RESIDUES OF MONOCROTOPHOS AND CYPERMETHRIN IN OKRA AND THEIR TERATOGENICITY IN THE RATS

BY

M.RAMA DEVI
M.Sc.(H.Sc.)

THESIS SUBMITTED TO THE
ANDHRA PRADESH AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

DEPARTMENT OF FOODS AND NUTRITION
PG AND RESEARCH CENTRE
FACULTY OF HOME SCIENCE
ANDHRA PRADESH AGRICULTURAL UNIVERSITY
RAJENDRANAGAR, HYDERABAD-500 030

February, 1991

CERTIFICATE

Mrs.M.RAMA DEVI has satisfactorily prosecuted the course of research and that the thesis entitled **RESIDUES OF MONOCROTOPHOS AND CYPERMETHRIN IN OKRA AND THEIR TERATOGENICITY IN THE RATS** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any university.

Date: 14 -1-1991

(Dr.(Mrs).P.GEERVANI)
MAJOR ADVISOR

CERTIFICATE

This is to certify that the thesis entitled **RESIDUES OF MONOCROTOPHOS AND CYPERMETHRIN IN OKRA AND THEIR TERATOGENICITY IN THE RATS** submitted in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY IN HOME SCIENCE** of the Andhra Pradesh Agricultural University, Hyderabad, is a record of the bonafide research work carried out by **Ms.M.RAMA DEVI** under my guidance and supervision. The subject of the thesis has been approved by the Student Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma or has been published. All the assistance and help received during the course of the investigation has been duly acknowledged by the author of the thesis.

P. Geervani

(Dr.(Mrs).P.GEERVANI)
Chairman of the Advisory Committee

Thesis approved by the Student Advisory Committee

Chairman :	(Dr.(Mrs).P.GEERVANI)	P. Geervani
Member :	(Dr.(Mrs).SAROJINI)	G. Sarojini
Member :	(Dr.D.D.R.REDDY)	D.D.R.Reddy
Member :	(Dr.B.NARASIMHA RAO)	B. Narasimha Rao
Member :	(Dr.A.JANARDHAN)	A. Janardhan

Contd..

Serial No.	Particulars	Page No.
V	DISCUSSION	
5.1	Survey	
5.2	Dissipation of insecticides	
5.2.1	Monocrotophos	
5.2.2	Cypermethrin	
5.3	Effcet of home processing on residue levels	
5.4	Toxicity studies	
5.4.1	Monocrotophos	
5.4.2	Cypermethrin	
	SUMMARY	
	LITERATURE CITED	
	APPENDICES	

LIST OF TABLES

Table No.	Title	Page No.
1	Absorbance values of monocrotophos at various concentrations	
2	Recovery of monocrotophos from okra fruits	
3	Frequency distribution of farmers regarding source of information on pesticides to be used on vegetables	
4	Frequency distribution of farmers source of information on dose of pesticide	
5	Frequency distribution of farmers on pesticide usage during different stages of plant growth	
6	Frequency distribution of farmers uisng pesticides at harvest and the time interval between harvest and last insecticides spray on vegetable crops	
7	Frequency distribution of farmers according to source of information regarding method of application	
8	Frequency distribution of farmers regarding awareness of expiry date of pesticide	
9	Residues of monocrotophos on okra	
10	Residues of cypermethrin on okra	
11	Effect of methods of processing on monocrotophos residues in okra sprayed @ 0.25 kg ai/ha	
12	Effect of method of processing on monocrotophos residues in okra sprayed @ 0.38 kg ai/ha	
13	Effect of method of processing on cypermethrin residues in okra sprayed @ 60 g ai/ha	
14	Effect of method of processing on cypermethrin residues in okra sprayed @ 100 g ai/ha	

Contd..

Table No.	Title	Page No.
15	Mortality of adult female rats due to acute dosing with monocrotophos	
16	Effect of sub acute doses of monocrotophos on body weight of rats during pregnancy	
17	Effect of monocrotophos on neonatal and postnatal development of pups born to rats treated during organogenesis and post partum	
18	Effect of monocrotophos on brain weights of pups at weaning	
19	Brain AChE activity of pups born to monocrotophos treated dams	
20	GABA, DNA and protein levels in brain of 21 days old pups born to monocrotophos treated dams	
21	Na^+ K^+ ATPase levels in rat brain at different doses of cypermethrin	
22	Effect of sub acute doses of cypermethrin on body weight of rats during pregnancy	
23	Effect of cypermethrin on neonatal and postnatal development of pups born to rats treated during organogenesis and post partum	
24	Effect of cypermethrin on brain weights of pups at weaning	
25	Brain AChE and Na^+ K^+ ATPase activities in pups born to cypermethrin treated dams	
26	GABA, DNA and protein content of brain of pups born to cypermethrin treated dams	

LIST OF FIGURES

Figure No.	Title	Page No.
1	Standard calibration curve for monocrotophos	
2	Standard curve for cypermethrin	
3	Standard curve for phosphorus	
4	Standard curve for GABA	
5	Standard curve for DNA	
6	Standard curve for protein	
7	Dissipation of monocrotophos on Okra	
8	Dissipation of cypermethrin on Okra	
9	Per cent removal of monocrotophos residues due to processing from okra sprayed @ 0.25 kg ai/ha	
10	Per cent removal of monocrotophos residues due to processing from Okra sprayed @ 0.38 kg ai/ha	
11	Per cent removal of cypermethrin residues due to processing from okra sprayed @ 60 g ai/ha	
12	Per cent removal of cypermethrin residues due to processing from okra sprayeed @ 100 g ai/ha	
13	Body weights of monocrotophos treated rats during pregnancy	
14	Per cent inhibition of AChE activity in monocrotophos treated rats	
15	Body weights of cypermethrin treated rats during pregnancy	
16	Per cent inhibition of brain AChE activity in cypermethrin treated rats	

LIST OF APPENDICES

Appendix No.	Title	Page No.
1	Interview schedule for information regarding insecticide usage on vegetable crops	
2	Calculation of residue half life and safety interval	
3	Calculation of LD_{50} (monocrotophos)	

ACKNOWLEDGEMENTS

The author wishes to express her immense gratitude to Dr.(Mrs.) P.Geervani, Professor of Foods and Nutrition & Dean of Home Science, Andhra Pradesh Agricultural University for her most valuable guidance, and encouragement throughout her period of study.

She expresses her sincere gratitude to Dr. D.D.R. Reddy Professor, Dept of Entomology, College of Andhra Pradesh Agricultural University and Dr.A.Janardhan, Professor, Dept of Pharmacology, College of Veternary Science, Andhra Pradesh Agricultural University, for their guidance, concern, co-operation and encouragement throughout the conduct of the study. She is extremely grateful to Dr.B.Narasimha Rao, Head All India Coordinated Research Project on Pesticides Residues, Department of Entomology, College of Agriculture, for his guidance and co-operation in extending the Gas chromatograph facilities for completing her experimental work. She is grateful to Dr.G.Sarojini, Head of All India Coordinated Research Project (H.Sc) Project, Post Graduate and Research Centre, Andhra Pradesh Agricultural University for her help and guidance.

She is highly grateful to Dr. N.C. Joshi, Director, Central Plant Protection Training Institute, Rajendranagar, Hyderabad for making possible 3 months of training in pesticide residue analysis at the CPPTI.

The author is grateful to the ICAR for the award of Senior Research Fellowship for a period of three and a half year. She is grateful for the assistance received by her from the staff, Department of Food and Nutrition, Post Graduate and Research Centre, Andhra Pradesh Agricultural University. She extends her special thanks to friends and colleagues for their concern, co-operation during her study period.

The author wishes to record her profound gratitude to her husband and son for their constant support, patient understanding and continuous encouragement throughout the period of her study.

M. Rama Devi

(M.RAMA DEVI)

...

LIST OF CONTENTS

Serial No.	Particulars	Page No.
I	INTRODUCTION	
II	REVIEW OF LITERATURE	
2.1	Pesticide residues in vegetables	
2.1.1	Studies on pesticide residue levels in controlled experiments	
2.1.1.1	Monocrotophos	
2.1.1.2	Cypermethrin	
2.2	Effect of processing on residues	
2.3	Biological effects of insecticides	
2.3.1	Effect of organophosphate insecticides	
2.3.1.1	Acetyl choline esterase	
2.3.1.2	Gama amino butyric acid	
2.3.1.3	DNA and protein	
2.3.1.4	Mutagenicity	
2.3.1.5	Other effects	
2.3.2	Effect of cypermethrin	
2.3.2.1	Sodium potassium adenosine triphosphatase	
2.3.2.2	Acute, short term and chronic toxicity studies	
2.3.2.3	Persistance in tissues	
2.3.2.4	Reproduction, embryotoxicity and teratogenicity	

Contd..

Serial No.	Particulars	Page No.
2.3.2.5	Protein metabolism	
2.3.2.6	Studies on human subjects	
III	MATERIALS AND METHODS	
3.1	Survey on pesticide usage in vegetable crops	
3.2	Dissipation studies	
3.2.1	Test host	
3.2.2	Insecticides and their applications	
3.2.3	Estimation of insecticide residues on/in okra fruits	
3.2.3.1	Monocrotophos	
3.2.3.1.1	Standard calibration curve	
3.2.3.1.2	Recovery, extraction, cleanup and determination of insecticide	
3.2.3.1.3	Monocrotophos residues on/in okra fruits	
3.2.3.2	Cypermethrin residues	
3.2.3.2.1	Recovery of insecticide	
3.2.3.2.2	Cypermethrin on okra fruits	
3.2.4	Half life value (RL_{50})	
3.2.5	Safety interval (T-tol)	
3.3	Processing procedures	
3.3.1	Washing	

Contd..

Serial No.	Particulars	Page No.
3.3.2	Washing and stir frying	
3.3.3	Washing and boiling in tamarind juice	
3.3.4	Washing and boiling in tamarind juice with turmeric	
3.3.5	Washing in sodium bicarbonate water and stir frying	
3.4	Toxicity studies	
3.4.1	Experimental subjects	
3.4.2	Insecticides and mode of administration	
3.4.3	Acute toxicity study	
3.4.4	Selection of doses for testing the effect on brain	
3.4.5	Schedule of feeding the insecticides	
3.4.6	Acetylcholine esterase enzyme	
3.4.6.1	Principle	
3.4.6.2	Reagents	
3.4.6.3	Procedure	
3.4.7.4	Calculation	
3.4.7	Sodium potassium ATPase	
3.4.7.1	Principle	
3.4.7.2	Reagents	
3.4.7.3	Procedure	
3.4.8	Gama amino butyric acid	

Contd..

Serial No.	Particulars	Page No.
3.4.8.1	Reagents	
3.4.8.2	Procedure	
3.4.8.2.1	Standard graph	
3.4.8.2.2	Estimation in sample	
3.4.8.3	Calculation	
3.4.9	DNA	
3.4.9.1	Isolation of DNA	
3.4.9.1.1	Principle	
3.4.9.1.2	Reagents	
3.4.9.1.3	Procedure	
3.4.9.2	Estimation of DNA	
3.4.9.2.1	Principle	
3.4.9.2.2	Reagents	
3.4.9.2.3	Procedure	
3.4.9.2.4	Calculation	
3.4.10	Protein	
3.4.10.1	Principle	
3.4.10.2	Reagents	
3.4.10.3	Procedure	
3.4.10.4	Standard graph	
3.5	Statistical procedures	
IV	RESULTS	
4.1	Survey	
4.1.1	Use of pesticides on vegetable crops	

Contd..

