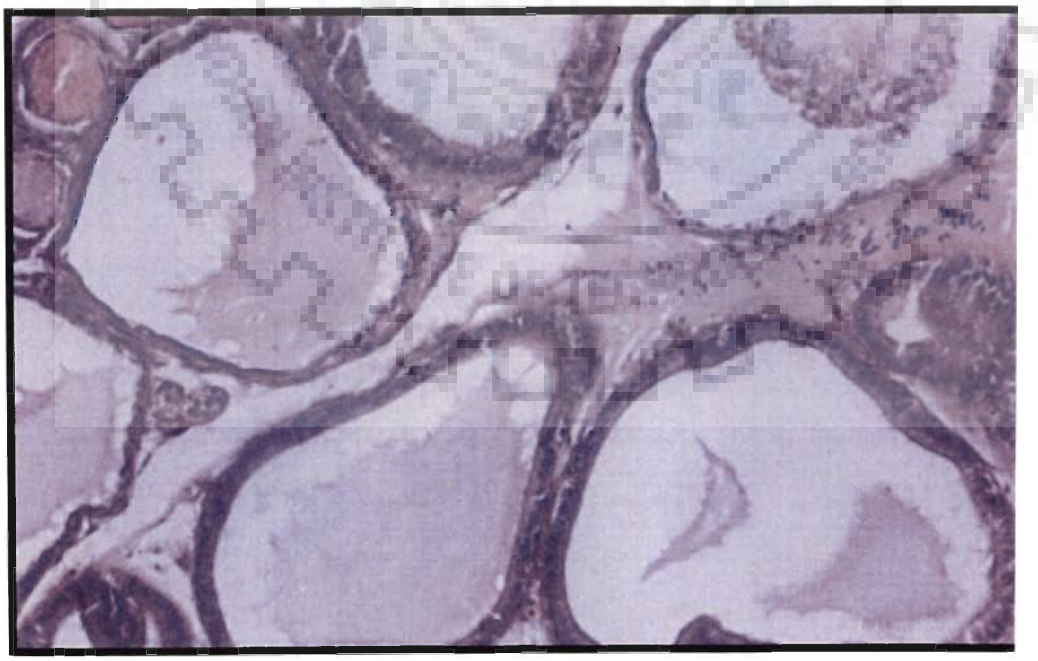
Fig. 33 Monocrotophos 14 mg/kg.b.wt./ day for 5 Weeks.

Microphotograph showing alveoli of reduced size. Degenerated stroma in increased interstitial spaces and little secretion in lumen are also visible.

(H & E 100 X)



32



33

PLATE XXXI

VENTRAL PROSTATE

Fig. 34 Monocrotophos 22 mg/kg.b.wt./ day for 2 Weeks

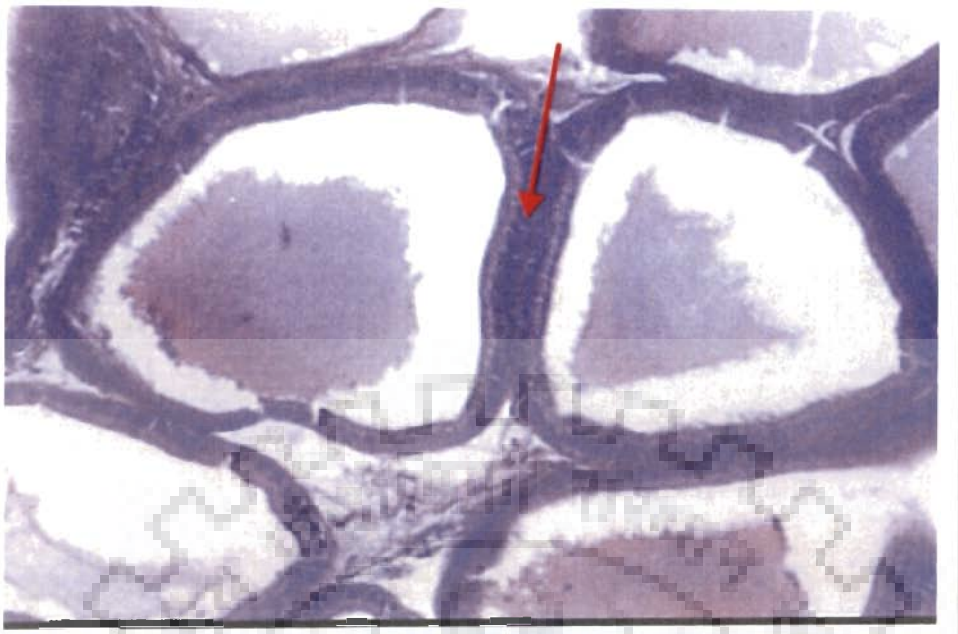
Microphotograph showing degenerated epithelium fused at several places(Shown by arrow). Cellular debris in intertubular stroma and less secretion in the lumen of alveoli could be seen.

(H & E 100 X)

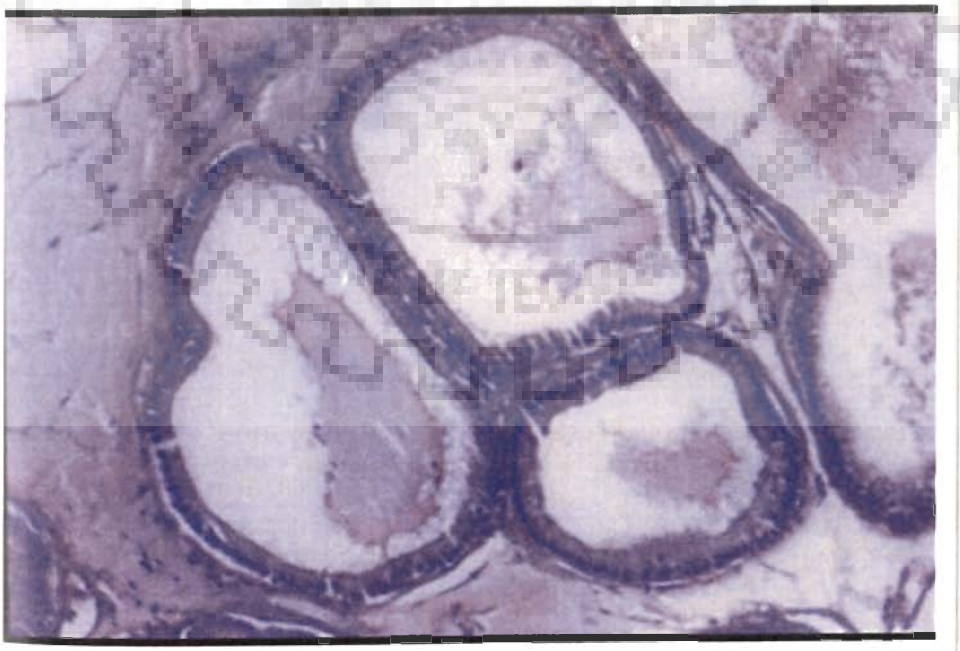
Fig. 35 Monocrotophos 22 mg/kg.b.wt./ day for 5 Weeks.

Photograph of prostate showing ruptured epithelial lining of alveoli. Intertubular stroma is highly damaged and lumen contains less secretion.

(H & E 100 X)



34



35

PLATE- XXXII

VAS DEFERENS

Fig.36 Control

Microphotograph of control rat showing vas deferens lined with columnar epithelium containing long stereocilia and surrounded by muscular coat of inner circular and outer longitudinal muscle fibre.

(H & E 100 X)

Fig.37 Monocrotophos 6 mg/kg.b.wt./ day for 2 Weeks.

Microphotograph showing slightly thickened epithelium and reduced lumen with debris. Muscular coat is detached at several places (shown by arrow).

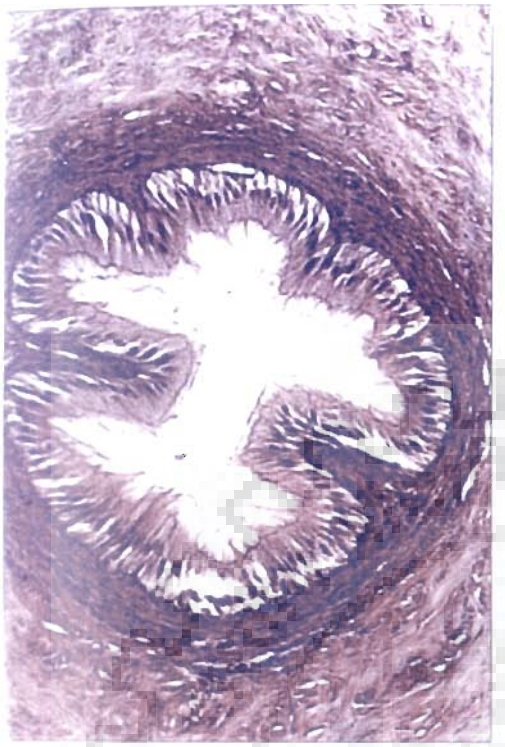
(H & E 100 X)

Fig.38 Monocrotophos 6 mg/kg.b.wt./ day for 5 Weeks.

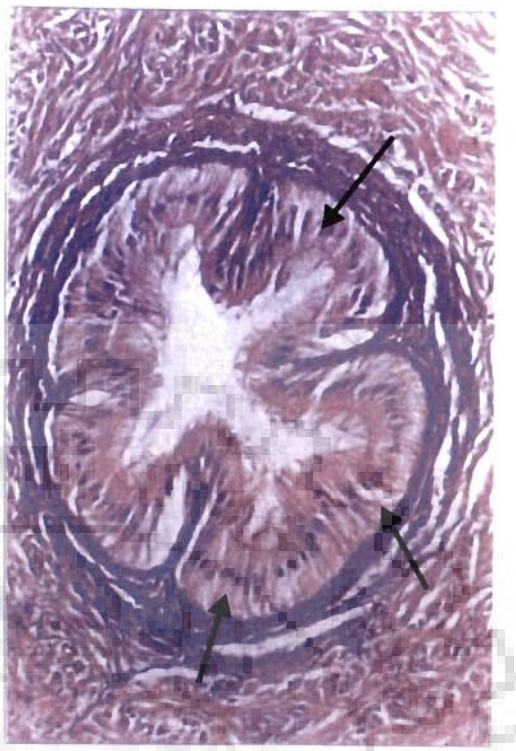
Microphotograph showing degenerated muscular coat with reduced epithelial folds. Lumen contains cellular debris.

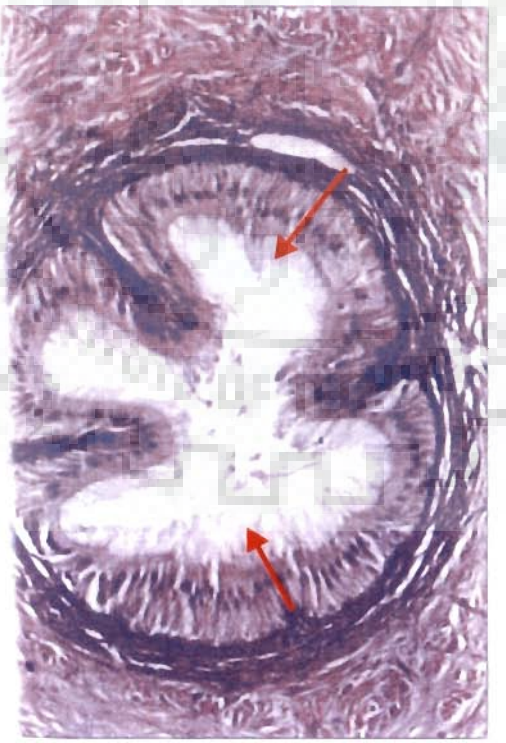
(H & E 100 X)

36



37





38

PLATE – XXXIII

VAS DEFERENS

Fig.39 Monocrotophos 14 mg/kg.b.wt./ day for 2 Weeks.

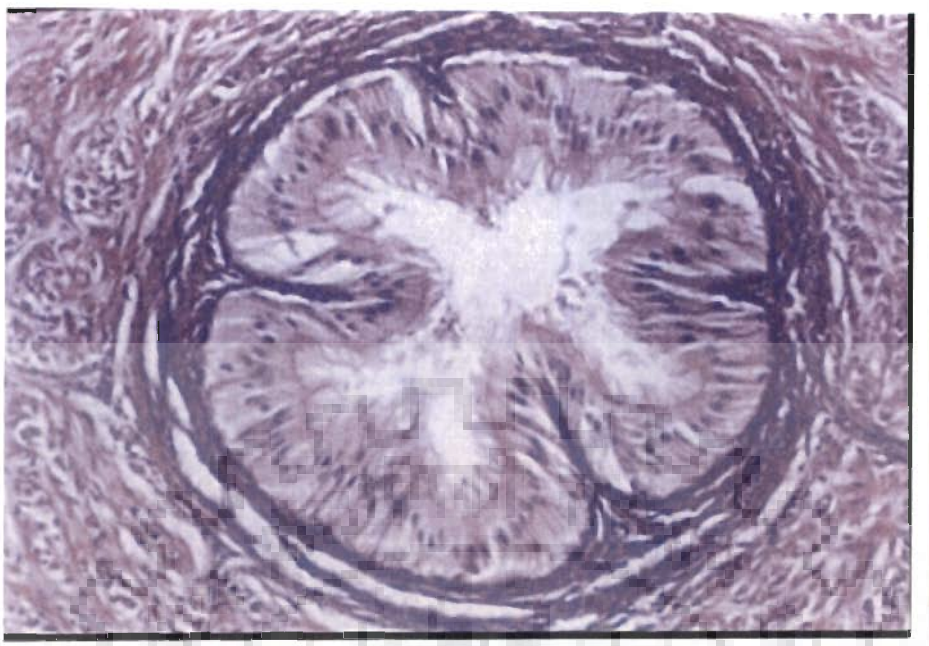
Microphotograph showing damaged and thickened epithelial cells with fused stereocilia. Muscular layer is disorganized and detached. Lumen could be seen with debris.

(H & E 100 X)

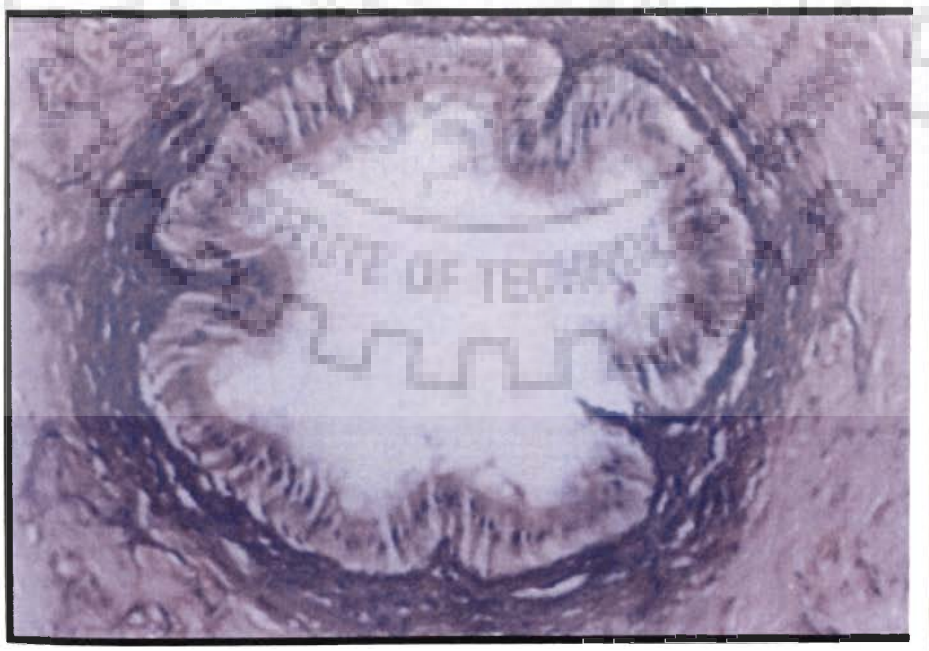
Fig.40 Monocrotophos 14 mg/kg.b.wt./ day for 5 Weeks.

Photograph of vas deference showing epithelium with short and fused stereocilia and wide lumen is devoid of spermatozoa.

(H & E 100 X)



39



40

PLATE – XXXIV

VAS DEFERENS

Fig. 41 Monocrotophos 22 mg/kg.b.wt./ day for 2 Weeks.