Serial No.	Particulars	Page No.
4.2	Residues of monocrotophos and cypermethrin in okra	
4.2.1	Residues of monocrotophos in okra fruits	
4.2.2	Residues of cypermethrin on okra fruits	
4.3	Effect of home processing on residue levels	
4.3.1	Monocrotophos	
4.3.2	Cypermethrin	
4.4	Toxicity studies	
4.4.1	Monocrotophos	
4.4.1.1	Acute toxicity study	
4.4.1.2	Teratogenicity testing	
4.4.1.2.1	Effect on pregnant dams	
4.4.1.2.1.1	Body weight	
4.4.1.2.2	Effect on postnatal development	
4.4.1.2.2.1	Effect on biochemical parameters in brain	
4.4.2	Cypermethrin	
4.4.2.1	Range finding test	
4.4.2.2	Teratogenicity testing	
4.4.2.2.1	Effect on pregnant dams	
4.4.2.2.1.1	Body weight	
4.4.2.2.2	Effect on postnatal development	
4.4.2.2.2.1	Effect on biochemical parameters in brain	

DECLARATION

I, **Mrs.M.RAMA DEVI**, hereby declare that the thesis entitled **RESIDUES OF MONOCROTOPHOS AND CYPERMETHRIN IN OKRA AND THEIR TERATOGENICITY IN THE RATS** is a result of the original research work done by me. I further declare that the thesis or part thereof has not been published earlier elsewhere in any manner.

M Rama Devi.

Date : 14-2-1991

(Ms.M. RAMA DEVI)

Name of the Author	:	M. RAMADEVI
Title of the thesis	:	RESIDUES OF MONOCROTOPHOS AND CYPERMETHRIN IN OKRA AND THEIR TERATOGENICITY IN THE RATS
Degree to which it is submitted	:	DOCTOR OF PHILOSOPHY IN FOODS AND NUTRITION
Faculty	:	HOME SCIENCE
Department	:	FOODS AND NUTRITION
Major Advisor	:	Dr.(Mrs.) P. GEERVANI
University	:	ANDHRA PRADESH AGRICULTURAL UNIVERSITY
Year of Publication	:	1991

ABSTRACT

Studies were undertaken on the survey of pesticide usage on vegetable crops in three representative vegetable growing areas of Andhra Pradesh, field experiments on the dissipation of monocrotophos and cypermethrin residues on Okra and effect of various cooking procedures in minimising the residues in Okra fruits. Toxicological effects of monocrotophos and cypermethrin on test subjects (albino rats) were also investigated under laboratory conditions. Pesticide usage survey in the selected vegetable growing areas indicated that at Guntur region (high pesticide usage area) farmers usually apply higher doses as well as more number of applications than the recommended, where as at Anakapalli (moderate pesticide usage area) and Ranga Redd, (low pesticide usage area) regions, except few farmers, most of them use less than the recommended doses. In general farmers mostly were guided by the pesticides dealers for pesticide use rather than official sources and none of the farmers observed any safety intervals for harvest of vegetable crops.

Field experiment on Okra for dissipation studies with monocrotophos and cypermethrin applied at recommended and higher doses recorded higher initial deposits. Half life periods ranged 4.03 to 4.14 days and 2.41 to 3.5 days for monocrotophos and cypermethrin respectively. Safety periods were recorded for monocrotophos (17-19 days) compared to cypermethrin (2.5 to 5.0 days). Washing in running water was found to be best and reduced the residues monocroptophos by 60% where as boiling with tamarind extract with or without turmeric proved highly effective in decreasing the cypermethrin initial deposits. Only marginal

difference increase in the per cent removal of residues as initial deposits or subsequent days after application was found in other processing procedures like washing and stirfrying washing in alkaline water and stirfrying both in the case of monocrotophos as well as cyperemthrin.

Toxicological studies, with monocrotohpos and cypermethrin applied orally at 0.5, 1 and 2 mg in the case of former 25, 50 and 75 mg/kg bw/day in the later for the albino rats during organogenesis period indicated distinct effects on the growth and biochemical parameters. Monocrotophos affected weight gain in rats during pregnancy, reduced birth weight, crown rump length and increased neonatal deaths, per cent survival of pups and litter weight. Cypermethrin effects were more apparent only in reducing the mean litter weight, crown-rump legnth and survival of pups at later stage. Adverse effects of monocrotophos and cypermethrin on brain size (weight), AChE, DNA, protein/DNA ratio, GABA and number of cells in brain and Na^+ K^+ ATP ase (only in case of cypermethrin) in albino rats were also investigated.

...

INTRODUCTION

CHAPTER I

INTRODUCTION

In view of ever increasing population, massive food production is a necessity which is only possible with the use of pesticides. The risks involved with the use of pesticides are occupational poisoning, as well as the dangers of pesticides residues in the food products that reach the consumer. Though small quantities are involved these risks cannot be ignored. Concern has been expressed about the effect of pesticides on the environment, arising from the agricultural use. Most of the problems associated with residues can be overcome through their judicious use, but indiscriminate use can endanger the health of human population.

The pesticide usage may vary depending on the region where the crop is grown, type of crop, economic status and awareness of the farmer. The problem of residues is further complicated by the pattern of usage, dose applied, dissipation pattern, harvest time and prevailing climatic conditions.

The residue levels in perishable food commodities such as vegetables and fruits are likely to be more since economic considerations are of prime importance to the farmer than consumer safety. The average farmer in India is not aware of the health hazards of the residues. Some of the pesticides have been shown to be carcinogenic, teratogenic and mutagenic and several

clinical studies have indicated a possible drug and chemical induced functional abnormalities.

Availability of pesticides free food on a scale, large enough to feed the entire world's population is not possible in the near future. Even after processing, pesticide residues are bound to be present in the food. Under such circumstances it is of interest to know the relevance of such levels of residues present in the food.

In the present study two commonly used pesticides viz., monocrotophos, an organophosphate and cypermethrin, a pyrethroid have been selected to study the biological effects. The major objectives of the present study are:

1. Survey on pesticide usage on vegetable crops

2. To investigate the dissipation pattern of monocrotophos and cypermethrin residues on okra

3. To study the effect of different cooking methods in reducing monocrotophos and cypermethrin residues in okra

4. To study the teratogenicity of monocrotophos and cypermethrin with special reference to brain development using rats as experimental subjects.

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Pesticides, besides contributing to increased farm productivity, also add one more entry to the long list of environmental pollutants. When used indiscriminately pesticides cause acute and long term toxic effects in humans, useful animals, fish and birds. Even when properly used, because of the persistence and tendency of some compounds to concentrate in organisms as they move up the food chain, may increase their toxicity to fish, birds and other forms of life including man.

In view of the hazard and toxic effects of pesticides the importance of environmental monitoring of pesticides was recognised and codex "Maximum residue limits" (MRLS) are recomended by the joint FAO/WHO meeting on pesticide residues (JMPR) on the basis of appropriate residue data obtained mainly from supervised trials. The residue data thus obtained reflect registered or approved usage of pesticide in accordance with "good agricultural practices" (GAP). These may vary considerably among different regions owing to differences in local pest control requirements, which are due to variety of reasons. Consequently residues in food, particularly at a point close to harvest may also vary. In establishing codex MRLS, these variations in residue due to differences in GAP are taken into account. The primary purpose of setting MRL's for pesticides residues in food and feed stuffs is to protect the health of the consumer. The acceptability of codex MRL's is judged on the basis of a

comparison of the acceptable daily intake (ADI) established by the JMPR, with the estimated intake, i.e., the total amount of a given residue actually ingested daily per person, as determined on the basis of suitable intake studies in individual countries. Intake data from such studies, compared with ADI help in determining the safety of foods in respect of pesticide residues. The CCPR, the UNEP and FAO/WHO, through appropriate expert consultations, have developed guidelines to assist governments in carrying out appropriate pesticide residues and contaminants intake studies (Lacomery, 1986). Regulation of pesticides has been at the forefront of environmental concerns in developed nations since mid-sixties. In the united states, the requirements for determining tolerance limits of crop contaminated by pesticide residues are contained in the Food-Drug and Cosmetic Act, and the Federal food and drug administration sets these limits. The environmental protection Agency is also authorised under Section 408 of the Food Drug and Cosmetic Act to establish tolerances for pesticide residues in raw commodities.

In India the limits for tolerance limits for pesticide residues in food and food commodities are approved by central committee for food standards under PFA rules, 1955 Published in the Gazette of India (PFA, 1976). Maximum residue limits, however have been set for only 31 pesticides out of the 125 pesticides registered. In India systematic monitoring is not done to evaluate the levels of pesticide residues and their biological effects. Surveys carried out by Punjab Agricultural

University, Ludhiana, Indian Agricultural Research Institute, New Delhi, 'Central Food Technology Research Institute, Mysore', Haryana Agricultural University, Hissar etc. (Gupta 1965) and some of reviews and compilations (Bindra and Kalra, 1973, Agnihotrudu and Mithyanta, 1978, Edward et al 1978, Gupta 1980, Kalra and Chawla, 1981;1983 and Gupta 1986) have clearly brought out the wide spread occurance of pesticide residues in food commodities, the levels of which are frequently above the legally permitted level. Consequently, the level of residue in animal tissues and human bodies is reported to be very high.

While in the west intensive monitoring is done to estimate the levels of pesticide residues in both abiotic and biotic components of the environment, such a programme is lacking in India. As a step in this direction ICAR has initiated an All India Co-ordinated programme on pesticide residues during the sixth five year plan during the year 1983 with seventeen centres in Agricultural Universities and two in ICAR institutes. Pesticides migrate and persist in soil, air and water. Therefore pesticide residues are present in almost all the food groups including those of animal origin. However pesticide residues present in vegetables are reviewed in this section.

2.1 PESTICIDE RESIDUES IN VEGETABLES

The residue levels of organochlorine pesticides, HCH, DDT, endrin and dieldrin in Kitakyushu district, Japan were monitored from 1971-74. Agricultural use of these insecticides

was banned in 1970, in Japan. HCH isomers, α, β, γ and δ BHC were detected in all vegetable samples taken; BHC residue appeared in the highest levels. The proportion of each BHC isomer in total BHC residues were much different from those in the technical product. Average residue levels of α, β, γ and δ BHC, dieldrin, endrin and DDT were 0.007, 0.042 and 0.010, 0.008, 0.021, 0.010 and 0.041 ppm in radishes and 0.004, 0.007, 0.009, 0.003, 0.087, 0.031 and 0.009 ppm in cucumbers. Levels found in 1974 were 0.002, 0.003, 0.001, 0.001, 0.005, 0.006 and 0.001 ppm in radishes and 0.001, 0.001, 0.001, 0.001, 0.008, 0.009 and undetectable in cucumbers (Suzuki et al. 1976).

These residues are translocated from the insecticide contaminated soil to the vegetable through the roots. Residue levels of dieldrin and endrin frequently exceeded the pesticide tolerance limits of Japan, but DDT residues were only slightly above the specified levels. Narayama et al (1986) carried out extensive survey of pesticide (organophosphate and organochlorine pesticides, acaricides and fungicides etc) residues during 1984-86, in fruits and vegetables from Japanese markets; Radish, spinach and celery appeared to be the vegetables most prone to residues and oranges and strawberry, the fruit most affected.

Data obtained in Japan between 1972 and 1980 on contamination of foods, revealed that of a total of 4396 fruit and vegetable samples examined, 188 vegetable samples and 427 fruit samples contained detectable levels of organophosphorus

pesticides. Most of the pesticide residues occured at levels below 0.01 ppm, but some occured at levels above those permitted in Japan. The pesticides most frequently detected in fruit were fenitrothion and diazinon, with peaches, pears, grapes, apples and cherries being most frequently contaminated while the pesticides predominantly present in vegetables were dichlorvos, diazinon, EPN with parsley, udo, Shungiku and spinach being most frequencly contaminated. Tejada et al (1983) reported that the pesticide residues detected in vegetables from Phillippines markets were within maximum residue limits or tolerance limits set by other countries.