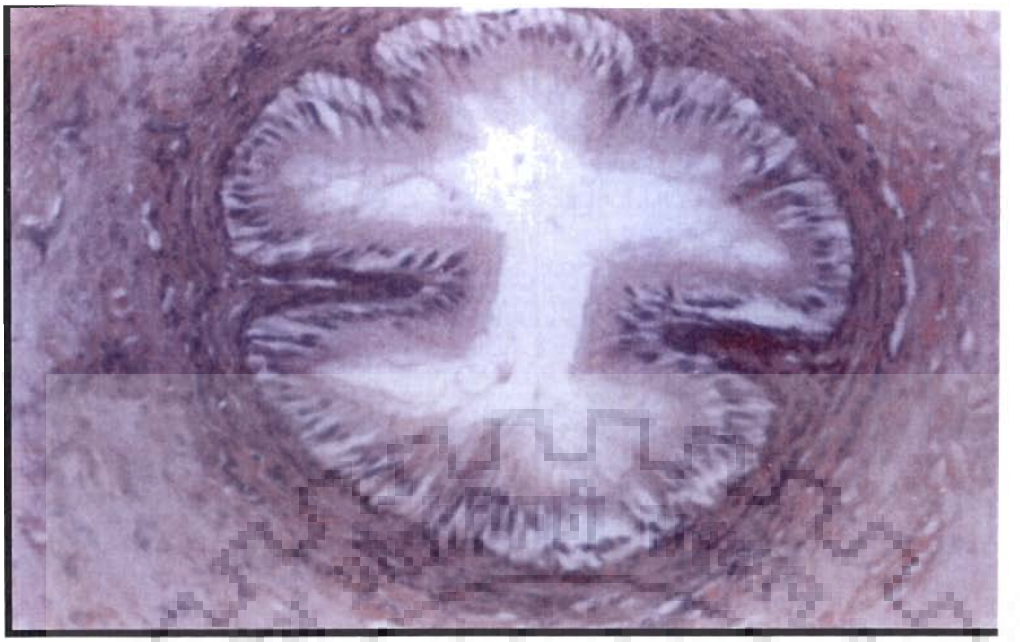
Microphotograph showing degenerated epithelium with fused stereocilia. Muscular coat is intact and lumen is almost empty.

(H & E 100 X)

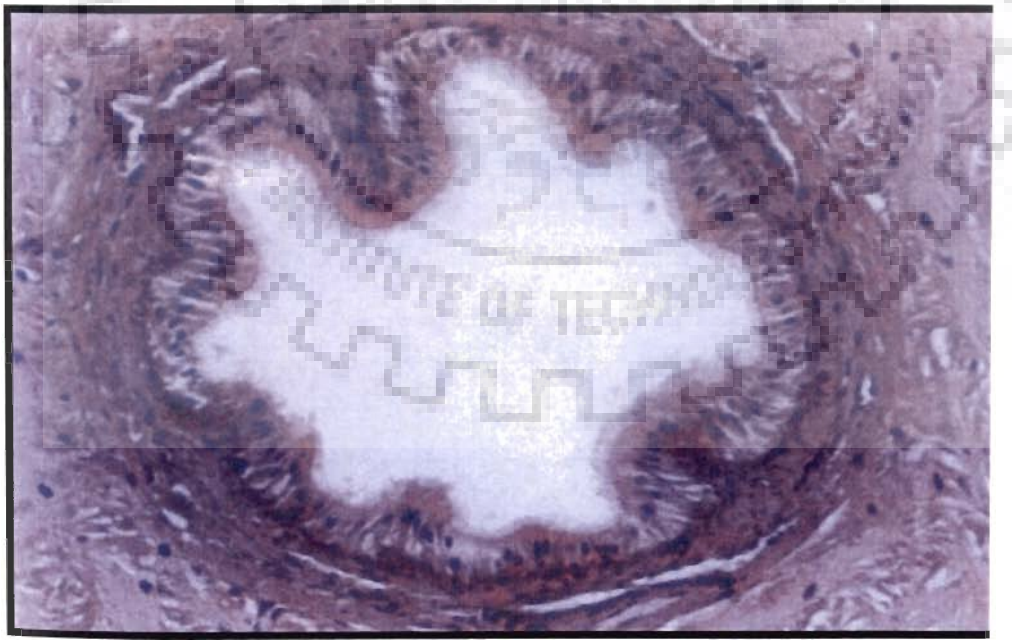
Fig.42 Monocrotophos 22 mg/kg.b.wt./ day for 5 Weeks.

Vas deferens showing reduced folds of epithelial lining and few stereocilia.

(H & E 100 X)



41



42

PLATE – XXXV

LIVER

Fig.43 Control

Microphotograph of control rat liver showing hepatic lobule with a central vein and hepatic sinusoids radiating from the central vein. Small nucleated endothelial cells are present in the sinusoids.

(H & E 100)

Fig.44 Monocrotophos 6 mg/kg.b.wt./ day for 2 Weeks.

Microphotograph showing dilation of sinusoidal spaces and degeneration of hepatocytes to some extent.

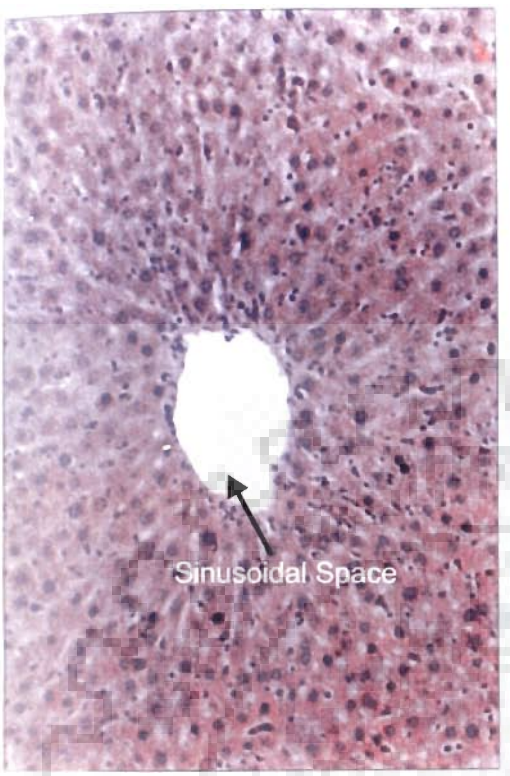
(H & E 100 X)

Fig.45 Monocrotophos 6 mg/kg.b.wt./ day for 5 Weeks.

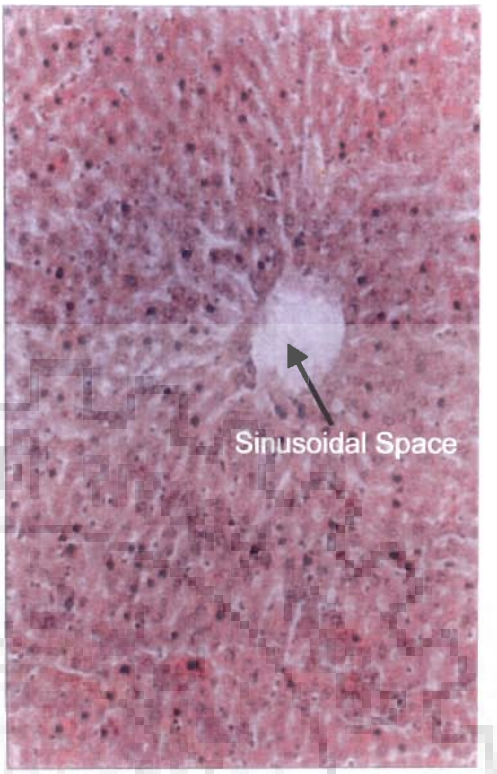
Photograph of liver showing reduction in the size of central vein. Binucleated and multinucleated hepatocytes with various nuclear aberrations are seen.

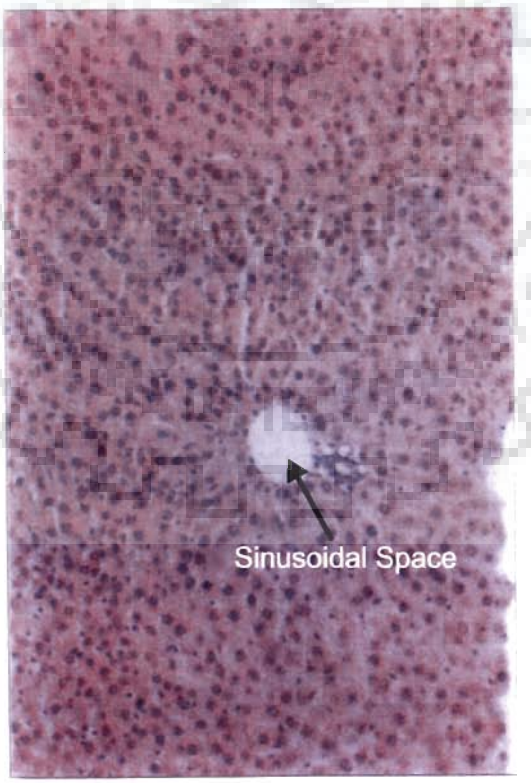
(H & E 100 X)

43



44





45

PLATE XXXVI

LIVER

Fig.46 Monocrotophos 14 mg/kg.b.wt./ day for 2 Weeks.

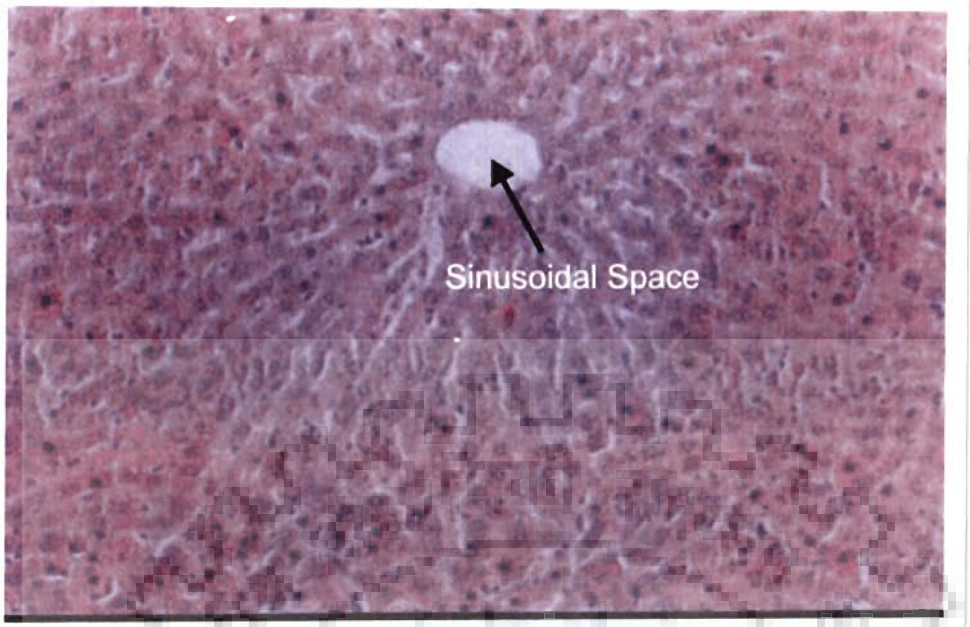
Microphotograph showing greater sinusoidal space dilation along with various nuclear aberrations.

(H & E 100 X)

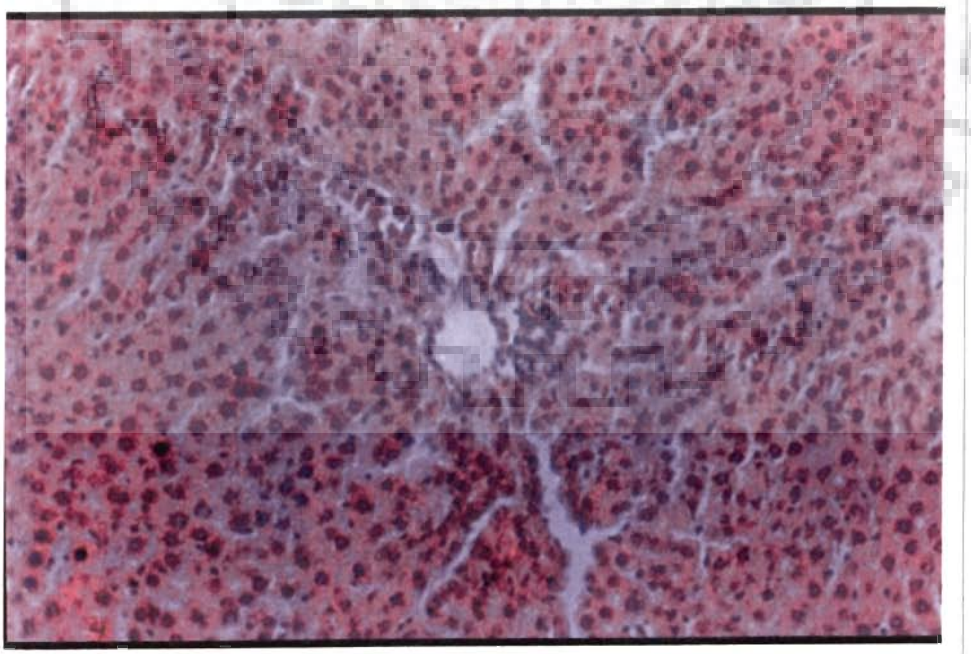
Fig.47 Monocrotophos 14 mg/kg.b.wt./ day for 5 Weeks.

Microphotograph of liver showing disorganized hepatocytes and widened sinusoidal space. Nuclear aberrations are also conspicuous.

(H & E 100 X)



46



47

PLATE XXXVII

LIVER

Fig. 48 Monocrotophos 22 mg/kg.b.wt./ day for 2 Weeks.

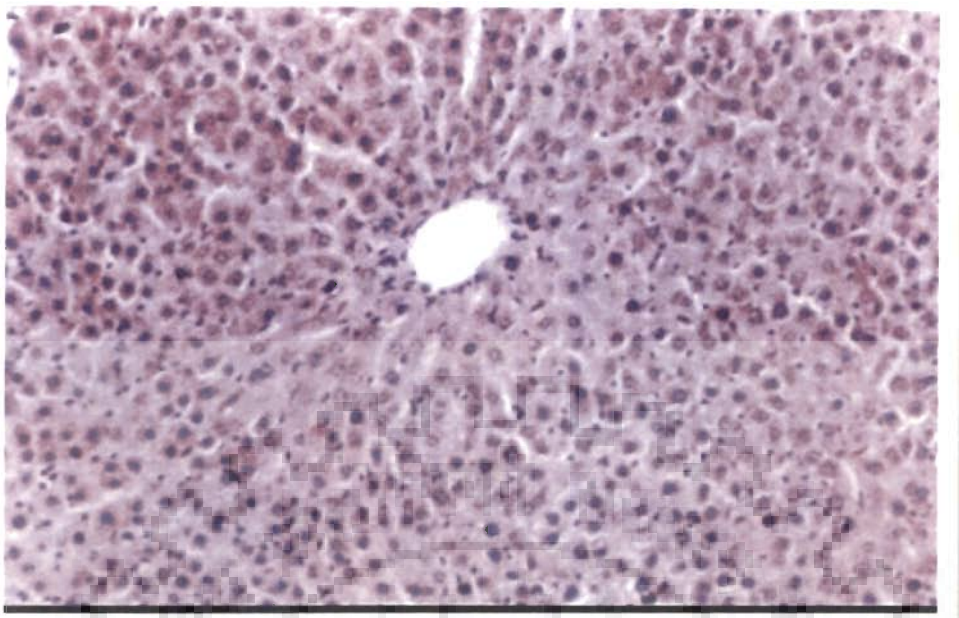
Microphotograph showing hepatocytic degeneration, vacuolization of sinusoids to some extent and presence of deformed nucler in endothelium.

(H & E 100 X)

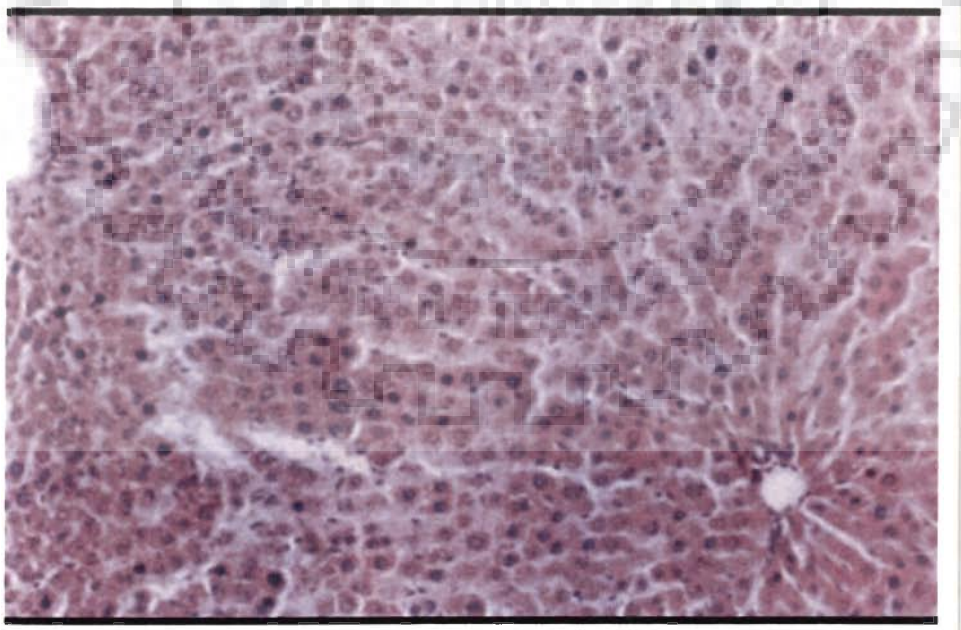
Fig.49 Monocrotophos 22 mg/kg.b.wt./ day for 5 Weeks.