Anderson et al (1982) analysed approximately 12000 Swedish market samples of vegetables, fruits, berrries, juices and marmalades for the purpose of pesticide residues. 275 samples had pesticide residues at levels exceeding Swedish tolerance; and most of the samples exceeding tolerances were imported produce. Imported apples, pears, citrus fruits, carrots and lettuces were relatively frequently contaminated. One sample of Italian carrots exceeded tolerance for HCH by a factor of 22. In another study a total of 1500 samples of 20 fruits and vegetable types obtained from Swedish markets were analysed for organophosphate pesticides. Twenty one pesticides were detected. Residues most frequently found were chlorpyrifos, Methidathion, azimphos-ethyl, azimphos-Methyl, parathion, parathion-Methyl and ethion. Residue concentrations exceeding 20 per cent of the acceptable limit were detected mainly in imported apples, pears

and citrus fruits, twenty eight samples (27-imported and one Swedish) exceeded the 1961 Swedish tolerances, the tolerances were exceed by a factor of upto 4.1.

Analysis of market samples of vegetables from various parts of India showed wide spread contamination with pesticide residue. Agnihotri et al (1974 a,b) have analysed sixty samples of okra, cowpea, brinjal, cauliflower, cabbage, mustard, spinach and radish top, drawn from eight different markets of Delhi, for malathion and organochlorine insecticides (DDT, DDE, Lindane, other isomers of BHC, Heptachlor, aldrin, Dieldrin, endrin, and endosulfan). None of the samples were found to contain any detectable residues of these insecticides except DDT and BHC. In very few samples even DDT and BHC were not present. Twenty per cent of the samples were positively above the tolerance limit. The samples showed as high as 50 ppm or more of BHC residues, suggesting a rather reckless use of chemical perhaps at or close to the time of picking of the produce for marketing in certain situations.

Three hundred and fifty samples of vegetables were tested at Andhra Pradesh Agricultural University, Hyderabad (Murthy and Reddy, 1978). DDT was detected in 65, HCH in 52, Endrin in 8, endosulfan in 6 and lindane in 14 of these samples. Six of the 75 potato samples and 6 okra samples had the residues above tolerance limit.

In 1969, 83 samples of various vegetables from Hyderabad markets were analysed and 58 samples showed the presence of organochlorine insecticide residues. (Lakshminarayana, Menon 1969). Of these the residues in 21 samples exceeded the tolerance limits, indicating a wide spread use and non adherence to recommended with holding periods. Similar market sample surveys were carried out in subsequent years (Lakshminarayana and Menon, 1975). The samples were drawn from Hyderabad markets. In case of leafy vegetables 7 out of 35 (20%) had high residue, 111 out of 192 samples of starchy vegetables had residues, with high residues in 62 cases (32.3%). Seven different types of insecticides (aldrin, BHC, DDT, dieldrin, endrin, heptachlor, Lindane) were present in these vegetables and in several cases more than one insecticide was noted on one sample. In the case of other vegetables (beans, okra, brinjal, cucurbits and tomato) 206 (147%) out of 442 had residues, yet it was above tolerance limit only in 19 (4.3%). Though the chemical control of insects was popular in these vegetables, the application seemed to be well regulated and properly used.

Market samples of tomato, carrot, cucumber, onions and radish were analysed at pesticide residue laboratory, AICRP, Rajendranagar during 1986-89 for HCH, DDT, monocrotophos, carbaryl, malathion, cypermethrin, fenvalerate and deltamethrin. The levels of residues were ranging from 0.5-12 for HCH, 0-10 for DDT, 0-3 for monocrotophos, 0-10 for carbaryl, 0-3 for melathion,

0-0.5 for cypermethrin, fenvalerate and deltamethrin (AICRP, 1990).

In Bombay, a total of 313 samples of fourteen vegetables were assessed and nearly half of the samples (153) were found to be contaminated with residues of BHC, lindane, aldrin, dieldrin, heptachlor, endrin and DDT. Leafy vegetables had a higher incidence (58.6%), compared to other vegetables (56.19%), Potatoes were found to be most contaminated and brinjals the least, while bottle gourds and bitter gourds were free of contaminants (Khandekar et al., 1982 cited by Lal et al., 1989). In 92 samples the residue levels were above FAO/WHO (1977) limits. Nornha (1978) also reported high level of insecticides in potatoes from Bombay markets which ranged from 0.3-7.04 ppm. Besides DDT, residues of lindane, dieldrin and endrin have been reported in these samples.

Dahiya and Chauhan (1982) screened summer and winter vegetables (okra, bittergourd, bottlegourd, brinjal, cabbage, carrot, cauliflower, tomato etc.) from Hissar market for organochlorine pesticide residues. Only 33 of the 140 samples showed DDT, BHC and endosulfan. The highest contamination was in okra followed by samples of tomato and chilli. Verma (1980) also reported wide spread contamination of vegetables from Hissar market with BHC and DDT. But in most of the samples, the residues were bleow the tolerance limits. Similarly samples of vegetables from Punjab showed negligible residues of organochlorine insecticides (Binara and Kalra, 1973).

However, all the 300 samples of leafy vegetables collected from a Mysore market were found to contain excessive amounts of bHC (10.5 to 20 ppm, Vishweswariah and Jayaram, 1972 cited by Lal et al., 1989). The samples analysed included, Chenopodium albunm, Trigonella foenunigraceem, Spinacia oleracea, A. Polygamurs A. gangeticus. These studies clearly showed a high level of pesticide residues in samples collected from large metropolitan cities of India like Bombay, Delhi etc., where vegetables fetch good price. It seems that the cultivators are using pesticiues recklessly and injudiciously without observing the safe waiting periods, otherwise such high residues are not possible.

One step towards the avoidance of environmental pollution from pesticides through food chain is to ensure that all plant protection schedules are as far as possible free from hazards due to toxic residues. Agnihotri et al. (1980) have reviewed almost 150 insecticide schedules of various field crops, food grains and vegetables, that have been evaluated from toxic residue angle upto 1976. In most of the cases, if recommended practices were followed, the residues fell below the maximum residue limits. Their results, clearly show that required safety waiting period was not observed. Not only this, sometimes residues were reported much above the initial deposit obtained in the supervised trials, indicating that cultivators were not using the proper dosage.

2.1.1 Studies on pesticide residue levels in controlled experiments

Residues data from supervised trials are the primary source of registration of pesticides and for setting of maximum residue limits (MRL is set at a level necessarily left after a pesticide has been used according to good agricultural practice). Such data are being generated by various agricultural universities and ICAR institutions in the country (Bindra and Kalra, 1973; Agnihotrudu and Mithyantha, 1978, Edwards et al, 1978; Agnihotri et al, 1983; Krishna Murthy, 1984). Apart from fixing MRL's these experiments are also conducted to study the dissipation pattern of the pesticides. Since such studies are numerous, only those studies where monocrotophos and/or cypermethrin were used on common vegetables have been reviewed here.

2.1.1.1 Monocrotophos

Dissipation of monocrotophos from chickpea sprayed at the pod formation stage at concentrations of 0.04, 0.06, 0.08 and 0.1 per cent was studied (Singh and Gupta, 1981) and samples were taken at 0, 1, 3, 7, 10, 20 and 44 days after the spray. Deposits from 0.04, 0.06, 0.08 and 0.1 per cent concentration were 9.42, 14.53, 18.59 and 21.94 ppm respectively. The rate of dissipation was rapid in the begining. On the 10th day after application, the per cent reduction in the residues showed values 91.3, 86.4, 83.3 and 80.72. The maximum time required for the

residues to reach 0.5 ppm tolerance limit was 10.31, 19.00, 19.88 and 25.26 days for the four concentrations.

When monocrotophos was sprayed on brinjal seedlings at the rate of 0.025 per cent the residues persisted for 8 days (Veeravel and Bhaskaran, 1979). Srinivasan and Lingappa (1986) studied the dissipation of monocrotophos residues in onion crop, sprayed at the rate of 0.05 and 0.075 per cent concentration. The initial deposits were 1.02, 1.05 and 1.40 ppm on onion tops, during winter, summer and monsoon seasons respectively at 0.05 per cent spray concentration. The corresponding values for 0.075 per cent spray concentration were 1.94, 2.20 and 2.23 ppm. In 20 days these levels fell below the detectable limits in winter and summer seasons, while 15 and 20 days were required for low and high concentrations in rainy season. Monocrotophos residues in the bulbs after 24 hours after spray on foliage was in the order of 0.34-0.44 ppm for low concentration and 0.61 and 0.71 ppm for high concentration. Though there was depletion of residues in the tops with lapse of time the monocrotophos residues in the bulb did not increase more than the level present on first day. Minimum intervals of 14-20 days for onion tops and 6-15 days for bulbs were recommended as waiting period for the safe use of these after spraying monocrotophos.

Monocrotophos residues in coriander leaf, plant and green grains collected from field, sprayed at the rate of 0.04 and 0.06 per cent were studied. The samples were collected 0, 1, 3, 7, 10, 15 and 20 days after spray. Dry grain samples were

collected after 35 days of application. An initial deposit of 7.82 and 11.59 ppm resulted from 0.04 and 0.06 per cent application of monocrotophos respectively. The residues reached the tolerance limit of 0.2 ppm in 14 and 15 days, respectively indicating a waiting period of 15 days for safe use of the plant. The residue half lives were 3 and 2.7 days respectively. The leaves had an initial deposit of 5.82 and 8.22 ppm at 0.04 and 0.06 per cent application respectively. The residues reached the tolerance limit of 0.2 ppm in 14 and 15 days and it was below detectable levels after 16.6 and 16.82 days and half life values were 3.01 and 2.73 days for 0.04 and 0.06 per cent application respectively. The residue in green grain was below detectable level with 0.04 per cent spray on 10th day after application. However, 0.23 ppm residue was present with 0.06 per cent spray. The residue reached below detectable level at 15 days after application of both the doses. At harvest no detectable residues of monocrotophos at both concentrations were found in dry grains (Jain et al., 1987).

The chilli crop was protected with 4 sprays of 0.08 per cent monocrotophos at an interval of 15 days starting from 20 days after transplanting. The initial residue was 16.82 ppm which reached below tolerance limit in 9.6 days, indicating a waiting period of 10 days. The residue fell below detectable limit by 15 days (Patil and Dethe 1984).

Nandihalli and Thondadarya (1986) also applied monocrotophos on chilli crop at 2, 5, 8 and 11th week after transplanting at the rate of 0.45 kg ai/ha which left a residue of 0.632 ppm after 2nd week of last spray. After fourth week of last application the residues were not detectable. Further young fruit had more insecticidal residue than matured fruits.

Narkhede et al, (1977) sprayed Nuvacron at the rate of 0.08 per cent on chilli crop. The initial deposit on chilli fruit was 11.97 ppm, which fell to 2.44 and 0.76 by first and fifth day respectively.

Metasystox (0.025%) and monocrotophos (0.025%) were sprayed on soybean crop at the flowering stage and the spraying was repeated after 20 days. Pod samples were collected at 0, 3, 7, 10 and 15 days after the second application and soybean seeds at harvest. The initial deposits were 3.3 ppm for metasystox and 2.5 ppm for monocrotophos. The monocrotophos residues were below detectable limits at 15 days after application (Awasthi et al, 1978).

Dixit et al, (1981) sprayed monocrotophos at 0.03 and 0.05 per cent concentrations, at the rate of 1100 l/ha on okra during fruit bearing stage. A repeat application was done 20 days after the first spray. Samples of fruit were analysed after one hour, 3, 7, 10 and 15 days for each of the sprays. The deposits from 0.03 and 0.05 per cent concentrations were 2.8 and 6.25 ppm after the first spray. The percentage reduction of

these residues was 36.8 and 37.6 in 3 days 71 and 63.2 per cent in 7 days and 81.5 and 82.4 in 10 days. Further no detectable residues of monocrotophos could be found from 0.03 per cent spray treatment after 15 days. After the second spray, the deposits from 0.03 to 0.05 per cent concentration were 4.2 and 7.0 ppm. They were reduced to 2.42 and 4.3 ppm in 30 days, 0.8 and 1.3 ppm in 10 days and to below detectable limits and 0.06 ppm in 15 days.