Microphotograph of liver showing binucleated hepatocytes with extensive vacuolization. Lymphacytic infiltration around central vein could be seen.

(H & E 100 X)



48



49

PLATE XXXVIII

KIDNEY

Fig.50 Control

Microphotograph of control rat kidney showing Bowman's capsule with parietal and visceral layers separated by capsular space. Distal and proximal convoluted tubule are also visible along with collecting tubule.

(H & E 100 X)

Fig.51 Monocrotophos 6 mg/kg.b.wt./ day for 2 Weeks.

Microphotograph showing degenerated glomerulus and capsular space filled with degenerating cells. Cytoplasm of epithelium is granular and vacuolated.

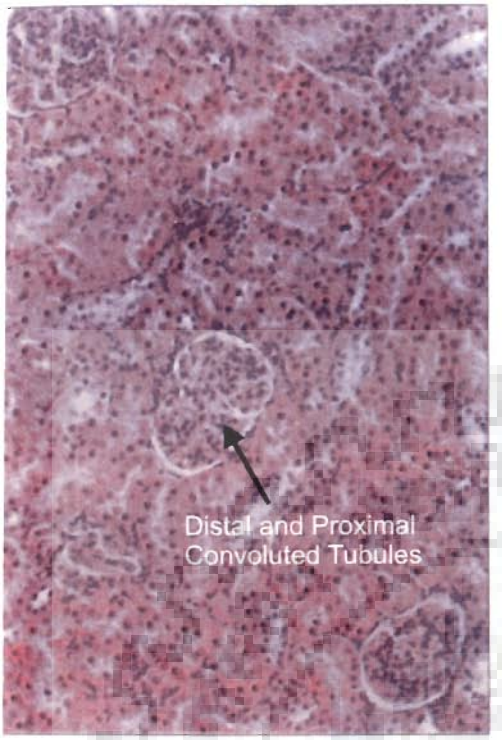
(H & E 100 X)

Fig.52 Monocrotophos 6 mg/kg.b.wt./ day for 5 Weeks.

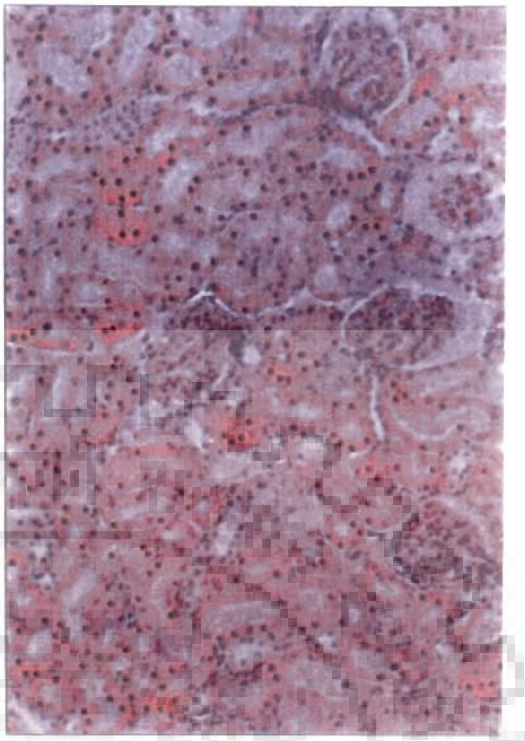
Kidney photograph showing vacuolization of collecting tubule. Damaged distal and proximal convoluted tubules with cellular debris are visible.

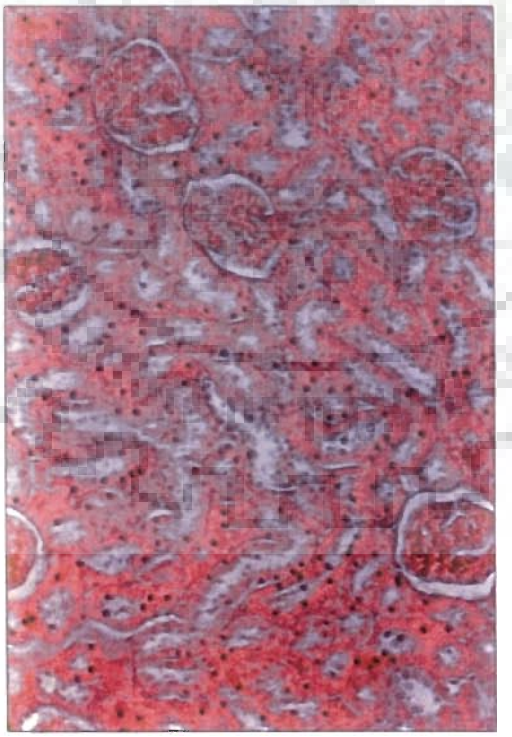
(H & E 100 X)

50



51





52

PLATE – XXXIX

KIDNEY

Fig.53 Monocrotophos 14 mg/kg.b.wt./ day for 2 Weeks.

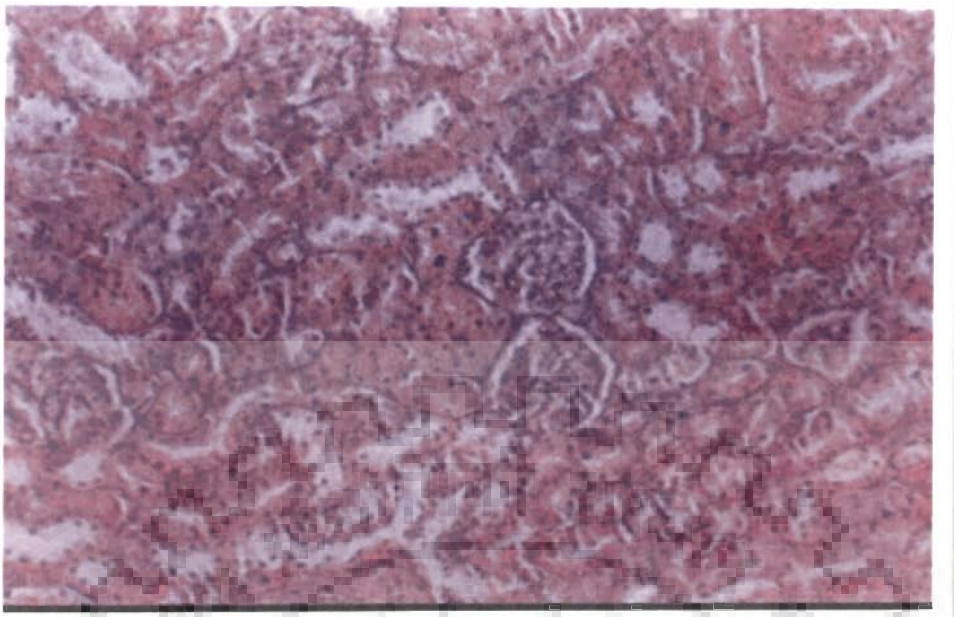
Microphotograph showing highly degenerated glomerular tufts. Convoluted tubules have thick wall and much reduced lumen.

(H & E 100 X)

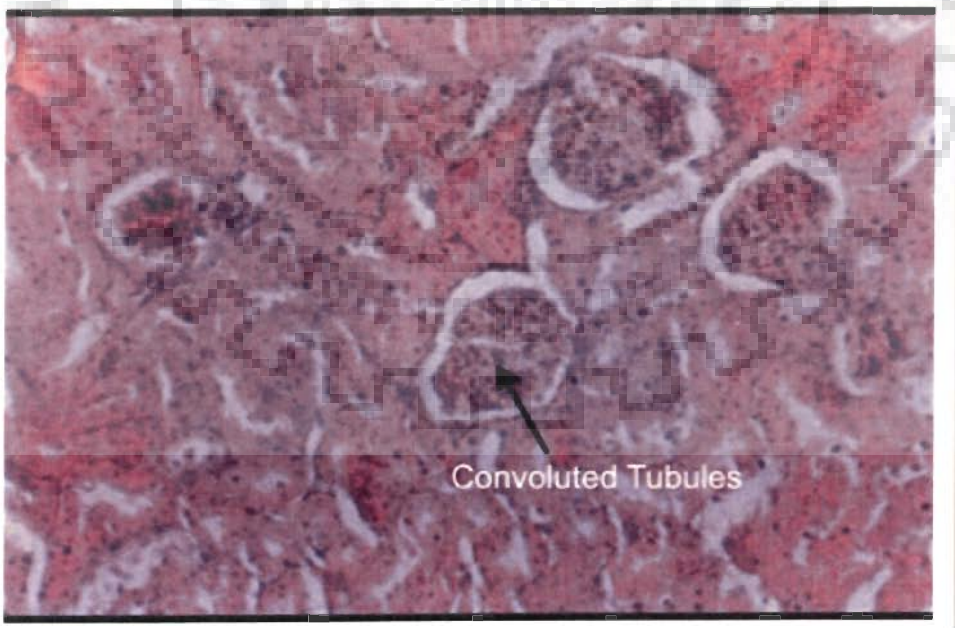
Fig.54 Monocrotophos 14 mg/kg.b.wt./ day for 5 Weeks.

Microphotograph of kidney showing sclerosed glomerulus with greatly reduced cellularity and increased capsular space. Convoluted tubule has very much reduced lumen.

(H & E 100 X)



53



54

PLATE – XXXX

KIDNEY

Fig.55 Monocrotophos 22 mg/kg.b.wt./ day for 2 Weeks.

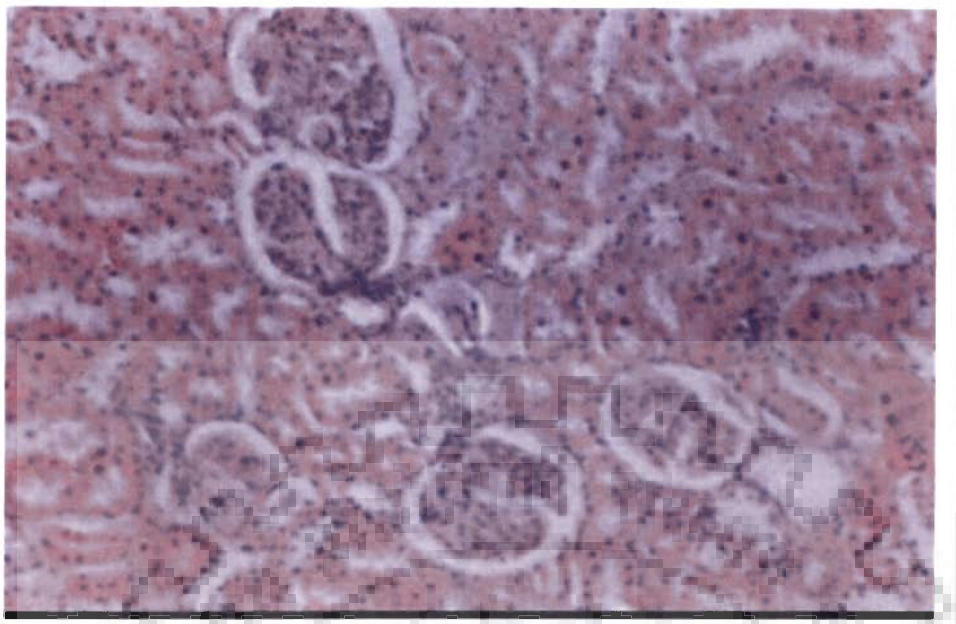
Microphotograph showing deforamed glomerular tufts with increased capsular space. Epithelium is also damaged and vacuolated.

(H & E 100 X)

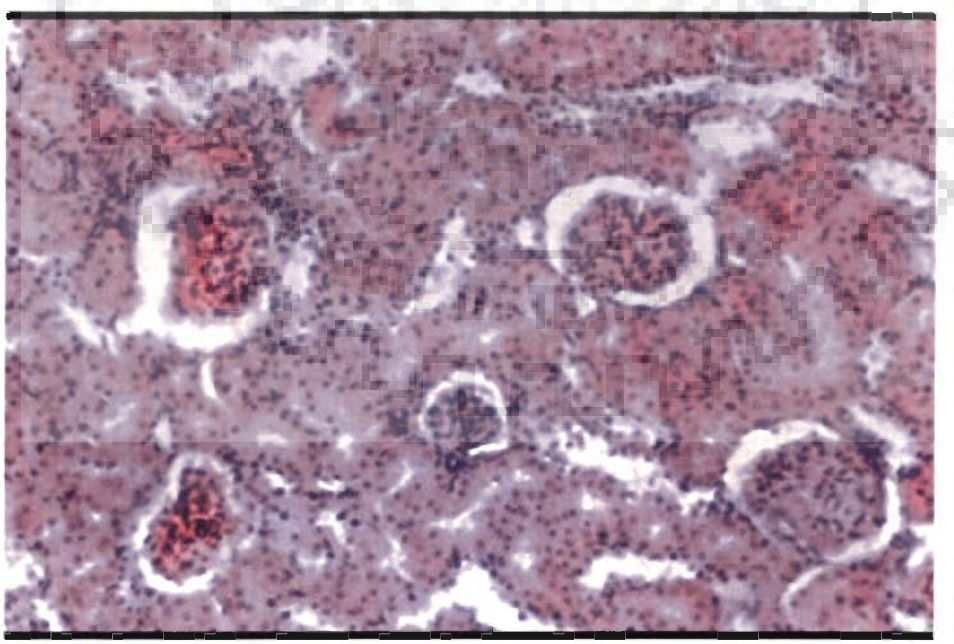
Fig.56 Monocrotophos 22 mg/kg.b.wt./ day for 5 Weeks.

Microphotograph showing sclerosed and reduced glomerulus. Increased capsular space and vacuolar degeneration of tubular epithelium could be seen.

(H & E 100 X)



55



56

4.3 Result of Hershberger Assay:

(Anti-) androgenic effect of chlordane and monocrotophos in castrated male rats:

4.3.1 Androgen dependent accessory sex organ weights:

The weight of sex organs was affected. Weight of seminal vesicle was significantly decreased in group treated with monocrotophos.There was significantly increased in prostate gland weight as compared to control. No significantly difference was observed in vas deference. There was significantly increased in epididymis in both treated group.

Table 11 : Body weight and reproductive organs weights.

Treatment	Body weight gain (gm)	Vas deference (gm)	Epididymis (gm)	Seminal Vesicle (gm)	Prostate Gland (gm)	Liver (gm)	Kidney (gm)	Adrenal Gland (gm)	Spleen (gm)
Control	97.5 ±4.2	0.2376 ±0.0096	1.328 ±0.021	0.457 ±0.189	0.166 ±0.036.	10.54 ±.462	1.047 ±0.026	0.03 ±0.001	0.783 ±0.022
Chlordane	107 ±4.7	0.18 ±0.007	1.08 ±0.136*	0.211 ±0.179	0.248 ±0.0018*	6.94 ±0.17	0.72 ±0.023	0.023 ±0.0008	0.640 ±0.014
Mono-crotophos	102 ±3.8	0.163 ±0.0097	0.729 ±0.006*	0.252 ±0.0057*	0.199 ±.005*	5.84 ±0.092*	0.47 ±0.001*	0.0144 ±0.0012	0.642 ±0.035

* Significantly different from vehicle/control at $p<0.05$

Values are mean ± SEM (n=5)

4.3.2 Liver, kidney, adrenal gland weight

Weight of kidney and liver was significantly decreased only in group treated with Monocrotophos.

Table 12 : Feed and water consumption

	Control	Chlordane	Monocrotophos
Feed consumption(g/g bw/day)	0.085 ±0.003	0.083 ±0.001	0.085 ±0.004
Water consumption ml/g bw/day	0.157 ±0.019	0.152 ±0.015	0.140 ±.025

4.4 Result of courtship behavioral study:

Number of approaches were recorded for 60 minute for chlordane and monocrotophos administered rats at higher dose level (chlordane dose was 600mg/kg b.w. and monocrotophos dose was 22 mg/kg b.w.for 2 week and 5 week respectively). In our present study, after administration of insecticides like chlordane and monocrotophos for 2 weeks and 5 weeks respectively showed significantly decrease in libido at higher dose (4.4).

	Number of approaches after 2 week	Number of approaches after 5 week
Control	44	48
Chlordane	38*	21*
Monocrotophos	30**	16***

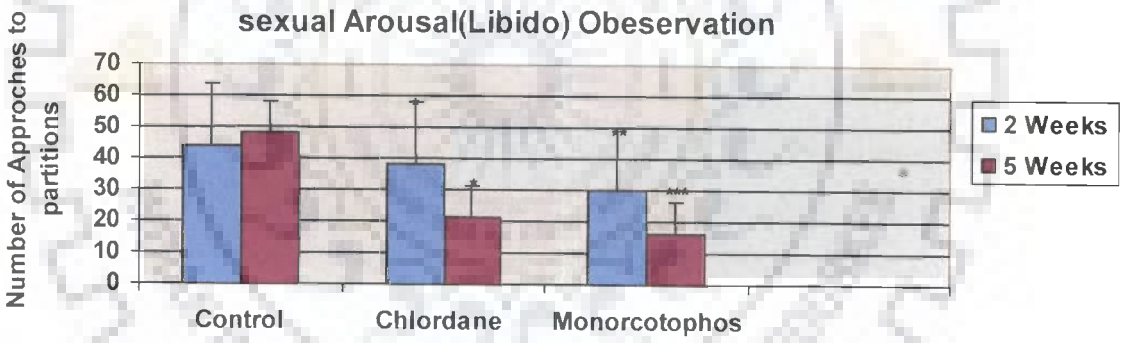


Figure: 4.24

Effect on sex arousal after 2 and 5 weeks administration of chlordane and monocrotophos treated rats.

CHAPTER – 5
DISCUSSION

The administration of chlordane and monocrotophos insecticides at various dose levels was studied and highlighted their toxic effects on :

1 Fertility and sperm dynamics.

2 Morphology and histopathology of male reproductive organs and vital organs.

3 Biochemical changes in the reproductive organs and vital organs.

4 Alteration in the blood and serum profile.

5 Serum testosterone level.

5.1 Weight response

Marked reduction in the weight of testis and accessory sex organs was observed after oral administration of chlordane and monocrotophos insecticides. The reduction in the testicular weight reflects regressive changes in semniferous tubules, which are associated with azoospermia and germinal aplasis. The change in testicular weight has also correspond to the presence or absence of post meiotic germ cells (Nelson and Patenelli, 1965).

Germinal cells and tubules account for approximately 98% of the testicular mass. A reduction in the number of germinal cells leads to reduction in the weight of testis (Sherins and Hawards, 1978.) Similar reduction in the testis weight was also observed. (Ansari et al, 1984, Naqvi and Vaishnavi, 1993, Sinha et al, 1995).

Reduction in the weight of accessory reproductive organs directly supports the reduced availability of androgens (Mills 1990, Mukherjee et atl. 1992, Patil et al. 1998). Further decrease in the sperm reserve appears to be a reasonable cause for reduction in weight of epididymides (Akbarshah et atl. 1995, Sarkar et al. 1997). Decrease in seminal vesicle and prostate gland weight after treatment reflects interference on testosterone output (Shaikh et al. 1993, Malarvizhi and Mathur, 1995).

5.2 Sperm dynamics

Sperm motility has always been considered as an important functional measurement to predict sperm fertilizing capacity (Aitken et al. 1984). The motility of sperms in cauda epididymides was reduced markedly after oral administration of chlordane and monocrotophos at various dose levels.

Sperm motility may be affected by altered enzymatic activities of oxidative phosphorolytic process. Oxidative phosphorylation is required for ATP production, a source of energy for the forward movement of spermatozoa. Full ATP pool is crucial for normal spermatozoal movement and a slight deprivation of ATP leads to reduction in motility which may cause decrease in sperm motility. This may further be enhanced by androgen deprivation effect of the drug. The epididymal spermatozoa are highly dependent on testosterone and epididymal protein for their final maturation and development of progressive motility and fertilizing capacity (Vawda and Davies, 1986). Gonadotropin-Releasing Hormone-Agonist Inhibits Synthesis of Nitric Oxide and Steroidogenesis by Luteal Cells in the Pregnant Rat (Hyunwon, Y et al, 2003).

5.3 Sperm density

In the present study, treatment with chlordane and monocrotophos caused a significant reduction in density of sperms in cauda epididymides as well as in testis. Reduction in sperm counts in testis may be due to altered gonadotrophins. Biologically active LH and FSH are necessary for normal sperm production, development and maturation in testis (Baker et al. 1975, Bastias et al. 1993). The suppression of gonadotrophins may decrease sperm density in testis (Reuber 1981, Sinha et al. 1995). Decline in sperm density in cauda epididymides may be due to alteration in androgen metabolism and or its biosynthesis. The physiological and biochemical integrity of epididymis are dependent on androgens (Brooks 1979). Evaluation of rat sperm by flow cytometry was carried out for the analysis of sperm count and viability (Yamamoto T et al. 1998).

5.4 Serum testosterone

In the present study, the reduction in the serum testosterone, at higher dosages clearly demonstrated the inhibitory effect of chlordane and monocrotophos like other insecticides on the secretion of pituitary gonadotrphins (FSH and LH) and in turn on the testosterone biosynthesis in the testis of rat (Belanger et al. 1980, Singh and Pandey 1990). The depletion of testosterone not only arrest spermatogenesis but this state leads to reduction of physical energy, sexual dysfunction and various metabolic process (Franchi et al. 1978, Horst et at. 1978). Alteration in testosterone level has also been reported due to endocrine disrupting activities by fungicides (Camilla T et al. 2007).

Their may be two mechanism by which insecticides could reduce circulating levels of testosterone, firstly by enhancing its degradation, excretion or tissue uptake or secondary by depressing circulating LH levels and thereby reducing LH dependent testicular steroidogenesis (Mc Lachlan et al. 1994 and Padungtod et al. 1998, Kumar et al. 2008). A dose and exposure dependent depletion in testosterone content in association with highly reduced circulating levels of this hormone in chlordane and monocrotophos fed animals confirmed alteration in the reproductive physiology of rat. Cumulative effect of plasticizers is also responsible for altered steroid hormones and genes (Kembra L et al. 2007)

5.5 Hematology

Blood is a sensitive index of the physiological changes of animals in response to any environmental pollutants and it is well known that toxic stress of any nature would show conspicuous and significant changes in the hematological parameters. Hence, hematology is used as a clinical tool to know the physiological and metabolic status of rat intoxicated with the insecticides. It is also known to constitute a very important diagnostic tool in the toxicological studies.

5.6 Total Erythrocyte Count (TEC)

The decrease in erythrocyte count recorded in the present study may be due to either inhibition of erythrocyte production (erythropoiesis in bone marrow) or destruction of erythrocyte due to poisoning. Two factors are essential for the liver and its deficiency is characterized by disturbance in erythropoiesis. Deficiency of vitamin B_{12} leads to impaired synthesis of nucleic acid resulting into defective maturation of erythrocytes and their nuclei (Lynch et al. 1969). Decrease in RBC, WBC, MCV, MCH, Platelet and hemoglobin are there due to insecticide consumption. (Celik I, Suzek H, 2008).

This is supported by the histopathological destruction of liver and kidney which leads to reduced availability of erythropoietin, which is produced in the juxtaglomerular apparatus in the kidney and is secreted in the plasma for the utilization by stem cells of bone marrow. The destruction of erythrocytes is indicated by haemolysis and corresponding increase in bilirubin.

5.7 Total Leukocyte Count (TLC)

Pesticides have been reported to stimulate leukocyte production. The observed leucocytosis justified the assumption that these pesticide acts as chemical stressors. The stress causes a slight increase in adrenaline level and in consequence to neutrophil and lymphocytic leucocytosis. Increase leukocyte counts were also reported (Thakur and Bais, 2000, Padmaja et at. 2000).

5.8. Hemoglobin

The decrease in the hemoglobin content may be due to decrease in the rate of Hb synthesis or increase in the rate at which the Hb is destroyed.

Synthesis of Hb begins in the polychromatic norm oblast stage. The synthesis of Hb requires iron which is generally obtained from stored ferrit in and from dietary sources. The reduction in general food intake by intoxicated albino rats and absence of supplementary supply of extra iron might be the reasons for the iron deficiency (Whipple, 1942, Lynch et al 1969, Wintrob's 1998)

The second possibility for the reduction in hemoglobin content may involve an increase in the rate at which the Hb is destroyed. It is presumed that insecticides may enter into the red cells and interfere with enzymes that synthesize the Hb. Due to this; cells became fragile and may rupture during passage through some tight spot of circulation. Lewis (1970) reported that when the supply of iron is inadequate, the rate of hemoglobin synthesis during all the stages of maturation of erythrocytes is decreased. Haemotological toxicity was also reported (Thakur PB, Bais VS, 2000).

On the basis of above discussion, it can be concluded that the rate of the destruction increases due to destruction of erythrocytes by monocrotophos and chlordane and this leads to fall in Hb concentration. Similar results were also reported. (Reeves et al, 1981, Siddiqui et al 1987 and Qadri et al 1987).

5.9. Hematocrit Values

The decreases in the hematocrit percents may be due to either a decrease in the size of RBC or a decrease in the number of erythrocytes.

The first assumption for the decrease in hematocrit value may be due to the decrease in the size of erythrocytes by way of reduction in Hb synthesis which in turn regulates the maturation of erythrocytes by its accumulation in the cell.

The decrease in the number of erythrocyte may be the reason for reduction in hematocrit as per the present investigation. As discussed earlier, decrease in the number of erythrocyte was due to decrease in the rate of erythrocyte production and destruction of erythrocytes due to insecticide poisoning. (Omoyakhi J.M., Orheruata and Osinowo O.A., 2008).

5.10. Serum Biochemistry

5.10.1 Aspartate amino transferase and Alanine amino transferase

Serum enzymes are useful indicators for the assessment of liver function in the toxicity studies of chemicals and environmental pollutants. In the present study, exposure of monocrotophos and chlordane resulted in an increase in aspartate amino transferase and alanine transferase levels and this may be due to the pathological changes such as necrosis of hepatocytes which cause increase in the permeability of the cell membranes, resulting in the release of transferases into the blood stream. Present study finds support from the work of other toxicologists. (Gupta,1977; Chakravarty and Sreedhar, 1982; Young, Mehendale, 1986 and P.Joseph John, 2006).

5.10.2 Acid phosphatases and Alkaline phosphatases

Normally plasma contain small amount of acid phosphatases which apparently originates mainly in the liver and spleen. Enormous amount of its presence in adult prostate gland, where the prostatic epithelial cells forms it and passes into the prostatic secretion.(Kalavathy KA, Sirvakumar A, Chendran R 2001).

A significant increase in serum acid phosphatase activity in chlordane and monocrotophos treated animals was observed whereas a significant decrease in the levels of alkaline phosphates has been found in the present investigation. Reduction in alkaline phosphatase may be attributed to the decreased osteoblastic activity of bone, since the alkaline phosphatase is formed and present in the osteoblasts (Cantarow and Schepertz,1967). A decrease amount of alkaline phosphates reflects decreased

osteoblastic activity. Similarly decrease of alkaline aphosphatase due to intoxication of insecticides has also been reported (Galdhar et al. 1978; Emine Saglam, 1988; Neskovic et at. 1991; Naqvi and Vaishnavi, 1993).

5.10.3 Bilirubin

Bilirubin, endogenous compound, has been widely used to evaluate chemical induced hepatic injury. One of the normal functions of liver is to excrete the breakdown product of hemoglobin into the bile. Therefore the measurement of the liver ability to remove bilirubin from the blood and excrete bilirubin into the bile can be used as a liver function test. (G. Kumar et al. 2007).

Elevation of serum bilirubin concentration accompanies sufficiently severe parenchymal injury (Plaa and Hewitt, 1982) as also noticed in the present investigation. Subchronic exposure of chlordane and monocrotophos caused severe pathological damages to hepatocytes which resulted in a significant increase in bilirubin level.

5.10.4 Total cholesterol

Administration of chlordane and monocrotophos at various dose levels increases the total serum cholesterol as recorded in the present study. The changes in the serum cholesterol levels can be attributed to alteration in hepatic synthesis or rate of tissue utilization and degradation (Boyd and Oliver, 1958). The serum cholesterol is exclusively synthesized in the liver and the distribution of cholesterol between liver and plasma is affected by both endogenous and dietary factors (Cook, 1958). The damage of kidney and liver by these pesticides may be the reason for *hypercholesteremia* (Jalliffe CJ, et. al, 2006).

5.10.5 Blood glucose

The sub acute toxicity studies of monocrotophos and chlordane insecticides caused *hyperglycemia* increased glucose concentration in blood as well as an impairment in the uptake and utilization of glucose by body tissues.

Liver plays a key role in the glucose homeostasis. Liver causes hyperglycemia, firstly by converting the stored glycogen into glucose (glycogenolysis) and secondly by the toxicity on glucose control of alimentary canal and liver. (Singh and Shrivastava, 1997)

In the present study, it is presumed that the insecticides some how reduce the secretion of insulin and stimulates glucagons secretion. Inhibition of insulin secretion is may be due to selective destruction of ß-cells of islets of langerhans. Thus, in the absence of insulin and in the presence of glucagons the liver secretes glucose into the blood and vice versa.

Chlordane and monocrotophos in some way also affect the adrenal gland that increases the secretion of adrenaline and glucocorticoids which in turn lower the glucose tolerance (Murphy, 1966). Hyperglycemia and lowered glucose tolerance showing impairment in the uptake and utilization of glucose by the tissues in rats administered with insecticides.

5.10.6 Blood urea

The oral administration of chlordane and monocrotophos insecticides to male albino rats caused *uremia,* the condition in which there is a high concentration of urea in the body fluids, as reported in the present investigation. It may be due to the high concentration of non protein nitrogen and chemicals especially urea, creatinine and uric acid, resulting from failure of the body to excrete the metabolic end products of protein (Guyton 1998). The increased protein content in the blood has also been reported in the present investigation. Singh and Pandey (1989) reported the toxic effects of endosulfan on kidney and found that endosulfan decreases lipid peroxide and increases protein levels and activities of membrane bound ATPases. A similar case of acute renal failure following organophosphorus poisoning has also been reported, (Betrosian et al. 1996 and Panda et al. 1999). Blood urea nitrogen, serum creatinine and uric acid levels are markedly elevated after 24 hours of fenthion pesticide administrations (Mustafa Kerem et al, 2007).

5.11. Histopathology

Administration of chlordane and monocrotophos insecticides to male albino rats, resulted in many degenerative changes in the testis, epididymis, vas deferens, seminal vesicle, prostate gland and various pathological alterations in the kidney and liver.

5.11.1 Testis

The mammalian testis is divided into two compartments, the tubular seminiferous and vascularized interstitial cells. The seminiferous tubules account for over 90% of the volume of the testis. The interstitial compartments consist of leydig cells, blood and lymph vessel and a few other cell types with variable amount of connective tissues. The spermatogenesis involves the transformation of an undifferentiated stem cell (spermatogonia) into a highly differentiated immature spermatozoa, a process which take place in close association with or even embedded in the cytoplasm of sertoli cells. Sertoli cells provide most of nutritional and physical support for the developing germ cells (Sharpe et al. 1983). Change in vascular dynamics in testis of adult rat may be there due to some chemicals (Jacqui Piner et al, 2002)

The decreased testicular weight and reduced seminiferous tubule diameter reflects wide spread cellular damage and androgen deprivation (Keel et al. 1980). The alteration observed in the testis of chlordane and monocrotophos exposed rats includes tubular shrinkage which occurs due to the cell death or sloughing of epithelial cells (Hess et al. 1993, Sinha et al. 1995, Chitra et al. 1999). Whereas the sloughing of germ cells may be due to inhibition of microtubules formation in the sertoli cell and in the mitotic apparatus of dividing germ cells as seen with single dose of other microtubule poisons (Russel et al. 1981).

Sertoli cells are the primary supportive cells of the testis creating the necessary structural and physiological environment for germ cell maturation. Sertoli cells form the blood testis barrier, isolating postpreleptotene spermatocytic germ cells from interstitial fluids (Nicander 1967 and Fawcett 1975) and secrete seminiferous tubule fluid which contains the nutrients proteins and hormones required by maturing germ cells. Sertoli cells dispose of residual germ cell cytoplasm through phagocytosis and

secrete systemic hormones which are involved in regulating testicular function, (Bardin et al. 1988). Thus, direct effects of toxicants on sertoli cell function have been shown to produce epithelial disorganization and subsequent tubular atrophy. The micro tubules in its apical cytoplasm, when disturbed, caused the seminiferous epithelium to become disorganized. The occurrence of giant cells in the testis is considered to be an expression of germ cell degeneration which may be due to non availability of androgen (Singh et al. 1990; Wango et al. 1997).

The leydig cells or interstitial cells are primary site of testosterone synthesis (Ewing et al., 1992). These cells are closely associated with the testicular blood vessels and the lymphatic space. The atropic state of the leydig cells in the testis of treated animals indicates a reduction of normal LH concentration. The insufficient intake of LH by leydig cells leads to loss of some steroidogenic enzymes, thereby depleting testosterone production nd resulting in atrophy of leydig cells (Thomas, 1975).