Pusa Sawani variety of okra crop was sprayed with monocrotophos during fruiting period at 0.5 kg ai/ha in three different sets of experiments. Medium and normal fruits were harvested at 0, 1, 3, 4, 7 and 10 days after spraying. Deposits of monocrotophos ranged from 0.23 to 0.49 ppm in different experiments. In all the three experiments it was observed that in four days the residues dissipated to levels below 0.2 ppm, the tolerance limit for monocrotophos on beans, cabbage, cauliflower and brussels sprouts (Krishnaiah and Prasad 1978).

The efficacy of monocrotophos in controlling Okra pests was studied by Bagle (1987). Three applications of monocrotophos at 0.5 kg ai/ha dose were done commencing from 15 days after sowing. Though monocrotophos was found to be effective in controlling pests of okra, residue levels were higher at fruiting stage, requiring a waiting period of 14 days.

Monocrotophos was sprayed at the rate of 0.05%, 0.25 kg ai/ha, 0.5 kg ai/ha and 0.07%; 0.07% on okra, beans, chillies

and tomato. The residues were estimated after 9, 7, 7 and 15; 7 days after the spray. The residues were 0.6, 0.26, 0.71 and 0.18; 6.57 ppm respectively (AICRP, 1980).

2.1.1.2 Cypermethrin

Rai et al. (1980) in their studies applied, cypermethrin (40-50g ai/ha), fenvalerate (500g ai/ha), permethrin (400g ai/ha) and malathion (500g ai/ha) on okra. The initial deposits of cypermethrin were low (1.1 and 1.3 ppm only) as compared to fenvalerate and permethrin because of low levels of application. The rate of loss of cypermethrin was 5.4, 12.5 per cent lower than fenvalerate and permethrin. In seven days 81.8 per cent was lost at 40g ai/ha of application and 76.9 per cent at 60g ai/ha rate of application during first spray and the corresponding loss in the second spray was 80 and 75 per cent.

The insecticides permethrin (0.017%), cypermethrin (0.015%), decamethrin (0.00375%), fenvalerate (0.0067%), DDVP (0.05%), carbaryl (0.005%) were sprayed on cabbage at the rate of 675 l/ha. The initial deposits of permethrin, cypermethrin, fenvalerate and decamethrin were 5.11, 4.8, 2.3 and 1.45 ppm respectively . The plants contained 0.035 ppm cypermethrin even on 10th day after application (Agnihotri et al, 1980).

Cypermethrin was one of the several synthetic pyrethroids tested for effectiveness in controlling pests of brinjal (Natekar 1967). It was found to be ineffective in controlling the brinjal pests. Initial deposits were low, ranging from 0.13

to 0.24 ppm and the rate of dissipation was fast, requiring a waiting period of one day.

Cypermethrin was sprayed on okra at the rate of 60 and 100 g ai/ha and analysed after 10 days of spray. The residue levels were 0.001 and 0.01 ppm, respectively (AICRP 1990).

2.2 EFFECT OF PROCESSING ON RESIDUES

Very few studies on the effectiveness of both home processing and commercial processing in reducing residues of monocrotophos and cypermethrin in vegetables have been reported in literature. Krishnaiah and Prasad (1978) found the percentage reduction of monocrotophos residues due to washing of okra to be 50 and 25 per cent at 0 and 3 days after spraying.

The effect of the various steps in the commercial canning of beans (Fahey et al, 1969) and tomatoes (Fahey et al, 1971) has been studied. Cold water washing of beans effected a 27 per cent reduction and blanching a further per cent reduction. Residues in the canned beans were only 2 per cent of the residues in the fresh beans. the process of cold washing and lye peeling reduced residues in tomatoes by 63 per cent and 87 per cent respectively. An 85 per cent reduction in residues of monocrotophos was achieved in tomato juice and a 91 per cent reduction in whole tomatoes by canning.

Awasthi et al. (1986) studied the effect of treating the brinjal with a number of chemical solutions and reported that dip

treatments of fruits with water, sodium chloride solution, Hcl solution, acetic acid solution or potassium permanganate solution were all found to ensure 30-35 per cent loss of the residues, sodium hydroxide solution removed 40-45 per cent and teepol solution removed 60 per cent. The efficiency of these treatments in reducing the residues was found to decrease progressively at the second and third harvests. As a result no significant difference in the effect of dip treatments in various chemical solutions was found in the later stages except with Teepol and NaoH solutions.

Very recently the removal of 42 and 47 per cent deposits of monocrotophos in beans was recorded by scrubbing and washing the fruits in hot 2% salt water and washing pods and cooking for 15 minutes respectively. It was also found 31 to 32% removal of deposits of monocrotophos from tomato fruits by washing in 2% salt water and cooking for 15 minutes or by washing and steam cooking for 15 minutes (AICRP, 1990).

Effect of cooking on loss of cypermethrin residues from okra was studied by Rai et al (1980). Three day old okra from cypermethrin treated plots were water washed and boiled. This resulted in 70-80 per cent los. of residues.

2.3 BIOLOGICAL EFFECTS OF INSECTICIDES

The ultimate aim of analysing levels of various pesticides in different ecosystems is to correlate their presence to man's well being. In an endeavour to have a better understanding

of the problem posed by the pesticide residues present in the environment, in addition to the metabolism and mode of action of pesticides, their interactions and resulting effects on various body tissues have been worked in several species of animals including man, wherever possible. These studies include acute, sub acute and chronic exposure studies, reproduction, embryotoxicity, mutagenicity, carcinogenicity and teratogenicity studies. In subacute and chronic studies the effects on AChE levels in brain and blood was the most common parameter studied. Since review of all such work done is beyond the scope of this study, only most relevant studies have been reviewed with specific reference to monocrotophos and cypermethrin.

2.3.1 Effect of organophosphate insecticides

2.3.1.1 Acetylcholine esterase (AChE)

The primary biochemical effect associated with toxicity caused by organophosphate pesticide is inhibition of AChE. The normal function of AChE is to terminate neurotransmission due to acetyl choline that has been liberated at cholinergic nerve endings in response to nervous stimulus. Loss of AChE activity may lead to a range of effects resulting from excessive nerve stimulation and culminating in respiratory failure and death (WHO, 1986).

Janardhan (1981) has reported a dose related inhibition of brain AChE activity in adult rats of both sexes and whole blood ChE activity in dogs treated orally for a period of 90 days

with monocrotophos at 0, 0.3, 0.6 and 1.2 mg/kg bw/day doses in case of rats and 0, 0.2, 0.5 and 1.0 mg/kg bw/day in dogs. Towards the end of experiment, some recovery in enzyme activity was observed, suggesting an increased production of ChE or an induction of detoxifying enzymes.

Groups of Japanese quail (12 males and 12 females per group) were fed monocrotophos at 0, 0.5, 5 and 50 ppm in the diet for three weeks. Blood cholinesterase activity was depressed at all feeding levels, while brain cholinesterase activity was depressed at 5 ppm and above (Shellenberger 1966).

The dietary components also seem to affect the efffects of pesticides. Indira and Kurup (1980) studied the effect of fibre isolated from Blackgram fed at a level of 30 per cent in the diet, on the toxic effects of malathion, in rats. It raised the AChE in the serum and brain, which is decreased to a low level by malathion. Retardation of growth of rats caused by malathion was also prevented by blackgram fibre. It was interpreted to be due to blackgram fibre which may adsorb malathion and decrease its absorption. This may be one of the reasons for the decreased toxic effects observed in the presence of fibre.

Naidu et al. (1978) noted a significant depression of brain ChE activity in chicks fed 5.0, 100 and 500 ppm of dichlorvos. Since the usual cause of death in mammals from organphosphate insecticide poisoning is respiratory failure resulting in part from a failure of the respiratory control

centre of the brain, Forsyth and chambers (1989) have investigated the ability of rat brain to activate and subsequently degrade two phosphorothionate insecticides, parathion and EPN. The findings demonstrate that brain possesses both phosphorothionate activation and oxon degradation abilities, both of which may be significant during exposure to organophosphates.

2.3.1.2 Gama Amino Butyric acid (GABA): GABA is formed in the central nervous system of vertebrate organisms by the decarboxylation of L-glutamic acid, a reaction catalysed by an L-glutamic acid decarboxylase (GAD-I) a B6 dependent enzyme. GAD-I is present largely in gray matter and is associated with pinched off nerve endings or synaptosomes (Salagani co and DeRobers 1965). In addition to GAD-I, the occurrance of a second GAD (GAD-II) has been reported. GAD II has no apparent B6 requirement and is associated with glia (Haber, et al, 1970) with the mitochondrial fractions of neural and non-neural tissues (Haber et al 1970 b, c). GAD-II is aclinated by anions and carbonyl trapping agents such as anunoxyacetic acid (AOAA) while GAD-I activity is inhibited by these agents (Roberts and Simonsen,1963). The reversible transamination of GABA with L-Ketoglutarate converts GABA to succinic semialdehyde and is catalysed by GABA L-glutaric acid transaminase (GABA-I), a B6 dependent enzyme found largely in grey matter (Salvador and Albers,1959).

Virtually all the data in the literature are consistent with the suppostion that steady state level of GABA in various

brain areas are governed by GAD activity and not by that of GABA-T (Roberts and Kuriyama, 1968). The curves representing the rates of change in GABA levels in the guinea pig, rat, mouse and dog brain were biphasic. Brain GABA levels have been measured during development in the mouse, rat, guinea pig, rabbit, cat, dog and chick. Neonatal cerebral GABA levels are generally low and increase with age. The time required to reach adult brain value is variable and species dependent. In the rat, mouse and rabbit brain, both glutamic acid and GABA increase dramatically during the first 21-30 days of life; at the same time GAD, amino butyrate amino transaminase (GABA-T) and succinate semialdehyde dehydrogenase (enzyme active in intermediary metabolism and TCA cycle) reach their highest levels of activity.

Bayer and McMurray (1967) report the GABA level in rat brain to be 0.03 and 0.02 moles/g wet weight of brain, at birth and at adult stages respectively.

Neal and Iversen (1969) studied the subcellular distribution of GABA in the cerebral cortex of rats. Their results support the theory of neuronal localisation of GABA and have shown that GAD may be localized mainly in synapatosomes. The large pool of glutamate consisting of presynaptic endings may be the primary source of GABA for synapatic function.

A reasonable interpretation of all the data obtained to date on adult cerebellum is that GABA is formed and stored in the presynaptic endings of the basket cells and upon stimulation is

released from them on the membranes of purkinje cells. The inactivation process probably consists of removal of GABA from the synaptic gap by transport processes. A part of the released GABA could be transported into the presynaptic endings, where most of it would be retained for subsequent release; or it could be taken up by the purkinje cell bodies, where it could be transported down the axon or metabolised via the GABA-T path way.

The possibility of glial uptake and metabolism also must be considered. The purkenje cells appear to be GABA neurons and both GAD and GABA are present in their axons, possibly in a state of transport from the cell body to the presynaptic endings of these cells on deitors' neurons in the vestibular nucleus and the cells of the intracerebellar nuclei. It is also possible that the golgi cells may employ GABA to mediate their inhibition (Fonnum et al., 1970).

It has been suggested that biochemical cross regulation may exist between an excitatory and inhibitory one (Roberts and Mattyssc, 1970). In this regard it should be noted that genetically induced high levels of AChE in the brain of ep mice, an inbred strain, known to be susceptible to convulsive seizures was paralleled by a proportionately increased level of GABA (Kurokawa et al., 1961, 1963).

Certain abnormal conditions such as administration of psychotropic drugs, hormones, hypoxia, environmental deprivation and exposure to ionizing radiation, may induce an alteration in

the level of one or more amino acids in brain tissue. Some of such induced changes have been extensively studied with special reference to GABA, glutamic acid, glutamine and aspartic acid in order to ascertain a direct role played by this group of amino acid in physiological functioning of the fully integrated nervous system (Hussein et al,1972,1965 a, b).

Hussein et al(1966) have estimated the levels of the amino acid GABA and glutamine in rat whole brain after daily injections of cyolane (an organo phosphate insecticide). With 1/2 LD50, an increase in the level of both GABA and glutamine was noted.

Ali and Hasan (1977) have studied the effect of organophosphorus insecticide Dichlorvos on the amino acid content of different regions of the rat brain and spinal cord. Their studies revealed a significant lowering of taurine, GABA, glycine, lysine, phenyl alamine and aspartic acid concentrations of different regions of the central nervous system. Dimunition of serine level was insignificant. The results suggest that dichlorvos-induced lowering of amino acid concentration varied in different regions of brain and spinal cord.

2.3.1.3 DNA and Protein : The protein content in the rat brain shows its most rapid increase from 6.27 per cent at 9 days to 7.56 per cent at 12 days followed by a reduced rate of accumulation to adult values at 30 days of the age (Pitts and Quick, 1967). The approximately 2 fold increase in the level of protein

in the brain of the rat from birth to maturity found by Bayer and McMurry (1967) was concomitant with the inversely proportional decrease in water content reported by Vernadakis and Woodbury (1962).

In the rat increments in the protein content of the central nervous system paralleled an increase in the activity of succinate semialdelyde dehydrogenase from 10 days onwards (Pitts and Quick 1967). Significant strain differences in protein concentration in rat brain were observed by Bennet et al (1962).

Oja (1967) studied protein metabolism in the developing rat brain and showed that although the breakdown rate of brain protein was relatively slow early in postnatal life, the catabolic rate rose until about 14 days of age and then diminished. While the rate of protein synthesis did not exhibit a clear cut trend, it seemed to be lowest in the adult rat.

Chanda et al (1973) investigated the cerebellar development in the rat after early postnatal damage by Methyl Azoxy Methanol (MAM). Cerebellum showed 50 per cent reduction in DNA, RNA and protein and wet weight.

Apholate interfered with nucleic acid and protein synthesis and alkaline phosphatase, GPT and 5' nucleotidase activities when administered at a dose rate of 0.05 mg/100g body weight in male albino rats. The normal levels were restored at 96 hour post-treatment. They opined that the interference of

apholate was due to its alkylaling action, primarily on DNA (Haqqui and Adhami, 1979).

Matsumol et al. (1972) treated rats with Methyl Azoxy Methanol (MAM). They observed reduction in foetal brain during the first 3 days after injection of the compound into the mother rat. And the MAM treated brain grew at almost normal rate after this period, but the reduction in DNA persisted through maturity of the animal. Complete or partial inhibition of DNA synthesis has been shown to be associated with but not necessarily responsible for, teratogenesis in the offspring of pregnanat rats treated with cytosine arabinoside (Ritter et al 1971, Scot et al. 1971).

Monocrotophos had no dysmorphogenic effect on the fetuses of both rats and rabbits. It could affect adversely by reducing fotal weight and mean litter weight of the pups at birth. The weight of live pups at the time of weaning was also reduced significantly due to the pesticide with a concomitant decrease in the number of live fetuses and the new borns and growth retardation at the time of weaning in the treated groups, is an indication of the embryotoxicity of the pesticide (Janardhan et al 1984).

When one or five mg/kg of carbaryl was administered to pregnant rats from 11th day to the last day of gestation, no significant decrease in ChE activity was noted in blood, brain and liver tissues of mother and new born pups. However, adminis-

tration of 50 mg/kg of carbaryl from 19th to last day of gestation showed a decrease in enzyme activity in dams and pups (Declume et al., 1978).

Azodrin interfered with normal embryogenesis in chickens, chukar patridge and bob white quail (Schom et al., 1972; and Schom and Abbott, 1977). Schom et al. (1979) studied the relationship between adult and enbryonic responses. Azodrin was applied to adult and embryo chickens, chukar patridge and bobwhite quail. Chronic exposure of adult birds to azodrin mixed in their feed indicated that no definite predictions could be made about one species based on the results of another; each had a different no effect (maximum acceptable toxicant concentration) level. The chicken adults were most resistant and the quail were least resistant to chronic exposure to azodrin. Yolk injected Azodrin caused the embryos of all three species to develop abnormally. The chicken and chukar embryos developed a generalised achondroplasia, the quail were amuscular only. In general, the 3 day quail embryos were most resistant to injected azodrin and the chicken embryo least resistant. The relationship between adult and embryo response was negative.

2.3.1.4 Mutagenecity: Bhunya and Behera (1988) have studied the mutagenecity of monocrotophos in mouse cytogenic test system. Cytogenic assays like somatic chromosome abberation, micronucleus test and sperm shape abnormalities were done. Comparison of acute and chronic treatments revealed that the chemical has no cumulative effect. Relative sensitivity of cytogenic assays has

been found to be as spermshape abnormality > chromosome aberrations > micronucleus. Monocrotophos has been found to be mutagenic in the present test system.

2.3.1.5 Other effects: Monocrotophos appears to be a strong nephral poison causing glomerular contraction, vasodialation and dissolution of lymphodial tissue to form blood-sinuses besides hydropic degeneration of tubules (Kumar and Panth, 1985).

Methoxychlor and Aldrin are reported to be much more lethal than monocrotophos to p-conchonius (Panth 1982). It is of interest to note that the renal pathologies registered by monocrotophos were more severe than those caused by methoxychlor and aldrin by sublethal poisoning in the same fish.

Chengli et al (1989) reported ten cases of pulmonary edema caused by accidental or deliberate ingestion of DDVP, a commonly used organophosphate insecticide.

Variations in blood parameters of the fish, channa punctatus when exposed to sublethal concentrations of phorate 10G (0.2 mg/l), elsan 20 per cent dust (2.0 mg/l) and bazanon 5 G (500 mg/l) for 15, 30, 45, 60, 75 and 90 days were reported by Chakrabarthy and Banerjee (1988). The rapid fluctuations of blood parameters indicated the toxic effects of these pesticides and their long term effects as the values did not return to control values even after 3 months. Elsan 20 per cent dust for

erythrocytes and thrombocytes and bazonon 5 G for leucocytes were most toxic.

Monocrotophos was administered to mice at a dose rate of 0.9, 1.8 and 3.6 mg/kg bodyweight over a period of five days and sacrificed at 35 days following the first dose. No histological changes were found in the testis. However, at all the three doses sperm abnormalities were observed (Vijaykumar and Janardhan 1988).

Roberts (1974) and Ottevanger (1976) found low evoked muscle potentials and low motor nerve conductive velocity in relation to organophosphate pesticide exposure in rats.

Electromyographic synapse testing was performed and neurological information was recorded for two groups of agricultural workers. One group was not exposed to pesticide sprays while the other group was exposed to organophosphate pesticides. No significant difference was found between the groups. The electrically evoked muscle potential series in exposed workers remained as constant as those recorded for the control group. The frequency of different types of amplitude changes was the same in exposed groups and in the control group. Neurological records showed no significant deviations from normal, for exposed workers. Changes in muscle action potential were, therefore, attributed to factors outside the synapse. The authors concluded that eletromyographical synapse testing and clinical neurological

examination are not sensitive enough for early detection of latent pesticide(s) intoxication (Jusic et al., 1980).

Kortz (1977) reported that dissociated behaviour and a decrease in ChE followed malathion exposure. Verberk and Salle (1977) reported that mevinphos, at low doses for one month caused a decrease in RBC ChE in man, and these low doses may cause disturbances of nervous system if exposure lasts longer.

Azodrin was reported to be toxic to adult birds, however, there appeared to be a species difference in maximum acceptable toxicant concentration (MATC) (Schom et al, 1972). The MATC's based on their hen day production, weight loss and mortality were between 25-100 ppm in feed for chicken, 5 and 25 ppm for chukar patridge and less than 1.25 ppm for bob-white quail. The embryos from birds fed with azodrin showed no abnormalities. This was not surprising as no azodrin residues were found in the eggs of birds fed with the pesticide. Therefore the authors conclude that exposure of female to the pesticide in feed represents no danger to humans eating eggs (Schom et al., 1979)

In 1982 there were reports of high death rate of birds in Metagorda county, Texas. On investigation, it was found that the birds died after ingestion of rice treated with monocrotophos and used as baits near paddy fields. The symptoms exhibited by the dying birds were loss of muscular coordination, prostration, tetany, outstretched wings and convulsions. The brain AchE

inhibition of affected birds ranged from 82-89 per cent. The rice ingested by these birds contained 950 ppm monocrotophos at the time of analysis (Flickinger et al., 1984).

In October, 1982 three patients of a hospital developed organphosphate poisoning symptoms after taking coconut water and copra. On analysis the coconut water as well as copra were found to contain monocrotophos residues at a level of 0.34 - 0.96 ppm. These residues are higher than the WHO recommended tolerance limit of 0.2 ppm. The reasons for such high residues being, use of monocrotophos at higher than the recommended dose and not plucking the mature coconuts before injecting the trunks with monocrotophos (Shanbhag, 1989).

Mammals given multiple doses of anti AChE organophosphate insecticides, develop a tolerance to the action of these compounds. The observations that agonist resistance attends tolerance to many anti AChE compounds has led to the hypothesis that subsensitivity of the cholinergic system might mediate the tolerance phenomenon. Particularly, the hypothesis that changes in post synaptic cholinergic receptors could mediate the development of tolerance has received the most direct empirical support. Other hypothesis eg., induced metabolism, non specific metabolic changes, neurochemical redundancy were not supported by experimental evidence. Assessment of risk of toxicity due to anti AChE compounds could be influenced by the existence of an inducible tolerance to their action. Tolerance might, on one hand, be considered a protective mechanism by which the organism

normalises function despite challenges from the external environment. However, its mechanism should not interfere with other physiological functions. The loss of muscarinic receptors found in brain of animals tolerant to organophosphates raises the possibility that the complicated and delicate balance of neuronal connections might have been altered and higher brain functions might be compromised. Taylor et al (1979) suggested that loss of muscarinic receptors occuring after prolonged stimulation with an agonist may represent a pathological phenomenon.

The cholinergic system is known to play an important role in certain behavioural functions and mental disorders (Davies et al, 1978, Weis et al 1976, Davis et al., 1978). It might be possible, then, that alterations of cholinergic receptors could be associated with subtle modifications that contribute to psychological and neurological impairment. Some of the symptoms reported by certain investigators, like memory impairment in man (Gershon and Shaw 1961, Stoller et al, 1965) might be related to alterations of the central cholinergic system. However, animal studies did not reveal any memory impairment in a single trial avoidance task in rats sub acutely treated with parathion.

2.3.2 Effect of Cypermethrin:

2.3.2.1 Sodium-Potassium Adenosine triphosphatase (Na^+K^+ ATPase): The active transport of sodium and potassium is a fundamental and major energy requiring cellular process underlyig

the maintenance of cell volume, absorption processes in kidney and intestine and exitability in nerve and muscle. In fact, much of the energy metabolism of a variety of cells is directed at supplying ATP for sodium transport (Whittam 1964). In the erythrocyte where the data are most accurate, three Na^+ are pumped outward and two K^+ inward for every ATP hydrolysed. The Na^+K^+ ATPase catalyses the hydrolysis of the r-phosphate of ATP if Mg^{2+}, Na^+ and K^+ are all present, and this hydrolysis is inhibited by cardiac glycosides, such as ouabain.