5.11.2 Epididymides

Anatomically the epididymis is arranged into three parts called the caput, corpus and cauda epididymides (Amann, 1989). The first two sections together (the caput, plus the corpus) are regarded as making up that part of the epididymis involved with sperm maturation, where as the terminal (the cauda) segment is regarded as the site of sperm storage and therefore it has wider luminal diameter and epithelial lining. The epididymis plays an integral role in male reproduction by providing a favourable fluid micro environment for sperm maturation and storage. The fluid secreted by epididymis is controlled by neurotransmitter substances that are allocrine and pracrine hormones (Brooks, 1979). The physiological and biochemical integrity of epididymis are dependent upon androgens. Deficiency of androgens caused a marked reduction in tubular diameter, regression of epididymal epithelium, decline in spermatozoal number in cauda epididymis and change in composition of epididymal plasma (Brooks, 1981 and Kasturi et al., 1995). Epididymal epithelium is sensitive to androgens and the toxic effects on epididymis possibly be due to reduced androgen level and thus reflects the anti androgenic effects of chlordane and monocrotophos.

5.11.3 Seminal vesicle

Seminal vesicles secrete substances which directly stimulate sperm motility and antigen that seems to prevent immune response against spermatozoa (Gonzales, 1989). Chlordane and monocrotophos treated rats resulted in the adverse effect on histoarchitecture of seminal vesicle with reduced secretion which may be due to anti androgenic effect of chlordane and monocrotophos insecticides.

5.11.4 Prostate gland

In rats treated with chlordane and monocrotophos insecticides, the secretary material of prostate gland was reduced and proliferation of epithelium with the crypts invaded in lumen has been observed. Prins (1991) reported non functional phase of the prostate due to decrease in sialic acid content. Waakies et al. (1982), observed the expression of metallothionein induced by cadmium in prostate gland. Some anti androgens have also been known to depress the uptake of testosterone in the prostate and reduce the binding of androgen to hormone receptor by competitive inhibition (Fang and Liao, 1969)

5.11.5 Vas deferens

Vas deferens, an organ of male reproduction, plays a key role in sperm transport and seminal emission. Histological aspect of Vas deferens has been widely studied in several mammals including man (Hamilton et al.1969). The cytological characteristics of the principle cells seem to indicate that the cells are responsible for both secretary as well as resorptive functions which are under the control of androgen (Amobi et al. 1986).

The toxic effect of chlordane and monocrotophos on histoarchitecture of vas deferens with degenerative changes in the epithelium along with the absence of spermatozoa in the lumen may be due to reduced androgen level.

5.11.6 Liver

The liver is the largest gland in the body which performs both exocrine and endocrine function. The marked vulnerability of the liver to chemical induced damage appears to be a function of (a) its anatomical proximity to the blood supply from the digestive tract (b) its ability to concentrate and biotransform foreign chemicals and (c)

its role in the excretion of xenobiotics and/or their metabolites into the bile. Liver enlargement in regulatory toxicity studies have been reported (Andrew D, 2005).

In the present investigation swelling in hepatocytes may be due to the disturbance of active transport of water from the cell (Nurbanu Ozer, 1993). The fatty degeneration characterized by vacuolization is due to local action of chlordane and monocrotophos on the liver cells with consequent interference in carbohydrate, lipid and protein metabolism (Boone L et al, 2005).

Lymphocytic infiltration around the central and portal vein and in sinusoids, observed in the present investigation indicates internal haemorrhage. It may be due to the inflammatory lesion which has increased the capillary fragility. It is believed to secrete neutralizing and lytic enzymes effective in detoxifying the toxic products of metabolism (Hayes, 1982). Pesticide chlordane and monocrotophos reaches the liver through the portal vein to the hepatocytes through sinusoids. This may cause dilation of sinusoids and vessels with increased permeability and fragility of membrane (Plaa and Hewilt, 1982). The nuclear aberrations viz karyoloysis, karyoschisis, pycnosis, necrosis reported to be present in the present investigation may due to the toxic effect of insecticides which prevent the cell to complete the mitotic cycle. Analysis of rat feeding studies with genetically modified maize reveal signs of hepatorenal toxicity (Serelini G-E, Cellier D and De Vendomois JS 2007).

All the above histological observations get support from the biochemical findings. Similar histopathological changes in the liver were also observed by (Gillete, 1975, Bhatnager et al. 1987, Singh, 1991, Muley, 1996, Singh and Shrivastava, 1998).

5.11.7 Kidney

Kidney plays an important role in maintaining the homeostasis and is important site for filtration and absorption. In the present study, alterations in the kidney structure have been found which caused the imbalance in the osmoregulation due to insecticide intoxication (Ernest, 1980). Histopathologically kidney treated with insecticides showed glomerulonephritis, glomerulescleroses, odenoma, pycnotic nuclei, glomerulus deposits shrunken glomeruli, necrosis of renal tubules. A similar

pattern of morphological deformations were observed by some earlier reports (Singh and Pandy 1989, Khillare and Wagh, 1990).

In view of the above findings recorded in the present investigation, chlordane and monocrotophos exert considerable effect on the sperm dynamics, hematology, biochemistry, histopathology and hormonal levels in male albino rats. Comparing chlordane and monocrotophos, the later reported to be more toxic. Further studies are needed to understand the exact mode of action of these two pesticides with special emphasis on monocrotophos, which in our study was found to be more toxic than the other chemical.

At this point, it is difficult to pin-point the exact mode of action of these two pesticides. However based on some earlier reports, it could be speculated that these particular chemicals act at various target sites like inhibition of androgen biosynthesis, gonadotropic hormone production as reported earlier (Kumar et al, 2008). Further it show the toxic effects by its direct action on various tissues, which has also been shown by several authors earlier.

In summary, the present work suggests that the non-judicious use of the pesticides can cause a severe threat to human being, especially in a developing country like India.

5.11.8 Discussion of Hershberger Assay:

Many studies have been focused on the estrogenecity of food preservatives, fragrance, plasticizer and phytoestrogen, to which human is easily exposed via diet and industrial products (Osamu Takahashi et al,2007, Zacharewski et al.,1998, Baker et al.,1999, Byford et al 2002). EDSTAC (2001) defined anti hormone which is involving following mechanism; can act via steroid hormone receptor, steroid hormone synthesis inhibition, reduction of bioavailability by reducing amount of free hormone level in serum etc. High level of estrogenic compounds may cross react with androgen receptor because the estrogen and androgen receptor are similar in structure and it reduces testosterone concentration in testis and serum and weight of prostate gland. (Gardner WA et al., 1997, Hwan Goo Kang et al, 2005).

5.11.9 Discussion of courtship behavioral study:

The initial component of male sexual arousal is elicited by pheromones of a receptive female. We have shown in our laboratory that introduction of a sexually receptive female to a sexually mature male, In addition, there is an increase in the amount of time that the males spend near the partition. This activity is indicative of male sexual motivation and arousal. We have shown that such partition tests are highly effective in activating both the sexual behavioral and hormonal components of sexual arousal in different strains of mice / rat (Sergei Ya et al. 1999, Laura Ricceri et al. 2006, Cooke PS et al. 1990)

In order to confirm reduction in libido of male rats, their testosterone levels were measured and it was concluded that at higher dose of these insecticides administration, causes reduction in the serum testosterone level evident by figure of serum testosterone level of chlordane and monocrotophos as given in observations /results (4.7 and 4.23).

CHAPTER – 6

SUMMARY AND CONCLUSION

The indiscriminate and injudicious use of insecticides particularly chlordane (organochlorine) and monocrotophos (organophosphate) in agriculture, animal husbandry and public health has considerably increased the risk of human health hazard. Pesticides are broadly acting toxic chemicals. As a result, we now face an unprecedented environmental crisis, which includes environmentally induced illness, cancers, genetic damage, reproductive toxicity etc. Now, its time that the methods employed must be such that their mode of action may be more understandable.

Keeping the above point in view, the present study was conducted in which chlordane and monocrotophos were used to evaluate their effects on reproductive toxicity in male albino rats. Chlordane was administered orally to male rats at the dose levels of 200, 400 and 600 mg/kg b.wt./day for 2 weeks and 5 weeks. Similarly Monocrotophos was administered orally to male rats at the dose levels of 6, 14 and 22 mg/kg b.wt/ for 2 weeks and 5 weeks respectively. The doses were based on LD_{50} values.

6.1 Weight response

Oral administration of chlordane and monocrotophos caused a significant reduction in the body weight of rats. The weight of testis and accessory sex organs were also decreased significantly, whereas the weight of the liver was increased significantly.

6.2 Effect on fertility

In order to test the effect of chlordane and monocrotophos on fertility, the treated male rats were allowed to mate with normal healthy female rats (1:2). Results showed 20%, 60% and 80 % negative fertility at 200, 400 and 600 mg. dose levels after 2 weeks of chlordane feeding. Whereas 20%, 60% and 100% negative fertility were observed in rats treated with chlordane at the dose levels of 200, 400 and 600 mg. dose for 5 weeks. Similarly monocrotophos at 6, 14 and 22 mg. for 2 weeks dose level caused 20%, 60% and 80% negative fertility and 20%, 60% and 100% negative fertility was observed at 6, 14 and 22 mg. dose level of monocrotophos after 5 weeks treatment.

6.3 Antispermatogenic effects

(1) Motility of sperms in cauda epididymides was decreased markedly after oral administration of chlordane and monocrotophos at all the dose levels. Maximum reduction was upto 79.95% in chlordane and upto 85.64% in monotocrotophos treated rats.

(2) Sperm density in the testis and cauda epididymides was decreased significantly ($P \leq 0.001$) in rats treated with chlordane and monocrotophos at all the dose levels.

(3) Reduction in the weights of testis, epididymides, seminal vesicle and prostate gland was also observed at various dose levels of chlordane and monocrotophos exposed rats.

6.4 Antiandrogenic effect

A significant decrease ($P \leq 0.05$ and $P \leq 0.01$) was observed in serum testosterone concentration in the chlordane and monocrotophos treated rats at higher dose levels. Degenerative changes in the histoarchitecture of testis and reduced amount of secretion in seminal vesicle and prostate was also observed.

6.5 Hematology

Total erythrocyte count (TEC), Hemoglobin concentration and Haematocrit values were significantly decreased at various dose levels of chlordane and monocrotophos treatment. Whereas the total leukocyte count (TLC) was increased in chlordane and monocrotophos exposed rats at all the dose levels.

6.6 Serum Biochemical Analysis

(i) Blood urea and blood sugar were increased significantly ($P \leq 0.001$).

(ii) Aspartate amino transferase in serum significantly increased ($P \leq 0.001$).

(iii) Serum acid phosphatase was increased whereas serum alkaline phosphatase was decreased significantly ($P \leq 0.001$).

(iv) Bilirubin concentration was increased significantly ($P \leq 0.05$).

(v) Serum protein was increased significantly ($P \leq 0.01$ and $P \leq 0.001$).

(vi) Serum triglyceride was increased at higher dose level, whereas at lower dose level it did not change remarkably.

(vii) Serum phospholipids level was found to be increased at higher dose levels.

(viii) Serum cholesterol, HDL-Chol, LDL-Chol and VLDL-Chol levels were increased at all the dose levels.

6.7 Histoarchitecture

(i) After oral administration of chlordane and monocrotophos at various dose levels, the histoarchitecture of testis was altered markedly. Inhibition of spermatogenesis, shrunken and damaged seminiferous tubules with increased interstitial spaces, degenerated and vacuolated sertoli cells, presence of vacuoles in the epithelium, disruption of leydig cells were conspicuous.

(ii) Epididymides showed reduced number of spermatozoa in the lumen of caput and cauda regions after the treatment with chlordane and monocrotophos. The epithelial lining was regressed.

(iii) Little or no secretion and altered epithelial lining was seen in prostate gland and seminal vesicle of chlordane and monocrotophos treated rats.

(iv) Histology of vas deferens was altered with few or no spermatozoa in the lumen.

(v) Histopathologically size of the liver was found to be increased (hypertrophy) along with sinusoidal dilation, pycnotic nuclei, cytoplasmic degranulation and various nuclear aberrations.

(vi) Various histopathological alterations were observed in the kidney which includes glomerulonephritis, glomeruloscleroses, odenema, pycnotic nuclei and glomerulus deposits.

6.8 Conclusion

The results of the present study revealed that the administration of chlordane and monocrotophos insecticides induces reproductive toxicity as well as hepato-nephrotoxicity in male albino rats.

(i) Mating test showed 100% negative fertility that is the state of sterility.

(ii) Low concentration of spermatozoa in testis and cauda epididymides and reduction in number of spermatogenic elements in testis reflects antispermatogenic nature of chlordane and monocrotophos insecticides.

(iii) Antiandrogenic nature is evinced by reduction in the weight of accessory sex organs, degenerative changes in histoarchitecture, reduced amount of secretion in seminal vesicle and prostate gland and low concentration of testosterone in serum.

(iv) Haematologica studies showed toxic effects of chlordane and monocrotophos on general body metabolism.

(v) Increase in acid phosphatase, aspartate, alanine transferase and bilirubin indicates the hepatotoxice action of insecticides due to cellular damage.

(vi) Increase blood urea and pathological alterations further suggests the chlordane and monocrotophos induced intoxication on the structure and function of kidney.

(vii) It can be concluded that toxic effect of monocrotophos is more pronounced than that of chlordane on mammals, taking rat as a biological indicator.

From these results, it can be conjectured that chlordane and monocrotophos not only disrupt sperm production or male hormones, the vital organs and general body metabolism were also altered from their toxic effects.

CHAPTER - 7
BIBLIOGRAPHY

REFERENCE

Adamssona, N.A., Brokken, L.J.S., Paranko, J., Toppari, J., 2008. In vivo and in vitro effects of flutamide and diethylstilbestrol on fetal testicular steroidogenesis in the rat. Reprod Toxicol. 25, 76–83.

Adeshina, F. and E.L. Todd. 1991. Application of biological data in cancer risk estimations of chlordane and heptachlor. Regul. Toxicol. Pharmacol. 14 : 59-77.

Adhikari N, Grover IS. Genotoxic effects of some systemic pesticides : In vivo chromosomal aberrations in bone marrow cells in rats. Environ Mol Mutagen 1988; 12: 235-42

Adilaxmamma K, Janardhan A, Reddy KS. Monocrotophos : reproductive toxicity in rats. Indian J Pharmacol 1994; 26 : 126-9.

Agrawal S.S, Sharma R.C, Arora R.B., 1969. anticestrogenic activity of root of abrus precatorius linn. Ind. Pharmac. 1969; 31:175.

Aitken RJ, Best FSM, Warner P and Templeton A (1984) : A prospective study of the relationship between semen quality and fertility in cases of unexplained infertility. J Androl. 5 : 297-303..

Aitken RJ, Best FSM, Warner P and Templeton A (1984) : A prospective study of the relationship between semen quality and fertility in cases of unexplained infertility. J Androl. 5 : 297-303.

Akbarshah MA. Stanley A and Averal HI (1995) : Effects of Vincristine on Leydig cell and accessory reproductive organs. Current Science. 68 (10) : 1053-57.

Al- Hachim, G.M. and A. Al-Baker. 1973. Effects of chlordane on conditioned avoidance response, brain seizure threshold and open-field performance of prenatally- treated mice. Br. J. Pharmacol. 49 : 480-483.

Alvarez, W.C. and S Hyman. 1953 . Absence of toxic manifestations in workers exposed to chlordane. Arch. Ind. Hyg. Occup. Med. 8 : 480-483.

Amann (1989) : Structure and function of the normal testis and epididymis. J. Am. Coll. Toxicol. 8 : 457-71.

Amobi NIB and Smith ICH (1986) : Differential effects of calcium channel blockers on response of rat vas deferens of intra nural nerve stimulation and exogenous drugs. Experimentia. 42 : 410-12.

Amstislavskaya TG, Osadchuk AV, Naumenko EV. Ways of the activation and time course of changes of testicular endocrine function caused by the effect of female presence. Prob Endocrinol 1989;35(6): 63–6 (In Russian).

Andersson, A.M., Jorgensen, N.K., Main, M., Toppari,_J., Rajpert-De Meyts, E., Leffers, H., Juul, A., Jensen, T. K., Skakkebæk, N. E., 2008. Adverse trends in male reproductive health: we may have reached a crucial 'tipping point'. Int J Androl. 31, 74–80.

Andersson, S. W., Skinner, J., Ellegård, L, Welch, A. A., Bingham, S., Mulligan, A., Andersson, H., Khaw, K.T., 2004. Intake of dietary plant sterols is inversely related to serum cholesterol concentration in men and women in the EPIC Norfolk population: a cross-sectional study. 58(10), 1378-1385.

Andrew D (2005) PSD Guidance document : Interpretation of liver enlargement in regulatory toxicity studies. York, England, Pesticide Safety directorate.