The products of ATP hydrolysis, ADP and Pi inhibit the reaction, whereas AMP does not, although Na^+ and K^+ both are required to activate the enzyme, high concentrations of either ion will inhibit activation by the other. Optimal enzyme activity has been observed at Na^+/K^+ ratios between 10:1 and 5:1. The requirement for Na^+ is absolute, but numerous other ions can substitute for K^+ and do so with varying degrees of efficiency (Dahl and Hokin, 1974).

2.3.2.2 Acute, short term and chronic toxicity studies: Signs of intoxication, indicature of an action on the central nervous system consist of sedation, ataxia, splayed gait, tip-toe walk, with occasional tremors and convulsions. These signs of toxicity appear within a few hours following dosing. Survivors show clinical recovery within 3 days. Factors known to influence the oral LD_{50} value of cypermethrin include concentration, vehicle, temperature, age of the animals and the animal strain used (Coombs et al, 1976).

The acute toxicity of cypermethrin was approximately 3 times greater in 3 weeks old, than in 12-week old rats (Rose and Dewar, 1978). Signs of intoxication, such as hyper sensitivity and abnormal gait were observed in 1600 mg cypermethrin/kg feed for 3 months.

Coombs et al (1976) have conducted short term oral feeding trials for 5 weeks with cypermethrin in rats. At 1500 mg/kg they observed reduced body weight gain and food intake, piloerection, nervousness, in coordinated movement, increased liver weight, and increases in blood urea and the Hb concentration, but no pathological changes.

Groups of charles river rats, 12 of each sex were fed with dietary concentration of 0, 25, 100, 400 or 1600 mg cypermethrin/kg diet for 3 months. Signs of intoxication such as hypersensitivity and abnormal gait were observed in 1600 mg/kg group, during the first 5 weeks and one animal died. The survivors showed clinical recovery in the second half of the study. However, body weight gain was reduced, liver, kidney weights increased and there were increases in plasma-urea concentration and plasma-alkaline phosphatase activity and decrease in Hb concentration and RBC count in females and in the kaolin-cephalin clotting in males and pcv were observed. Two out of 4 male rats, killed prematurely, showed axonal breaks and vacuolization of myelin in the sciatic nerve. None of the survivors showed nerve lesions and no other pathological effects were found. In the 400 mg/kg group of rats, males showed increased weights but no histo-

pathalogical changes. No effects were found in the 100 mg/kg body weight group. Male and female rats were fed cypermethrin at dietary levels of 0, 75, 150 or 1500 mg/kg diet, for 90 days. Hematology and the results of urine analysis were normal. Both males and females receiving 1500 mg/kg showed reduced body weight and reduced food consumption during the first month of the study. Increased liver mono oxygenase activity was noted at 1500 mg/kg diet in both males and females and only in males at 150 mg/kg diet. These changes were substantially reversed within 4 weeks. Gross microscopic examination of tissues and organs did not reveal any significant difference between the treated group and the controls. There were no changes in the sciatic nerve that could be attributed to cypermethrin even at 1500 mg/kg diet (Glaister et al 1977).

Hend and Butterworth (1976) in their experiments fed cis cypermethrin to male and female rats at levels of 0, 30, 100, 300, 750, 1500 mg/kg diet for 5 weeks. Mortality as well as neurotoxic signs of poisoning were observed at 1500 mg/kg diet level. Growth was reduced at 750 mg/kg level. Food intake reduced during the initial phase at 300 mg/kg and more doses. Liver weight increased at doses of 750 mg/kg and kidney weights increased significantly at 300 mg/kg or more dose. There was substantial degeneration of liver and sciatic nerve at 1500 mg/kg. No lesions were observed in brain or spinal cord.

Beagle hounds were fed diets containing 0, 5, 50, 500 or 1500 mg/kg cypermethrin/kg diet for 3 weeks. At 1500 mg/kg severe signs of intoxication consisting of diminished food intake, weight loss, diarrhoea, anorexia, licking and chewing of the paws, whole body tremors, a stiff exaggerated gait, ataxia, incoordination and hyperaesthesia were observed. No changes in haematology, organ weight and histo pathology were observed and despite the severe signs of intoxication no lesions of the sciatic nerve were observed in the dogs. No effects were seen at 500 mg/kg feed (Buckwell and Buttwerworth, 1977).

McAusland et al(1978) conducted 2 year feeding trial with cypermethrin in rats. At 100 mg/kg dietary dose there was a significant reduced growth rate in both males and females. The other parameters like survival, biochemical, clinical, hamatology, histo pathology were not affected. 100 mg/kg diet did not produce any toxic effects.

Analysis of liver microsomal enzyme activity in 6 rats of each sex fed 0 or 1000 mg cypermethrin/kg feed for 2 years showed, cypermethrin to be a weak inducer of the microsomal enzyme, hepatic P-nitroanisole O-dimethylase (PNOD) used as an index of monoxygenase activity (Potter and McAusland 1980).

Five groups of wistar strain rats, each composed of 52 males and 52 famales were given diets containing 0, 20, 150 or 1500 mg/kg diet (equivalent to 0, 1, 75 and 750 mg/kg body weight) for 2 years. After 104 weeks at highest dose level, body

weight loss increased, liver weight increased, smooth endoplasmic reticulum in hepatocytes and some haemetological and other slight clinical changes were observed. No other changes or increases in the incidence of tumours were found. The dose of 150 mg/kg diet was considered to be no-observed adverse effect level in this study (US EPA 1984).

Rats when fed 1500 ppm showed reduced body weight gain and poor food consumption over the first three weeks but there after gained weight and ate as well as controls. The actual body weights remained depressed throughout the study including the recovery period (Glaister et al 1977). When rats were fed 1500 ppm of cypermethrin there were no haematological changes other than a slight increase of the myeloid-erythroid ratio in the bone marrow in female rats on the top dose. There was proliferation of hepatocyte smooth endoplasmic reticulum, increased hepatic aminopyrine demethylase activity at 1500 ppm cypermethrin in male and females and 150 ppm in males. These changes were considered to be due to physiological responses of liver to detoxify the chemical more effectively and not to have any toxicological significance.

Lindsay et al. (1982) fed mice with cypermethrin at 0, 100, 400 or 1600 mg/kg feed level. The body weight gain was reduced at 1600 mg/kg diet. Several haemetological changes, consistent with a mild anaemia were found in 1600 mg/kg group of animals at the interim kill i.e., 52 weeks after initiation of the experiment (decreased Hb, haematocrit and RBC counts in

males, fixed mean cell volume and mean cell Hb concentration in females) but were not found at termination. Thrombocytosis and increased liver weights were also observed in this group, at both interim and terminal kills. There were no accompanying histopathological changes. No effects were observed at dose levels of 400 mg/kg diet or less.

Cypermethrin (dissolved in corn oil) was administered to 4 groups of 8 beagle dogs of each sex at dose levels of 0, 1, 5 or 15 mg/kg body weight per day, by capsule, for period of 52 weeks. The purity of the cypermethrin was 90.6 per cent. (Cis-trans ratio, 54:46). The dogs in the highest dose group exhibited loss of appetite, tremors, gait changes, incoordination, disorientation, and hypersensitivity. No other abnormalities were found in the composition of the blood or urine or in organ weights. Dogs receiving 5 mg/kg or more showed liquid stools by this mode of administration. However, this effect was not found when cypermethrin was fed in the diet for 2 years. No effects were seen at 1 mg/kg (US-EPA, 1984).

Groups of 4 male and 4 female Beagle dogs were fed diets containing 0, 3, 30 or 300 mg cypermethrin/kg feed for 2 years. An additional group received 1000 mg/kg feed but, because of severe intoxication signs, the dose was decreased to 750 mg/kg. The signs of intoxication consisted of licking and chewing of the paws, a stiff high stepping gait, whole body tremors, head shaking, incoordination, ataxia and convulsions. After 3 weeks

of feeding at 750 mg/kg, the animal received the control diet, until signs of intoxication could no longer be seen. The dogs were then fed a diet containing 600 mg cypermethrin/kg from week 8 until termination at 2 years. No signs of intoxication were observed in dogs fed diets containing 0, 3, 30 or 300 mg/kg during the course of the study. There was reduced body weight gain in male dogs in the 600 mg/kg group. Clinical chemistry and haematological investigations performed at 6-week itervals for 2 years, did not reveal any consistent difference between treated groups and controls. The minor changes in absolute organ weight observed in the brain and thyroid in the 300 mg/kg group were not present when the differences were corrected for terminal body weight. Furthermore, a dose relationship was not apparent, and there were no accompanying histopathological changes.

Opthalmological observations performed during the course of the study did not reveal any ocular differences between treated groups and control. No abnormalities were found in the sciatic nerves, brain, or spinal cord in any of treated groups. The feeding of diets containing upto 600 mg cypermethrin/kg diet to dogs for 2 years did not reveal any treatment related gross or histopathological effects, although the dogs receiving diets containing 1000 mg/kg decreased to 600 mg cypermethrin/kg showed reduced body weight gain. Cypermethrin did not produce any toxicological effects in dogs fed dietary concentrations of 300 mg/kg feed or less, over 2 years (Buckwell, 1981).

The effects of synthetic pyrethroid pesticide cypermethrin on the immune system were studied by Desi et al (1985). Both humoral and cell mediated immune response of rabbits and rats was suppressed due to the cypermethrin treatment.

2.3.2.3 Persistence in tissues : Male albino rats were treated by oral or intra peritoneal (ip) route with pyrethroids dissolved in 125 ml of glycerol formal. Food was withheld for 24 hr. before oral treatments with a mixture of cis-permethrin, trans-cypermethrin, deltamethrin and (2-RS, α-RS) fenvalerate (each at 3 mg/kg) in one series and a mixture of trans permethrin, cis-cypermethrin, deltamethrin and (2-RS, α-RS) fenvalerate (each at 3 mg/kg) in another treatment. Residues in fat were higher and more persistant with the cis than with the trans isomers of permethrin and cypermethrin.

Another investigation utilized administration with a mixture of (1R)-cis and (FR) or with a mixture of cis-permethrin, cis-cypermethrin, deltamethrin and trans permethrin each of 5 mg/kg wt. Brain analysis were carried out at 3 hours, 3 and 5 days after treatment with the permethrin isomers and at 1, 3, 5 and 7 days after treatment with the other pyrethroids. On ip administration the brain levels were remarkably higher with (

IS-permethrin than with (1R) trans-permethrin, (1R) S-cis-cypermethrin, deltamethrin and (2S,αS) fenvalerate. The high levels and persistence of cis-permethrin was due to both extensive entry to slow dissipation between 1 to 7 days (Marei et al., 1982). Male rats were given 1.2 mg/g body weight and females were given 2.1 mg/kg body weight of cis/trans mixture of 14 cyclo propyl labelled cypermethrin, as a single oral dose. Three days after dosing, low concentration of radioactivity was found for both sexes in the kidneys, muscle, brain and blood. The highest levels were found in liver for males and fat in case of females. Concentration in muscle, brain, were less than 0.5 mg/kg, urinary excretion was rapid in both sexes, approximately 50-65% of the dose being excreted in 48 hours (Crawford, 1977).

When cows were fed diets containing 0, 2, 5, or 10 mg cypermethrin/kg feed with 14 c-benzyl and 14 c-cyclo propyl labelling, for 7 or 21 days, residues in the milk were 0.0006, 0.012 or 0.03 mg/l of milk mostly in cream phase (Croucher et al., 1985). Swaine and Sapiats of FAO/WHO (1982) dosed cows daily with 0.2, 5 or 50 mg cypermethrin for 29 days. Residues in milk were comparable to those reported by Croucher et al. (1985).

2.3.2.4 Reproduction, embrytoxicity and teratogenicity: Groups of pregnant female sprague dawley CD rats (25 animals per group) were administered cypermethrin orally as 1% solution in corn oil at doses of 0, 17.5, 35 or 70 mg/kg body weight per day, from days 6 to 15 (inclusive) of gestation. Cypermethrin at 17.5 mg/kg body weight, per day did not affect maternal performance or

fetal survival and development. At the higher doses of 35 and 70 mg/kg body weight, respectively, slight and significant retardation of meternal body weight gain was recorded. In addition, at 70 mg/kg per day slight to severe neurological disturbances were observed in nearly half of the females, including slight splaying of the hind legs while walking ranging to severe splaying of all limbs, involuntary movements of the jaws, convulsive spasms, and hypersensitivity to noise. Dispite this maternal toxicity, there were no indications of any embryotoxic or teratogenic effects of cypermethrin (Tesh et al., 1978).

Ahmed and Gupta (1988) studied reproduction toxicity of cypermethrin in rats. The female fertility index was significantly reduced at 15 mg and 30 mg/kg of cypermethrin. The pregnancy index, average litter size, live birth index, average gestation period and day 1, 4, 12 and 21 survival indices did not show any significant difference after cypermethrin. At the highest dose level (30 mg/kg) the lactation index was significantly reduced. The body weight gain was significantly reduced at day 4, 14, and 21 of lactation.

Groups of pregnant female banded dutch rabbits (30 controls and 20 for each dose group) were dosed orally with 0, 3, 10, or 30 mg cypermethrin/kg body weight per day in corn oil (by gelatin capsule) during days 6-18 (inclusive) of gestation. No influence was found on growth, pre-implantation losses, resorptions, fetal deaths, or numbers and sizes of fetuses. The

incidence of fetal visceral and/or skeletal abnormalities was comparable to that in the vehicle control group, except for a slight increase in the mean percentage of fetuses showing visceral and/or skeletal abnormalities in the group receiving 30 mg/kg body weight per day. No teratogenic effects were found in this study (Dix, 1978).

Cypermethrin was fed to wistar rats (30 male and 30 female per group) at a dietary concentration of 0, 10, 100 or 500 mg/kg for 5 weeks, after which the males and females (10 weeks of age) from each treatment group were mated. Two successive litters were produced from each pair. The first of these litters was discarded, and randomly - selected male and female pups of the second litters were mated to produce the next generation. The study was continued until 2 litters from each of 3 successive generations had been bred. The parent animals in the 500 mg/kg group consumed less food than the controls and this was accompanied by a reduction in body weight. Otherwise, the parent animals of control and treatment groups behaved similarly. Cypermethrin did not cause any adverse effects on the reproductive performance of the rats or on the survival of the offspring.

No consistent changes were observed in mean litter weight between birth and weaning in any treatment group, with the exception of a reduction in total litter weights in the 500 mg/kg diet of F1a litters on days 4, 14 and 21. There was also a statistically significant decrease compared with controls in total litter weights and size in the F1b litters at 500 mg

cypermethrin/kg diet. In the 500 mg/kg diet F0 group, one animal showed a squamous cell carcinoma of the skin. However, this was considered not to be related to the compound, because no increase in tumour incidence was found in the long-term studies in rats and mice. No changes were observed in rats administered 100 mg cypermethrin/kg diet (Hend et al., 1978).

2.3.2.5 Protein metabolism : Reddy and Bashamohidden (1988) have studied the toxic impact of cypermethrin on the protein metabolism in the branchial tissue of fish. The total, structural and soluble proteins were significantly decreased where as the neutral protease activity levels were significantly raised with a concomitant increment in the Aspartate and alamine aminotransfarase activity which showed significant elevation in all the exposure periods. Ammonia content decreased but the urea and the glutamine levels increased.

2.3.2.6 Studies on human subjects : Male volunteers were each given a single oral dose of 0.25, 0.5, 1 or 1.5 mg cypermethrin in oil in a capsule. Urinary excretion of cypermethrin was rapid. The ester cleavage was a major route of metabolism. As reported in other animal species, the trans-isomer was metabolised more readily than the cis-isomer (Eadsforth and Baldwin, 1983).

Vansittert et al. (1985) carried out experiments by feeding cypermethrin over five day period and concluded that no accumulation in the body occured.

In another field study in India, 18 workers, including spraymen (spraying an emulsifiable concentrate formulation by mist-blower or knap-sack), mixers, and loaders, handled cypermethrin daily for 5 consecutive days. Medical examination, with special attention to the sensory function of the peripheral nervous system, was carried out before, during, and after spraying (Suthers and Marlow, 1981). No compound-related adverse clinical effects of peripheral neuropathy were noticed in any of the workers. The urinary excretion of the cypermethrin metabolite (methyl ester of the cyclopropane carboxylic acid moiety) increase from day 1 to 5 of the study, but decreased 24 hours after spraying had ceased. On the fifth day, concentrations of up to 0.18 mg (average 0.1 mg) were found in the 24 hr. urine of workers using the mist-blower. Concentrations were lower in workers using the knap-sack sprayer.

A field study was carried out on Indian workers in the Satara district in India prior to, during, and after spraying cotton with the synthetic pyrethroid formulations - permethrin (Ambush) and cypermethrin (Cymbush). The formulations were applied using a mistblower or knap-sak sprayer. Exposure of 7 spraymen and 2 loader mixers was monitored by measuring the 24-h urinary excretion of 3-(4'-hydroxyphenoxy)- benzoic acid, and medical assessments were carried out by 4 medical doctors. The sensory function of the peripheral nervous system was also assessed. The formulations were applied at the recommended rates; permethrin, 150 g, a i/h a and cypermethrin, 70 g ai/ha.

Average exposure was approximately 8 hours per day for 5 days. No compound related adverse clinical effects were noticed. The average urinary excretion of 3-(4'hydroxyphenoxy)-benzoic acid increased from day 1 to 5, but then decreased 24 hour after spraying had ceased (Hart et al., 1982).

He et al. (1989) have reviewed 573 cases of acute pyrethroid poisoning reported in the chinese medical literature during 1983-84. Of these 45 cases were of acute cypermethrin poisoning. The clinical manifestations of acute poisoning induced by the major kinds of pyrethorid are very similar. Apart from the irritative symptoms of the skin and respiratory tract (or digestive tracts in intestine poisoning) acute pyrethroid poisoning is clinically characterised by abnormalities of nervous exitability.

Several studies of ocupational exposure to pyrethroids indicate that pyrethroids cause abnormal sensations of the face and irritative symptoms of the skin and upper respiratory tract in some exposed subjects (Le quesne and Maxwell, 1980; Kolmodin-Hedman et al., 1982; Tucker and Flannigan , 1983; Knox et al., 1984; He et al., 1989).

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

The investigations formed three parts viz., (1) Survey on the pesticide usage in three vegetable growing areas of Andhra Pradesh; (2) Dissipation pattern of monocrotophos and cypermethrin on okra and effect of selected processing procedures on the deposits of these insecticides in okra fruits and (3) effect of monocrotophos and cypermetnrin on some bio-chemical parameters of rat brain.

3.1 SURVEY ON PESTICIDE USAGE IN VEGETABLE CROPS

To elicit information on the pesticide usage on vegetable crops in the major vegetable growing areas, a survey was undertaken in Guntur, Visakhapatnam and Ranga Reddy Districts of Andhra Pradesh, which are identified as maximum, medium and minimum pesticide usage areas respectively.

At random about 40 farmers from four villages of each district were selected and interviewed with the help of a schedule formulated in the Appendix I.

3.2 DISSIPATION STUDIES

3.2.1 Test Host

Pusa Sawani variety of Okra (Abelmoschus esculentus) was grown in the students farm of College of Agriculture, Andhra Pradesh Agricultural University, Rajendranagar during August-November, 1987. The crop was raised in sub-plots of 8x5 m. The

plants were spaced at 30 cm within the row and 60 cm between rows. There were 13 rows per plot. In total there were 200-210 plants in each plot.

3.2.2 Insecticides and their applications

The dissipation was studied for two insecticides viz., monocrotophos (phosphoric acid, dimethyl 1-methyl 3 (methyl amino-3-oxo-1-propenyl ester), an organo phosphate and cypermethrin (RS-alpha-cyano-3-phenoxy benzyl (1 RS)-Cis-Trans-3-(2, 2-dichlorovinyl)-2, 2-dimethyl cyclopropane carboxylate)a synthetic pyrethroid, which are commonly used against the pests of okra. Monocrotophos in the form of Nuvacron 36 per cent EC and cypermethrin in the form of Ripcord 10 per cent EC were supplied by Hindustan CIBA-GIEGY Limited, Bombay and National Organic Chemical Industries Limited (NOCIL), Bombay respectively for experimental use.

Monocrotophos @ 0.25 and 0.38 kg ai/ha and cypermethrin @ 60 and 100 g ai/ha were applied as sprays. Each insecticide was applied thrice, the first spray starting at the fruit initiation stage (55 to 58 days after germination). The second and third sprays were given 15 and 30 days after first spray. Spraying was done using knapsac hand compressed sprayer. There were five treatments including untreated control and were replicated thrice. To minimise drift during spraying one meter distance was maintained between the plots and the okra fruits from the border plants were not included for sampling.

3.2.3 Estimation of insecticide residues on/in okra fruits

The residues of monocrotophos and cypermethrin present in/on okra fruits were determined by colorimetric and gas chromatographic methods respectively. After the third spray optimally mature okra fruit samples (5 to 10 cm in length) from the control and treated plots were collected at 0 (one hour), 1, 3, 7, 10 and 15 days and analysed for the residue content.

3.2.3.1 Monocrotophos

3.2.3.1.1 Standard curve : Technical grade monocrotophos of 97.6% purity obtained from Environmental Protection Agency, Bombay was used for preparation of standard solution. While preparing standard solutions, corrections were made for the per cent purity. A quantity of 102.5 mg of monocrotophos was dissolved in 100 ml of acetone to obtain a concentration of 1 mg/ml (1000 ppm).

A series of working standard solutions containing 5 to 250 mg monocrotophos were pippeted out into clean dry getz tubes. The colour was developed by modified Getz and watts (1964) procedure, reported by Jain et al. (1974). The volume in the getz tubes was made upto 5.0 ml with chloroform A drop of propylene glycol was added to each tube and the solvent was evaporated. Then 0.4 ml each of 2% 4-(P-nitro benzyl) pyridine and cyclohexylamine were added and air condensers were fixed. The tubes were placed in an oil bath for 3 minutes at 175-180°C and subsequently cooled in ice water bath for half minute. The

condensors were removed and the contents of the tubes were diluted by adding 3.0 ml of distilled ethyl acetate to each tube. A blank was also run without the insecticide solution. The absorbance of the coloured solutions was read at 540 u in Spectronic-21 against the blank (Table 1). A straight line was obtained by plotting the absorbance values against the corresponding insecticide concentrations (Fig. 1).

Table 1 : Absorbance values of monocrotophos at various concentrations

Monocrotophos concentration (mg)	Absorbance value
5	0.08
10	0.17
15	0.28
20	0.37
25	0.47

3.2.3.1.2 Recovery, extraction, cleanup and determination of insecticide : A representative 25.0 g of sample of okra fruits were collected from the untreated control plots and cut into small bits. To this sample 1.0 ml of 100 ppm technical grade insecticidal solution was added and mixed thoroughly and left for two hours to facilitate absorption of the insecticide. Later the samples were subjected to extraction, clean up and the insecticide residues were estimated.