Ansari RA, Siddiqui MKJ and Gupta PK (1984) : Toxicity of endosulfan. Distribution of α and β isomers of raqcemic endosulfan following oral administration in rats. Toxicol. Letters. 21-29..

Astoor AM and King EJ (1954) : Simplified colorimetric blood sugar method. *Biochem. J* 56:44.

B.Saleha Banu (2001) : Genotoxic effect of monocrotophos to sentinel species uring comet assay. Food and chemical toxicology 39 (4) : 361-366

Babu B. V, 2007. Effluent treatment : Basic and case study. Water digest, Vol.2 (No. 4), 14 - 26 .

Baker HWG, Santen RJ, Burges HG, Dekretsec DM, Hudsen B and Pepperell RJ (1975) : Rhythms in the secretion of gonadotropin and gonadal steroids. J . Steroid, Bio. Chem. 6 : 793-01.

Balaji M and Sasikala K (1993) : Chromosome damage occurs to human blood cells. *Mutation Research*. 301: 13-17

Bame H J B, Blaylock B L, Gandy J et al (1990): Alteration of fetal liver colony formation by prenatal chlordane exposure. Fundam. Appl. Toxicol. 15: 820-822.

Bardin CW, Cheng CY, Musto NA and Gunsalus GL (1988) : The Sertoli cell. In the physiology of reproduction. Raven Press, New York. p. 933-74.

Barnett, J.B., D. Holcomb, J.H. Menna, and L.S.F. Soderberg. 1985a. The effect of prenatal chlordane exposure on specific anti- influenza cell-mediated immunity. Toxicol. Lett. 25 (3): 229-238.

Barnett. J.B., B.L. Blaylock, J. Gandy, J.H. Menna, R. Denton, and L.S. soderberg. 1990b. Alteration of fetal liver colony formation by prenatal chlordane exposure. Fundam. Appl. Toxicol.15 (4) : 820-822.

Barnett. J.B., L.S.F. Soderberg. And J.H. Menna. 1985b. The effect of prenatal chlordane exposure on the delayed hypersensitivity response of BALB/C mice. Toxicol. Lett. 25 (2) : 173-183.

Bastias MC, Kamijo H and Povlou SN (1993) : Sperm motion parameters after suppression of spermatogenesis with gonadotropin- releasing hormone antagonist plus testosterone supplementation. Fertil. Steril. 59 (6) : 1261-65.

Bedford CT, Robinson J. The alkylating properties of organophosphates. Xenobiotica 1972; 2:307-37.

Belanger A, Caron Sa and Picard V.(1980) : Simultaneous radioimmunoassay of progestins, androgens and estrogens in rat testis *J. of Steroid. Biochem.* 13 : 185-90.

Bergstrom R, Adamic H O, Mohner M (1996) : Increase in testicular cancer in six European countires : a birth cohort Phenomenon. J Natl Cancer Inst; 88 : 727-33

Betrosain A, Ballami et al. (1996) : Multiple system organ failure from organo phosphorus poisoning. J. Toxicol Clin. Toxicol. 34 (2).

Bhatnagar MC, Bana AK and Dalela RC (1987) : Histopathological alterations in liver of Channa gachua exposed to endosulfan. Acad. Environ. Biol. 126 : 200.

Bhunya SP, Behera BC. Mutagenicity assay of an organophosphate pesticide, Monocrotophos in mammalian in vivo test system Cytologia 1988 ; 53 : 801-7.

Bhunya SP, Jena GB. Studies on the genotoxicity of Monocrotophos, an organophosphate insecticide, in the chick in vivo system. Mutat Res 1993; 292 : 231-3.

Bindra OS and Kalra RL (1973) : A review of work done in India of pesticide residue. Proc. SPRA.p.1-44.

Bloomquist, Jeffrey R. 1996. *Insecticides: Chemistries andCharacteristics.* University of Minnesota. http://ipmworld.umn.edu/chapters/bloomq.htm.This chapter in an online textbook on pest control (Radcliffe's IPM World Textbook) describes the chemistry and action of different classes of insecticides.

Bokare N (1995) : Assessment of pesticide burden and its sources in pregnant women of Jaipur : Ph.D. Thesis, University of Rajasthan, Jaipur.

Boone L, Meye D, Cusick P, Ennulat D, Provencher Bolliger EA, Ever N, Meador V, Elliot G, Bounous D and Jordan H (2005) : Selection and Intupretations of chemical

paterology indicator of hepatic jnyuries in pseclinical studies. Veterninary chinical pathology 34 (3) : 182-188.

Borders CK, Salamon CM, Mayhew DA. Technical Azodrin (SD9129) teratology study in SD CD rats. USA: Toxi-genics, Inc, 1983; unpublished report, cited in FAO92 and Skr 94.

Boyd GS and Oliver MF (1958) : The physiology of circulating cholesterol and lipoproteins. In : cholesterol. Cook RP (ed.) Academic press, New York.

Brooks DE (1979) : Biochemical environment of sperm maturation. In : The spermatozoa. DE Fawcett and J.M. Bedford (eds) Urban and Schwargenber Baltimore. p. 23-24.

Brooks DE (1981) : Androgen effects on epididymal metabolism. Physical Rev. 61 : 515-55.

Brown VK, Gellatly JBM. A long-term feeding study with Azodrin in rats to investigate chronic toxicity and oncogenicity (6, 12, 18 and 24 month necropsies). Sittingbourne, UK : Shell Research Ltd, 1983; rep no SBGR. 82.062; unpublished report, cited in SIP85 and Skr94.

Brown VK. A two-year oncogenicity study in mice fed Azodrin. Sittingbourne, UK: Shell Research Ltd, 1982; rep no SBGR 81.218; unpublished report, cited in SIP85 and Skr94.

Burnstein M, Scholink HR and Morfin R (1970) : Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *J Lipid Res.* 11 : 583-95.

Byford, Jr. Shaw. L.E, Draw, M.G., Pope, G.S., Sauer M.J. and Darbre, P.D. (2002) : Oestrgenic activity of Parabens in MCF 7 human breast cancer cells. Journal of steroid biochemistry and Molecular biology 80, 49-60

Camilla Taxvig, Ulla Hass et al (2007) : Endocrine- disrupting Activities invino of the fungicides Tebuconazole and Epoxiconazole. J. Toxicol . Sei. 100 (2) : 464-473.

Cantarow A and Schepertz B (1967) : Biochemistry 4th ed., Saunders company, Philladelphia.

Cassidy, R.A., C.V. Vorhees, D.J.Minnema, and L.Hastings. 1994. The effects of chlordane exposure during pre-and postnatal periods at environmentally relevant levels on sex steroid-mediated behaviors and functions in the rat. Toxicol Appl. Pharmacol. 126 (2) : 326-337.

Celik I, Suzek H (2008) : The hematological effects of methyl Parathion in rats. J Hazard Mater. 153 (3) : 1117-21.

Chakravarty I and Sreedhar R (1981) : Effect of malathion on some hepatic enzyme activities in developing rats. IRCS Med. Sci. 9 : 1115.

Chemical Corporation. MRID No. 00067568. Available from the U.S. Environ. Contam. Toxicol. 43 : 459-466.

Chitra KC, Latchoumy Candane C and Mathur PP (1999) : Chronic effect of endosulfan on the testicular functions of rat. Asian. J. androl. 1 : 203-06.

Chun Mei Li, Shinji Taneda, Akira k.Suzuki, Chie Furtura, Gen Watanabe and Kazuyshi Taya (2006). Antiandrogenic activity of 3-methyl-4-nitrophenol in diesel exhaust particles. European Journal of Pharmacology,543, Issue 1-3, pp 194-199.

Colborn T, Clement C,eds,1992. Advances in Modern Envitonmental Toxicology. Vol XXI. In: Chemically induced alteartion in sexual and functional development. The wildlife/Human connection. Princeton NJ. Princeton Scie.jpgic.

Cook RP (1958) : Cholesterol : Chemis by Biochemistry and Pathology. Academic Press, New York.

Cooke PS, Eroschenko VP. (1990) : Inhibitory effects of technical grade methoxychlor on development of neonatal male mouse reproductive organs. Biol Reprod; 42:587–98.

Costa LG, Murphy SD. 1983. [3H]Nicotine binding in rat brain: Alterations after chronic acetylcholinesterase inhibition. J Pharmacol Exp Ther 226:392-397

Crisp, T. M., Cregg, E. D., Cooper, R. L., Anderson, D. G., Baetcke, K. P., Hoffmann, J. L., Morrow, M. S., Rodier, D. L., Schaeffer, J. E., Touart, L. W., Zeeman, M. G., Patel, Y. M., Wood, W. P., 1997. U.S. EPA/630/R-96/012, 1–120.

Crissby WH, Munn Ji and Furth FW (1954) : Standarizing a method for clinical haemoglobinometry. US. *Armed. Force. Med. J.* 5 : 695-703

Dambska M et al: Blood brain barrier in young rabbit brain after dichlorvos intoxication. Neuropath Pol, 22:129-37, 1984

Darbre, P.D., 2006. Environmental oestrogens, cosmetics and breast cancer. Best practice & research. Clin Endocrinol Metab . 20, 121-143.

Das GP, Shaik AP, Jamil K (2006): Estimation of apoptosis and neurosis caused by pesticides in vitro on human lymphocytes using DNA diffusion assay. Drug Chem. Toxicol. 29(2): 147-156

Dayal, M., Parmar, D. Dhawan, A., Dwivedi, U.N and Seth, P.K (2003): Effect of cytochromeP450 modifiers on deltamethrin induced neurobehavioral toxicity. Food & Chemical Toxicology 41, 431-437.

Dearlove GE. Development toxicity study of Azodrin insecticide (technical) in New ealand white (NZW) rabbits. Horsham PA, USA: Argus Research Laboratories, 1987; proj no 619-005; unpublished report, cited in FAO94 and Skr94.

Desjardins, P. R. & Dodds, J. A, Abdalla, O. A., (1985). Survey of pepper viruses in California by the ELISA technique. *Phytopathology* 75, 1311

Dikshit, P., Chatterjee, M., Goswami, A., Mishra, A., Jana, N.R., 2006. Aspirin induces apoptosis through the inhibition of proteasome function. J Biol Chem. 281, 29228-29235.

Dix KM, Thrope E. A reproduction study in rats fed Azodrin. Sittingbourne, UK: Sittingbourne Research Centre, 1981 ; unpublished report, cited in SIP85 and Skr94.

Dureja P and Parmar BS (1994) : Minimising environmental hazard of agrochemicals-Indian approach and status. Society of pesticide science. India, New Delhi. P 57-65.

Ecobichon D. Organophosphorus ester insecticides. In: Pesticides and Neurological Diseases (Ecobichon DJ, Joy RM, eds). Boca Raton, FL:CRC Press, 1994;171-250

Ecobichon DJ (1991) Pesticides. In *Casarett and Doull's Toxicology*: *The BasicScience of Poisons* p. 580 Eds MO Amdur, J Doull and CD Klaassen. Pergamon Press, New York

Eertmans, F., Dhooge, W., Stuyvaert, S., Comhaire, F., 2003. Endocrine disruptors: effects on male fertility and screening tools for their assessment. Toxicol In Vitro. 17, 515-524.

Ellen Thom, Johannes C. G. Ottow and Gero Benckiser (1997): Degradation of the fungicide difenoconazole in a silt loam soil as affected by pretreatment and organic amendment Environmental Pollution Volume 96, Issue 3, 409-414.

Emine Saglam (1988) : The effect of endosulfan on serum alkaline phosphatase activity in Swiss Albino types mice (Musmusculus). Master thesis Ondokuz Mayis University. Turkey.

Ernest (1980) : Renal toxicology in Toxicology : Principles and Practices Vol. 2 fredric and sperling (2^{nd} ed) John Wiley and Sons, New York. p.145-58.

Eskenazi B, Wyrobek AJ, Sloter E, Kidd SA, Moore L, Young S, Moore D. The association of age and semen quality in healthy men. Hum Reprod (2003) 18:447–454

Ewing LL (1992): The Leydig cell, in Scialli AR, clegg (eds) : Reversibility in Testicular Toxicity Assessment Boca Raton, FL : CRL Press. p.89-126.

Exon JH, Kerkvliet NI and Talcott PA (1987) : Immuno toxicity of carcinogenic pesticides and related chemicals. *J. Environ. Sci. Health.* 5 : 73-120.

Fang SE and Liao S (1969): antagonistic action of antiandrogens on the formation of a specific dihydrotestosterone receptor protein complex in rat ventral prostate. Mol. Pharmacol. 41 : 87-172.

Fawcett DW (1975): Ultrastructure and function of the Sertoli cell. In hand book of physiology and Endocrinology. American physiologic Society Washington DC. p. 21-55.

Fishbein, W.I., J.V. White, and H.J. Isaacs. 1964. Survey of Workers exposed to chlordane. Ind. Med. Surg. 33 : 726-727.

Fisher, J.S., 2004. Environmental anti-androgens and male reproductive health: focus on phthalates and testicular dysgenesis syndrome. Reproduction. 127(3), 305-315.

Fleming, L.E. And W. Timmeny. 1993. aplastic anemia and pesticides : An etiologic association ? J. Occup. Med. 35 : 106-1, 116.

Flora, S.J.S., Gubrelay, U., Kannan, G. and Mathur R. (1998): Effect of zinc supplementation during chelating agent administration in cadmium intoxication in rats. Jour. Applied toxicol, 18,375-362.

Foster WG, Pentick JA, Mcmohan A and Lecaualier P (1993). : Body distribution and Endocrine toxicity of hexachlorobenzene (HCB) in the female rats. *J App. Toxicol.* 13 (2) : 79-83.

Franchi F (1978) : Long term study of oral testosterone undecanoate in hypogonadal males. Int. J. Androl. 1 : 270.

Freidwald WT, Levy RI and Freidrickson DS (1972) : Estimation of concentration of LDl-cholesterol in plasma without preparative of ultra centriguge. *Clin. Chem.* 28 : 449-502.

Freyberger, A., Ziegelbauer, H.E., Krtlinger, F., 2007. Evaluation of the rodent Hershberger bioassay: Testing of coded chemicals and supplementary molecular-biological and biochemical investigations. Toxicology. 239, 77–88.

Fuchs A. C1414tech., Oral (gavage) teratogenicity study in the rat. Munster, Germany: Hazleton Deutschland GmbH, 1992; unpublished report, cited in Skr94.

G. Kumar, G Sharmila Banu and M. Rayasekara Pandian (2007) Biochemical actininty of selenium and gluta thione on country wade liguor (CML) Induced hepatic dewage in rats. Indian Journal of clinical Biochemistry. 22 (1) : 105-108.

Galdhar NR, Fawade MM, Hade UK and Pawar SS (1978) : Acid and alkaline phosphatase activity during pesticide intoxication. Ind. J. Biochem. Biophys. 15 : 78.

Gardner WA Hypothisis (1995) The Prenatal origins of prostate cancer. Humpatnot 26 : 1291-2

Genevieve Bondy, Cheryl Armstrong, Laurie Coady, Josce Doucetet et al.(2003): Toxicity of chlordane metabolite oxychlordene in female rats: clinical and histopathological changes 41(2):291-301

Gillelte JR (1975) : Mechanism of hepatic necrosis induced by halogenated aromatic hydrocarbons. In the pathogenesis and Mechanism of liver cell necroses edited by D Kepplen VK.p.239-54.

Gonzales GF (1989) : Functional structure and ultra structure of seminal vesicles. Arch. Androl. 22 : 1-4.

Gore AC (2008) Developmental programming and endocrine disruptors effect in reproductive and neuroendocrine system. Frontiers in Neuroendocrinology. 29 : 358-374

Gottfried SP and Rosenburg B (1973) : Improved manual spectrophotometric procedure for determination f serum triglycerides. *Clin. Chem.* 19 : 1077-78.

Gray, L. E. Jr., Kelce, W. R., Wiese, T., Tyl, R., Gaido, K., Cook, J., Klinefelter, G., Desaulniers, D., Wilson, E., Zacharewski, T., Waller, C., Foster, P., Laskey, J., Reel, J., Giesy, J., Laws, S., McLachlan, J., Breslin, W., Cooper, R., Di Giulio, R., Johnson, R., Purdy, R., Mihaich, E., Safe, S., Colborn, T., 1997. Endocrine screening methods workshop report: detection of estrogenic and androgenic hormonal and antihormonal activity for chemicals that act via receptor or steroidogenic enzyme mechanisms. Reprod Toxicol. 11, 719-750.

Grutsch, J.F. and A. Khasawinah. 1991. Signs and mechanisms of chlordane intoxication. Biomed. Envrion. Sci. 4 (3) : 317-326.

Guerriero,G.,DiFinizio,A.,Ciarcia,G.,2002. Stress-induced changes of plasma antioxidants in aqua cultured sea bass, Dicentrarchus labrax.Comp Biochem Physiol A Mol Integer Physiol.132, 205-211

Guillette, L. J., 2006. Endocrine disrupting contaminants – beyond the dogma. Environ Health Perspect. 114, (Suppl. 1), 9-12.

Gupta PK (1985) : Carcinogenicity and Mutagenicity. In : Modern Toxicology Basis of Organ and Reproduction Toxicity. Vol. 1 Metropolitan Book Co. Pvt. Ltd., New Delhi. P 394-443.

Gupta PK and Kanan K (1984) : Immuno. Suppression : In Modern Toxicolgy, Immuno and Clinical Toxicology. Vol. III Metropolitian Book Co. Pvt. Ltd., New Delhi. P. 135-209.

Gupta RC (1977) : Biochemical and toxicological studies of malathion in a "Buffalo calves." Ph. D. Thesis, Punjab Agriculture University, Ludhiana, India.

Guyton AC and hall JE (1998) : Text book of medical physiology (9th ed.): WB saunders Company, Philadelphia.

Hall RJ and Clark DR (1982) Responses of the iguanid lizard *Anolis* carolinensis to four organophosphorus pesticides Environmental Pollution Series A 28: 45–52

Hamilton DW, Jones Al and Fawcett DW (1969) : Cholesterol biosynthesis in the mouse epididymis and ductus deferens : A biochemical and morphological study. Biol. Reprod. 1 : 167-84

Hartmann HR. C1414 tech.: Acute dermal toxicity in the rat. Stein, Swizerland: Ciba-Geigy Ltd, 1992; unpublished report, cited in Skr94.

Hassal KA: The Chemistry of Pesticides. Methuen, 1982

Hayes AW (1982): Principles and methods of Toxicology. Raven Press, New York. p.407-45.

Hertner T. Micronucleus test, mouse-in vivo study. Basle, Switzerland: Ciba-Geigy Ltd. 1992; unpublished report, cited in FAO94 and Skr94.

Hess RA and Moore BJ (1993) : Histological methods for evaluation of the testis. In Chapin B Heindel J. ed. Methods in Toxicology, Vol.3 Academic Press. p.52-85.

Hiroaki Takahashi, Takeshi Kojima, Takanori Ikeda, Shuji Truda and Yasuhiko Shirasee (1991). Diffrences in the Mode of Lethality produced through Intravenous and oral administration of organophosphorous insecticides in rats. Fundam. Appl. Toxicol.16,459-465.

Horst H and Holtye WJ (1978) : Lymphatic absorption and metabolism of orally administered testosterone undeconoate in man. Kli Woechenchr. 54 : 875-79.

Hwan Zoo Kang, Sang Hee Jeog, Joon Hyoung Cho, Dong Gyu Kim, Jong Myung Park and Myung Haing Cho (2005) : Evaluation of estrogenic and androgenic activity of butylated hydroxyanisole in immature female and castrated rats. Toxicology, Volume 213 : Issue 1-2

Hyunwon, Y., Bhat, G.K., Wadley, R., Wright, K.L., Chung, B.M., Whittaker, J.A., Dharmarajan, A.M., Sridaran, R., 2003. Gonadotropin releasing hormone-agonist inhibits synthesis of nitric oxide and steroidogenesis by luteal cells in the pregnant rat. Biol. Reprod. 68, 2222–2231

ICF-Clement. 1987. Pathology peer review of chlordane in F344 rats. Pathology review participants : Goodman, D.G., A.W. Mackin, R.R. Maronpot, J.A. Popp, R.A. Squire, J.M. Ward, and M.R. Anver.

Ipstein J. and F.Poly,1970. Banchroft's introduction to Biostatics.2nd Edn.(Harper International).pp 44-64.

IRDC (International Research and Development Corporation). 1973. Eighteen-month oral carcinogenic study of chlordane in mice. Unpublished report to Velsicol

ITRC (1999)s: Indian Toxicological Research Centre, Lucknow.

Jacqui Piner, Mar k sutherlanal et al (2002) : changes in vascules dynamics of the adult rat testis leading to transjent accumulation of Seminiferous tubule fhid after administrntion of a novel 5-HT agonist. Repro. Toxical 16 (2 : 141-150.

Jalliffe CJ, Javssen I (2006) : Distri Dution of lipoprotein by age and gender in adultes cents, circulations : 114 (10) : 1056-62 . EPub 2006 Aug 28.

Jena GB, Bhunya SP. Thirty day denotoxicity of an organophosphate insecticide, Monocrotophos, in the chick in vivo system. In Vivo 1992; 6 : 527-30.

Jendrassik L and Groff P (1958) : Method for estimation of bilirubin. *Biochem. Z.* 297 : 81-89.

Johnston CD, Thompson WM, Donoso J. Azodrin (C1414) safety evaluation by chronic feeding study in the rat for 2 years, final report. New Haven Ct, USA: Woodard Research Corporation, 1967; unpublished report, cited in SIP85.

Johnston CD. Azodrin (C1414) safety evaluation by chronic feeding studies in the rat and the dog for two years, interim report: 52 weeks. New Haven CT, USA: Woodard Research Corporation, 1966; unpublished report, cited in SIP85 and Skr94.

Jotz MM, Mitchell AD, Jones DCL, et al. An evaluation of mutagenic potential of Monocrotophos employing the L5178Y $TK^{+/-}$ mouse lymphoma assay. Menlo Park CA, USA: SRI International, 1980; unpublished report, cited in FAO82 and Skr94.

K. Kiaii, A. Österborg, A. Moshfegh,A., Mashayekhi K. Choudhury, H. Mellstedt. Karolinska(2007): Gene Expression Profiling of Peripheral Blood T Cells in Patients with Indolent B-CELL Chronic Lymphocytic Leukemia (B-CLL). Institutet.haematologica, the hematology journal, ISSN 0390-6078,Vol.92:94

Kalavathy KA, Sirvakumar A, Chendran R (2001) : Toxic effects of Pesticides dimethoate on fish Sarotherodon Massambicus. J Ecol. Res. Bisconserv. 2 (122) : 27-32.

Kasturi M, Manivannan B, Ahmed RN, Sheikh PD and Pathan KM (1995) : Changes in epididymal structure and function of albino rat treated with azardirachta indica. Ind. J. Exp. Biol. 33 (10) : 725-29.

Keel AB and Abney TO (1980) : Influence of bilateral cryptorchidism in the mature rat: Alteration in testicular function and serum hormonal levels, Endocrinology. 107 : 1226-33.

Keller, E. T., Ershler, W. B., Chang, C., 1996. The androgen receptor: a mediator of diverse responses. Front Biosci. 1, 59-71.

Kembra L. Howdeshell, Johnathan Furr et al. (2007) : Cumulative effects of dibictyl phthalate and Diethylhezxyl Phthalate on male rat reproductive tract Development : Altered Fetal Steroid Hormones and genes. J Toxicol. Sei. 99 (1) : 190-202.

Khasawinah, A.M. and J. Grutsch. 1989b. chordane : thirty-month tumorigenicity and chronic toxicity test in rats. Regul. Toxicol. Pharmacol. 10 (2) : 95-109.

Khillare YK and Wagh SB (1990) : Effects of long term Toxicity (Endosulfan, Malathion and Sevin) on Kidney of Freshwater Fish, Barbus Stigma Cham. : Proceeding of 8th annual session of academic of environment biology. p.71-76.

Kilburn, K.H. and J.C. Thornton.1995. Protracted neurotoxicity from chlordane sprayed to kill termites. Environ. Health Perspect. 103 (7-8) : 690-694.

King EJ and Jagathesan KN (1959) : Method for estimation of acid and alkaline phosphatases. *J. Clin. Path.* 12 : 85.

Kirkhart B, Jones DCL, Skinner WA. Micronucleus test onmonocrotophos. Menlo Park CA, USA: SRI International, 1980; unpublished report, cited in FAO92 and Skr94.

Kowley DL, Rab MA, Hardjotanojo W, Liddle J, Burse VW, Saleem M, Sokal O and Falk H (1987) : Convulsions caused by eldrin poisoning in Pakistan. *Pediatrics*. 79 : 928-34.

Kraybill HF (1969): Significance of Pesticide residues in foods in relation to total environment stress. *Con. Med. J.* 100: 204-07.

Kumar DV, Janardhan A. Mutagenicity of Monocrotophos in mice. Bull Environ Contam Toxicol 1988; 41 : 189-94.

Kumar, S., Saradhi, M., Chaturvedi, N.K., Tyagi, R.K., 2006. Intracellular localization and nucleocytoplasmic trafficking of steroid receptors: an overview. Mol Cell Endocrinol. 246, 147-156.

Kumar, V., Chakraborty, A., Gunda, V., Roy, P., 2007. Androgenic endocrine disruptors in wastewater treatment plant effluents in India: Their influence on reproductive processes and systemic toxicity in male rats. Toxicol Appl Pharmacol. 226(1), 60-63.

Laura Ricceri, Aldina Venerosi, Francesca Capone, Maria Francesca Cometa, Paola Lorenzini, Stefano Fortuna and Gemma Calamandri (2006) : Developmental Neurotoxicity of organophosphorous pesticides : Fetal and neonatal exposure to chlorpyrifos alters sex-specific behaviors at adulthood in mice. Toxicological Sciences. Volume 93. Number 1: 105-113.

Levin HS, Rodnitzky RL. 1976. Behavioral effects of organophosphate pesticides in man.*Clin Toxicol* 9(3):391–405

Lewis AE (1970); Principles of haematology : Meredith Corporation, New York.p.276-78.

Lin MF, Wu CL, Wang TC. Pesticide clastogenicity in Chinese hamster ovary cells. Mutat Res 1987; 188 : 241-50.

Lowry OH, Rosenbrough DJ and Farr AL and Randall R J (1951) : Protein measurement with folin- phenol reagent. *J. Biol. Chem.* 193 : 265-75.

Lynch JM, Raphel S S, Melier LD, Spare PD and In wood MJH (1969) : collection of blood sample and haemocytometry Red Cell count, White Cell Count. In : Medicinal laboratory Technology and Clinical Pathology. Pub. W.B. Saunders Company Igakar Sohim Ltd., Tokyo. 626-47.

M.Merhi, M.Raynal, E.Cahuzac et al(2007):Occupational risk of hematopoietic cancer:meta-analysis of case control studies.Cancer causes and control.18(10):1209-1226.

Maffini, M. V., Rubin, B. S., Sonnenschein, C., Soto, A. M., 2006. Endocrine disruptors and reproductive health: the case of bisphenol-A. Mol Cell Endocrinol. 25, 179-186.

Mahboob M, Rahman MF, Danadevi K, etal. Detection of DNA damage in mouse peripheral blood leucokytes by the comet assay after oral administration of Monocrotophos. Drug Chem Toxicol 2002; 25 : 65-74.

Malarvizhi D and Mathur PP (1995) : Effect of cisplastin on physiological status of normal rat testis. Ind. J. Exp. Biol. 33 : 281-83.

Mc Lachalan RI, Wrefor NG, Meachem Sj, Dekretser DM and Robertson DM (1994) : Effects of testosterone on spermatogenic cell population in the adult rat. Biol. Reprod. 51 : 945-55.

Michael C.R. Alavanja, Jane A. Hoppin et al (2004) : Health effects of chronic pesticide exposure. Cancer and Neurotoxicity. Annual Review of Public health. 25 : 155-197.

Mills NC (1990): Androgen effects on Sertoli cells. Int. J. Androl. 13 : 123-34.

Mlussalo- Rauharmaa, H. 1991. Partitioning and levels of neutral organochlorine compounds in human serum, blood cells, and adipose and liver tissue. Sci. Total Environ. 103 : 159-175

Mortelmans KE, Shepherd GF, Jones DCL, et al. In vitro detection of mitotic crossing-over, mitotic gene conversion and reverse mutation with Saccharomyces cerevisae D7 for seven pesticides. Menlo Park CA, USA : SRIInternational, 1980; unpublished report, cited in FAO92, SIP85, and Skr94.

Mukherjee M, Chattopadhyay S and Mathur PP (1992) : Effect of flutamide on the physiological status of epididymis and epididymal sperms. Andrologia. 24 : 113-16.

Mukherjee, K. L., 2003. Routine Biochemical Tests and Histological Techniques. In Medical Laboratory Technology (K. Mukherjee Eds.), Tata McGraw-Hill Publishing Company Limited, New Delhi. 1124-1190.

Muley DV, Kumble SB and Gaikwad PT (1993) : Endosulfan toxicity in the freshwater fish tilapia mosambica. Proc. Acad. Environ. Biol. 5 (1) : 49-55.

Munoz-de-Toro, M., Durando, M., Beldomenico, P.M., Beldomenico, H.R., Kass1, L., Garcia, S.R., Luque, E,H.,. Estrogenic microenvironment generated by organochlorine residues in adipose mammary tissue modulates biomarker expression in ERα-positive breast carcinomas. J Breast Cancer Research. 8(4), R47.

Murphy SD and Porters (1966) : Effect of toxic chemicals on some adaptive liver enzymes, liver glycogen and blood glucose in fasted rats. Biochem. Pharmacol. 15 : 1665-76.

Mustafa Kerem, Nurdan Bedirli Et Al (2007) : Turk J. Med. Sci : 37 (5) : 281-288.

Naqvi SM and Vaishnavi C (1993): Bioaccumulative potential and toxicity of endosulfan insecticide to non. Target animals. *Comp. Bio. Chem. Physiol.* 105 : 347-61.

Naqvi SM and Vaishnavi C (1993): Bioaccumulative potential and toxicity of endosulfan insecticide to non- target animals. Comp. Bio. Chem. Physiol. 105 : 347-61.

Naumenko EV, Amstislavskaya TG, Osadchuk AV. The role of the adrenoceptors in the activation of the hypothalamic-pituitary-testicular complex of mice induced by the presence of female. Exper Clin Endocrinol 1991; 97(1):1–12.

NCI (National Cancer Institute). 1977. Bioassay of chlordane for possible carcinogenicity. Technical Report Series No. 8. U.S. Department of Health, Education and Welfare; National Institutes of Health.PB 271 977.

Nelson WO and Patenelli DJ (1965): The chemical control of spermatogenesis. In : Agents affecting fertility. Austin CR and Perry JS (eds). Boston : Little Brown. P 78-82.

Neskovic NK, Karan VZ, Vitorovic SL and Vojinovic VD (1991) : Toxic effects of bound malathion residues in rats. Biomed. Environ. Sci. 41 SS4 : 423-32.

Nicander L (1967): An electron microscopial study of cell contacts in the seminiferous tubules of some mammals. Z. Zellforsch. Microsh. Arc. 83 : 375-97.

Norgil Damgaard, I.Main, K.M.Toppari, J. and skakkebaek N.E. (2002). Impact of exposure to endocrine disruptors in utero and in childhood on adult reproduction. Best Pract. Res.Cln.Endocrinol.Metab. 16,289-309.

Nurbanu Ozer (1993): Investigation of the effect of malathion on muse, liver parenchymal cells of light microscophycal level. Ph.D. Thesis, University of Yukrain.

Ohlson CG., Hardell, L. (2000) Testicular cancer and occupational exposure with PVC. Chemosphere, 40, 9-11

Omoyakhi J.M., Orheruata Osinowo O.A. (2008): Actellic 2 % dust as pesticide in feed ingredients : effect on haematological and serum metabolite in growing rabbits. Afrian Journal of Biotechnology. 7 (3) : 201-206.

Osadchuk AV, Naumenko EV. Role of genotype and some forms of social behavior in the regulation of the testical endocrine function in mice. Dokl Akad Nauk SSSR 1981;258(3):746–49 (In Russian).

Osamu Takahashi, Shinshi Oshi, Masako Yoneama, Akie Ogata, Hisashi Kamimur (2007) : Antiestrogenic effect of parachlorodibenzene in immature mice & rats. Archieve of toxicology : 81 : 505-517

P.Jaseph John (2006): Alteration of Certain Blood parameters of freshwater teleost Mystus Vittatus after chronic Enposwe to Metasystox and Sevin. Fish Physiol Biochem. 33 : 15-20.

P.N. Saxena, L.K.S. Chauhan and S.K. Gupta (2005): Cytogenetic effects of commercial formulation of cypermethrin in root meristem cells of Allium sativum: Spectroscopic basis of chromosome damage. Toxicology, Vol 216, Issue 2-3, 244-252

Padmaja ST, Reddy N, Uma P, Reddy M and Raju TN (2000) : Selenium toxicity in wistar rat. Haematological studies. J. Environ and Poll. 7 (4) : 295-98.

Padungtod C, Lasley BL, Christiani DC, Ryan LM and Xu M (1998): Reproductive hormone profile among pesticide factory workers. J. Occup Environ. Med. 40 : 1038-47.

Panda SK, Mohanty NK and Rathore PK (1999): Acute renal failure following organophosphorus poisoning. Ind. J. nephrology. 9 (2) : 61-64.

Patil S, Patil SR, Londonkar R and Patil SB (1998): Effect of pathidine on spermatogenesis in albino rats. Ind. J. Pharmacol. 30: 249-53.

Peitl PJ, sakamoto-Hojo ET, De Syllos Colus IM. Genotoxic activity of the insecticide Nuvacron (Monocrotophos) detected by the micronucleus test in bone marrow erythrocytes of mice and in CHO cells. Braz J Genet 1996; 19 : 571-6.

Plaa GL and Hewitt WR (1982) : Detection and evaluation of chemically induced liver injury.In "Principals and methods of Toxicology" ed. Hayes AW, Raven Press, New York. p.407-45

Popova NK, Amstislavskaya TG, Kucheryavy SA. Sexual motivation in male mouse produced by female exposure. Zhurnal Vishei Nervnoi Deyatelnosti 1998;48:84–8 (In Russian).

Prakash N and Venkatesh N (1996): Human Chronics Gonadotrophin (hcG) protects malathion induced PLA Leuteinizing hormone and testosterone changes in Rats. *Indian J. Pharmacol.* 28 : 257-60.

Prasad MRN (1972) : control of fertility in male In pharmacology and the future of man. Proc 5th Int. Cong. Pharmacology. San Francisco.

Prashanath MS, DavidM, Mathed SG (2005) : Behavioural changes in freshwater fishcirrhinus mrigala (Hamilton) exposed to cypermethrie J.Environ. Biol. 26 (1) 141-144.

Prins G, Birch L and Greenc G (1991) : Androgen receptor localization in different cell types of the adult rat prostate. Endocrinology. 129 : 3187-199.

Qadri SS, Usha G, Jabeen K, Rahman MF and Mustafa M (1987) : Effect of dermal application of phosphamidon on different tissues and haematobio chemical parameters in albino rat. J. Toxicol. Environ. Hlth. 20 (1553) : 273-89.

Rani, B.E., N.G. Karanth, and M.K. Krishnakumari. 1992. Accumulation and embryotoxicity of the insecticide heptachlor in the albino rat. J. Environ. Biol. 13 (2) : 95-100.

Rao RP, Kaliwal BB. Monocrotophos induced dysfunction on estrus cycle and follicular development in mice. Ind Health 2002; 40 : 237-44.

Rao VJ, Gunda VG, Srikanth K, Arepalli SK (2007): Acute toxicity bioassay using Paramecium Caudatuam, a key member to study the effects of monocrotophos on swinging behavior, morphology and reduction. Toxicol. Environ. 89(1): 1-10.

Rattner BA, Sileo L and Scanes CG (1982) Oviposition and the plasma concentration of LH, progesterone and corticosterone in bobwhite quail (Colinus virginianus) fed parathion Journal of Reproduction and Fertility 66:147–155

Reeves JD, Driggers DA and Vincent AK (1981) : Child leukemia and Aplastic anemia after malathion exposure. The Lancet. 300.

Reitman S and Frankel A S (1957) : A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. Clin. Pathol.* 28 : 56.

Reuber MD (1981) : The role of toxicity in the carcinogenicity of endosulfan, Sci. Total. Environ. 20 : 23-47.

Rogan, W.J : Ragan, N.B. (2003) Evidence ofeffects of environmental chemicals on the endocrine system in children, Pediatrics, ; 112, 247-252

Ronald K, Albright MD, Barry WK, Robert P and White MD (1983) : Kidney filure after man sprays malathion in home. *J. of the American Med. Assoc. (JAMA).* 250 : 18-20.

Roy, P, Salminen, H, Koskimies, P, Simola, J, Smeds, A, Saukko, P., Huhtaniemi I.T., 2004. Screening of some anti-androgenic endocrine disruptors using a recombinant cell-based in vitro bioassay. J Steroid Biochem Mol Biol. 88, 157-166.

Roy, P., Franks, S., Read, M., Huhtaniemi, I.T. 2006. Determination of androgen bioactivity in human serum samples using a recombinant cell based in vitro bioassay. J Steroid Biochem Mol Biol. 101(1), 68-77.

Roy, P., Pereira, B. M. J., 2005. A treatise on hazards of endocrine disruptors and tool to evaluate them. Indian. J Exp Biol. 43, 975-992

Ruijten MW, Salle HJ, Verberk MM, Smink M. Effect of chronic mixed pesticide exposure on peripheral and autonomic nerve function. Arch Environ Health 49:188-195 (1994)

Rupa DS, Laksman Rao PV, et al. In vitro effect of Monocrotophos on human lymphocytes. Bull Environ Contam Toxicol 1988; 41 : 737-41.

Russel LD, Malone JP and Maccuraly DS (1981) : Effect of the microtubule disrupting agents, coichicine and Vinblastine on seminiferous tubule structure in the rat. Tissue cell. 13 : 349-67.

Sanchez-Pena LC, Reyes BE, Lopez-Carrillo L, Recio R, Moran-Martinez J, Cebrian ME, Quintanilla-Vega B. Organophosphorous pesticide exposure alters sperm chromatin structure in Mexican agricultural workers. Toxicol Appl Pharmacol (2004) 196:108–113.

Sandhu SS, Waters MD, Simmon VF, et al. Evaluation of the genotoxic potential of certain pesticides used in Pakistan. Basic Life Sci 1985; 34:185-219.

Sarkar SN, Majumdar AC and Chattopadhyay SK (1997) : Effect of isoproturon on male reproductive system : Clinical histological and histoenzymological studies in rats. Ind. J .Exp. Biol. 35 : 133-38.

Sarmah, A.K., Northcott, G.L., Leusch, F.D.L., Tremblay, L.A., 2006. A survey of endocrine disrupting chemicals (EDCs) in municipal sewage and animal waste effluents in the Waikato region of New Zealand. Sci Total Environ 355, 135-44

Satoh, A. And H. Kikawa. 1992. Metabolic fate of cis-and trans-chlordane in mice. Nippon Eiseigaku Zasshi 47 (4) : 818-825.

Satoh, K., Nagai, F. Aoki, N., 2001. Several environmental pollutants have binding affinities for both androgen receptor and estrogen receptor. J Health Sci. 47, 495-501.

Serelini G-E, Cellier D and De Vendomois JS (2007) : New analysis of a rat feeding study with a genetically modified maize reveals signs of hepatorenal Toxicity. Archives of Environment Contamiuation and Toxicology. 52 : 596-602.

Sergei Ya. Amstislavskya, Tamara G. Amstislavskayab, Victor P. Eroschenkoc (1999) : Methoxychlor given in the periimplantation period blocks sexual arousal in male mice. Reproductive toxicology volume 13 issue 5, 405-411

Shaikh PD, Manivannan B, Pathan KM, Kasturi M and Ahmed RN (1993) : Antispermatic activity of Azardirarchta indica leaves in albino rats. Current Science, 64 (9) : 688-89.

Sharma S S and Gupta Y K, 1998. Effect of ginger(Zingiber officinale)against cisplastin induced delay in gastric empty in rats. J.Ethanopharmacol 62:49-55,

Sharpe RM (1983) : Local control of testicular function. Quaterly. J. Exp. Physiol. 68 : 265-87.

Sharpe, R. M., Irvine, D. S., 2004. How strong is the evidence of a link between environmental chemicals and adverse effects on human reproductive health? Br Med J. 328, 447-451.

Sherins RJ and Hawards SS (1978) : Male infertility. In : Campbell's urology, 4th ed. Harrison JH, Gaites RF, Perlmutlor AD, Stamey TA and Walsh PL (eds) WB saunders Co. Philadelphia vol. 1. p. 175-80.

Shewta Agarwal, Kashev C. Pandey(2007):Biochemical alteration induced by monocrotophos in blood plasma of fish,channa punctatus(Bloch).Pesticide Biochemistry and Physiology.88(3):268-272.

Shinjiro Ogita, Hirotaka Uefuji, Yuko Tatsumi, Masayuki Morimato, Pulla Kaothien-Nakayama, and Hiroshi Sano, 2005. Caffeine Production in tabacco plants by simultaneous expression of three coffee N-methyltransferase and its potential as a pest repellent. Plant molecular Biology Vol. 59, 221-227.

Siddiqui MKJ, rahman MF, Anjuman F and Syed Qadri (1987) : Effects of oral administration of endosulfan on some haematological parameters and serum enzymes in rats. Pesticides. 25-27.

Simmon VF, Mitchell AD, Jorgenson T. Evaluation of selected pesticides as chemical mutagens "in vitro" and "in vivo" studies. Menlo Park CA, USA: SRI International, 197; unpublished report cited in FAO94 and Skr94.

Singh S and Shrivastava K (1997) : Effect of endosulfan toxicity on glucose content of alimentary canal and liver of Heteropneustus fossilis. J. Ecotoxicol environment. 7 (4) : 247-51.

Singh S and Shrivastava N (1998) : Histopathological changes in the liver of the fish Nandus Nandus exposed ot endosulfan and carbaryl. J. Ecotoxical. Environ Monit. 8 (2) : 139-44.

Singh SK and Pandey RS (1991) : Ethanol potentiates in vivo hepatotoxicity of endosulfan in adult male rats.Ind. J. Exp. Biol. 28 : 1035-38.

Sinha N, Narayan R, Shanker R and Saxena DK (1995) : Endosulfan induced biochemical changes in the testis of rats. Vet. Hum. Toxicol. 37 : 547-49.

Sobti RC, Krishan A, Pfaffenberger CD. Cytokinetic and cytogenetic effects of some agricultural chemicals on human lymphoid cells in vitro: organophosphates. Mutat Res 1982; 102 : 89-102.

Society for Reproductive Biology & Comparative Biology (SRBCE). Translational Endocrinology & Reproductive Biology at Thiruvananthapuram, Kerala, India (2007): Toxicity of monocrotophos on tissues, protein and carbohydrate metabolism of esturine crab; Scylla Serrata.

Solleveld, S.P., J.K. Haseman, and E.E. McConnell. 1984. Natural history of body weight gain, survival and neoplasia in the F344 rat. J Natl. Cancer Inst. 72 : 929-940.

Soto, A.M., Lin, T., M., Justicia, H., Silvia, R.M., Sonnenschein, C., 1992. An "in culture" bioassay to assess the estrogenicity of xenobiotics (E-screen). In: Colborn T, Clement C (eds) Advances in modern environmental toxicology: chemically-induced alterations in sexual and functional development: the wildlife/human connection. Princetown Scientific, Princetown. NJ:Princeton Scientific Publishing; 295-309.

Spyker- Cranmer, J.M., J.B. Barnett, D.L. Avery, and M.F. Cranmer. 1982. Immunoteratology of chlordane. Cell-mediated and humoral immune responses in adult mice exposed in utero. Toxicol. Appl. Pharmacol. 62 (3) : 402-408.

Strasser F, Arni P. Chromosome studies on somatic cells of Chinese hamster. Basle, Switzerland: Ciba-Geigy Ltd, 1986; unpublished report, cited in FAO92 and Skr 94.

Stromberg. P.C. and L.M. Vogtsberger. 1983. Pathology of the mononuclear cell leukemia of Fischer rats. I. Morphologic studies. Vet. Pathol. 20 L 698-708.

Strumia MM, Sample AB and Hart ED (1954) : An improved micro haematocrit method. *Am. J. Clin. Pathol.* 24 : 1016-24.

Subramaniam A, Ohtake M, Kunisue T and Tanabe S (2007) : High level of organochlorines in milk of mothers. Chemosphere. 68 (5) :928-939.

T.S.Sreelaxmi and B.B.Kaliwal (2007) : Phosphamidone induced effects on estrous cycle, ovarian and uterine biochemicles parameters in Swiss albino mice. Pesticide biochemistry and physiology 87 (3) : 204-210.

Taguchi, S. and T. Yakushiji. 1988. Influence of termite treatment in the home on the chlordane concentration in human milk. Arch. Environ. Contam. Toxicol. 17 : 65-72.

Ternes, T. A., Stumpf, M., Mueller, J., Haberer, K., Wilken, R. D., Servos, M., 1999. Behavior and occurrence of estrogens in municipal sewage treatment plants--I. Investigations in Germany, Canada and Brazil. Sci. Total Environ. 225, 81-90.

Thakur PB and Bais VS (2000) : Toxic effects of aldrin and fanvalerate on certin haematological parameters of fresh water teleost heteropneustes fosilis. J. Environ. Biol. 21 (2) : 161-63.

Thakur PB, Bais VS (2000) : Toxic effect of eldrin and fenvelnate on certain Hematological Parameters of a freshwater heteropneustes fossisls (B.I.) J.Environ. Biol 21 (2) : 161-163.

Thomas JA (1975) : Effects of pesticides on reproduction. In : Molecular Mechanism of Gonadal Hormone. Thomas JA, Singhal RL (eds.) University Park Press. Baltimore. p.205-23

Thomas PI (1995) : Pesticides induced immunotoxicity; Are Great Lakes Residents at Risk ? *Environ. Health. Perspectives.* 103 : 55-61.

Tinwell, H., Friry, S.C., Rouquie, D., Belluco, S., Elies, L., Pallen, C., Bars, R., 2007. Evaluation of the antiandrogenic effects of flutamide, DDE, and linuron in the weanling rat assay using organ weight, histopathological, and proteomic approaches. Toxicol Sci. 100(1), 54-65.

U.S. EPA. 1997. Toxicological Review of Chlordane.

Vaidya VG, Patanakar N. Mutagenic effect of Monocrotophos –An insecticide in mammalian test systems. Indian J Med Res 1982; 76 : 912-7.

Varley H (1969) : Determination of blood urea by urease nesslerization method. In : Practical Clinical Biochemistry 4th ed. White Herrers Press Ltd., London. P. 158.

Vawda AI and Davies AG (1986) : Effect of cisplastin on the mouse testis. Acta Endocrinol. 112 : 436-41.

Vikas Kumar, Ajanta Chakaraborty, Gunda Viswanath and Partha Roy (2008) : Androgenic endocrine disruptors in wastewater treatment plant effluents in India : Their influence on reproductive precesses and systemic toxicity in male rats. Toxicology and Applied Pharmacology : 226, 1, 60-73

Vogel, J.M., 2004. Tunnel vision: The regulation of endocrine disruptors. DOI: 10.1007/s11077-005-1764-0 _C Springer 2005 Policy Sciences. 37, 277–303.

Waakjes MP, Donovan MP and Thomas JA (1982) : Cadmium induced prostate mettallothionein in the rabbit. Prostate. 3 : 23-25.

Wango LO, Onyango DW, Odongo H, Okido E and Mugweru J (1997) : In vitro production of testosterone and Plasma levels of leutenizing Hormone Testosterone and cortisol in male rats treated with heptachlor. Pharm-Toxico Endocrnology. 118 (3) : 381-86.

Warner R.E, Paterson K.K. And Borgman L (1966): Behaviouralpathology in fish:A Quantitative study of sublethal pesticide intoxination. *J.appl.Ecol.*, 3:223-247

Whipple GH (1942) : Haemoglobin and plasma protein, their production utilization and interrelation. Am. J. Med. Sci. 3 (203) : 477-87.

WHO 1999, World Health Organization.

Wintrobe's MM (1998) : Wintrobe's clinical hematology (10^{th} ed) Williams and Wilkins A Waverly Company, Maryland, USA

Wipawan Thangnipon,Wipa Thangnipon,Pinsuda Luangpaiboon and Surpranee Chinabut (1994). Effect of organophosphorous insecticides, monocrotophos, on acetyl-cholinesterase activity in the Nile Tilapia Fish Brain. Neurochemical Research, 2006 (5), 587-591

Witorsch, R. J., 2002. Endocrine disruptors: Can biological effects and environmental risks be predicted? Regul Toxicol Pharmacol. 36, 118-130.

Yamada, T., Kunimatsu, T., Miyata, K., Yabushita, S., Sukata, T., Kawamura, S., Seki, T., Okuno, Y., Mikami, N., 2004. . Enhanced rat Hershberger assay appears reliable for detection of not only (anti-)androgenic chemicals but also thyroid hormone modulators. Toxicol Sci. 79(1), 64-74.

Yamamoto T, (1998) : Evaluation of rat sperm by flow cytometry : Simleltameous analysis of sperm count and sperm motility. J. Toxicol. Sei. 23 (5). 373-378.

Young RA and Mehendale HM (1986) : Effect of endrin and endrin derivatives on hepatobilary function and carbon tetrachloride induced hepatotoxicity in male and female rats. Fund. Chem. Toxicol. 24 : 863-66.

Zacharewski, T., (1997) : In vitro bioassays for assessing estrogenic substances. Environ Sci Technol. 31, 613-623.

Zilversmit DB, Davis AK and Memphis BS (1950) : Micro determination of plasma phospholipids by trichoroacetic acid precipitation. *J.Lab. Clin. Med.* 35 : 155-60.

Zlatkis A, Zak B and Boyle AJ (1953) : A new method for direct determination of cholesterol. *J. Lab. Clin. Med.* 41 : 486-92.