For extraction, the sample was blended for 3 minutes with 50 ml of acetone and filtered through buckner funnel under

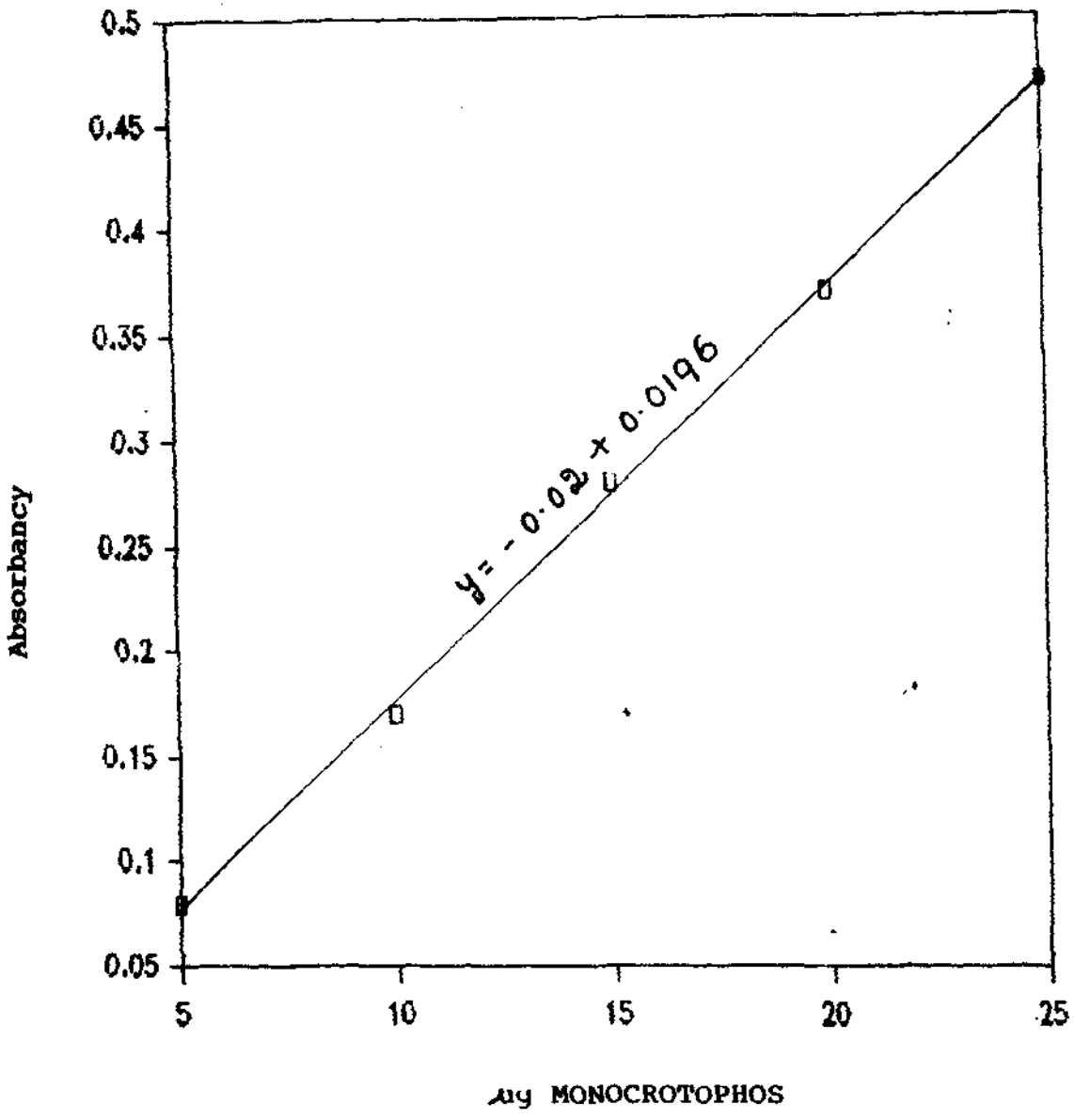


Fig.1: STANDARD CALIBRATION CURVE FOR MONOCROTOPHOS.

suction. The sample residue was transferred back to the blender and re-extracted twice with 50 ml acetone filtering each time. The filterate thus collected from the three extractions was condensed to 5.0 ml after adding a drop of propylene glycol in rotary vaccuum flash evaporator. This extract was quantitatively transferred to a 500 ml separating funnel and to it 10 ml distilled water, 5 ml chloroform, 10-25 ml of saturated sodium chloride solution were added. The contents for thoroughly mixed for one minute and the layers were allowed to separate for 5 minutes. The lower layer of chloroform was passed through a funnel containing anhydrous sodium sulphate over laid on a cotton plug. The aqueous phase was again extracted with fresh 10-15 ml chloroform and above procedure was repeated. The chloroform extract was evaporated to a volume of 5 ml.

For the clean up of the above extract 0.5 g of attivated charcoal was added and mixed well. A glass column of 40cm length and 2 cm diameter was taken and packed with a column packing consisting of 50 cm layer of anhydrous sodium sulphate over laid with 5.0 cm layer of adsorption mixture consisting of activated charcoal, magnesium oxide and cellite mixed in the proportion of 1:1:1. The elute was collected in a reagent bottle and residues were determined.

For the determination of residues 5.0 ml of the above clear elute was taken and same procedure was followed as per the standard cure. The absorbance values were interpolated on the standard curve and the residue content was expressed as mg of

pesticide residue per kg of the okra fruit. The percentage recovery of insecticide was calculated from this value (Table 2).

Table 2 : Recovery of monocrophos from Okra fruits

Level of insecticide* (mg)	Level of insecticide recovery. (mg)	Recovery (%)	Average recovery ± S.D (%)
25	24.0	96	
25	23.5	94	94.66 ± 1.15
25	23.5	94	
50	49.0	98	
50	48.0	96	97.00 ± 1.00
50	48.5	97	
Overall average recovery			95.83 ± 1.46

*Technical grade MCP was added to 25 g sample

3.2.3.1.3 Monocrotophos residues on/in okra fruits: A representative sample of 25.0 g of okra fruits treated with monocrotophos were taken for each treatment and were subjected to extraction and clean up as described earlier and the residues levels were determined. The insecticide residue values thus obtained were multiplied by the recovery factor to arrive at the exact levels of residues present in the sample.

3.2.3.2 Cypermethrin residues

The standard insecticide solution of cypermethrin was prepared by taking 0.1098 mg of technical grade material of 91.1%

purity obtained from Environmental Protection Agency (EPA) and dissolved in 100 ml hexane to give 1 ppm (1 mg/ml) solution.

3.2.3.2.1 Recovery of insecticide : Okra fruits from the untreated plots were collected and a sample of 25 g was taken and cut into small pieces and sprayed with 1.0 ml of 1 ppm solution of cypermethrin. The sample was mixed thoroughly and left for two hours to facilitate absorption of the insecticide by the fruit. The sample was then subjected to extraction, clean up and residues were determined by gas chromatography (Talekar, 1977; Baker and Bottomley, 1982; improved by Awasthi, 1985).

For the purpose of extraction the above sample was blended for 3.0 minutes with 5.0 g sodium sulphate and 50.0 ml of acetone: hexane (1:1) solvent mixture. The macerate was filtered through buckner funnel under suction. The residue was re-extracted twice as above. The combined filtrate was transferred to a separating funnel and to it 500 ml of water was added. The flask was shaken well and hexane layer was collected. The lower aqueous layer was washed twice with hexane and added to the hexane extracts. An aliquot of hexane extract representing 5.0 g of the fruit sample was taken and concentrated to 5.0 ml in a rotary flash evaporator.

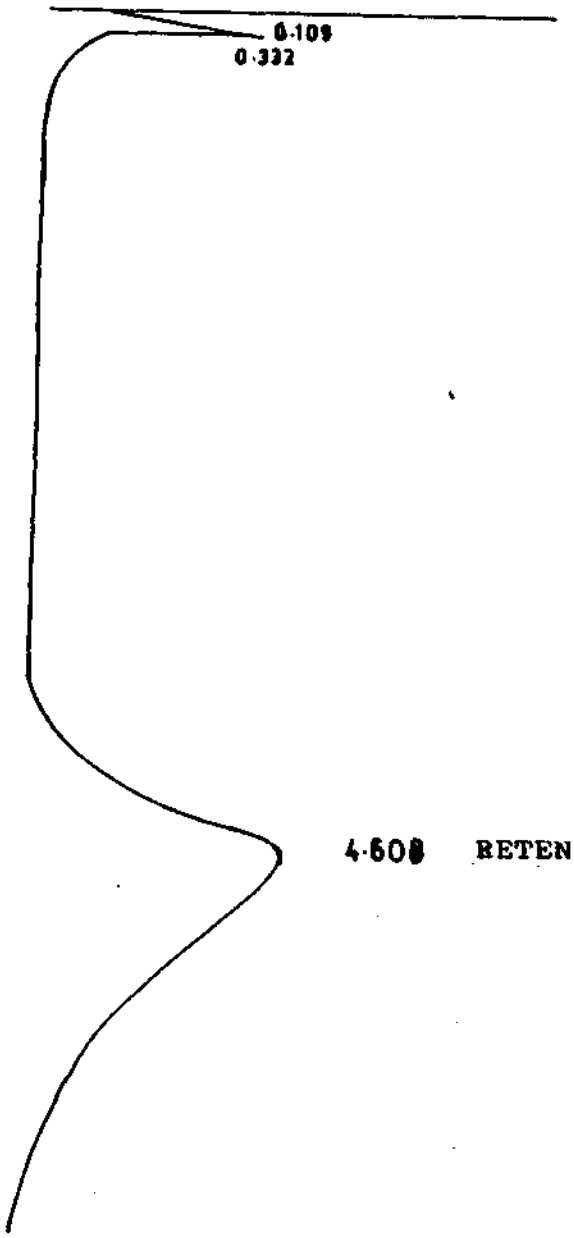
The clean up of the hexane extract was done by eluting through a glass column (10 x 300mm) containing 5.0 g florosil, 5.0 g alumina grade-III and 20 mm layer of anhydrous sodium sulphate using n-hexane : acetone (9:1) as the eluent. The eluate was concentrated to 10.0 ml.

Residues of cypermethrin were determined by gas liquid chromatography on a packard 437 A Gc model equipped with Ni 63 electron capture detector and fixed with a glass column packed with a 5% OV 101 on Gas chrom Q. Other GLC parameters were nitrogen as carrier gas at a flow rate of 100 ml/min; column temperature 240^{o}C, injection port temperature 270^{o}C and detector temperature 300^{o}C and attenuation 6, chart speed 10mm/min.

Two µl of 1 ppm standard solution of cypermethrin was injected into the GC and its retention time and peak area were noted (Fig. 2). Two µl of cleaned up sample was injected and the area of the peak eluted at the same retention time as that of the standard was noticed. The insecticide recovered was quantitated by comparing the peak areas of both the sample and standard. From this the per cent recovery of the insecticide was calculated.

3.2.3.2.2 Cypermethrin on okra fruits: Okra fruits treated with the two dosages of cypermethrin were collected separately and a representative sample of 25 g for each dose and also from untreated control plots was subjected to the extraction and clean up as described in recovery study. The residue level was determined by GLC. The values thus obtained were multiplied by the recovery factor to give the actual amount of residues present in the fruits.

Fig.2 : GAS CHROMATOGRAPH FOR CYPERMETHIN,COLUMN USED OV 101



3.2.4 Half life value (RL_{50})

Half life was calculated for both monocrotophos and cypermethrin using the formula given by Hoskins (1961). RL_{50} or t 1/2 = log 2/ki; where ki = slope of regression coefficient, (b) of log ppm residue, (y) on the number of elapsed days (x) and is calculated by the formula -

$$b = \frac{(x - \bar{x})\ (y - \bar{y})}{(x - \bar{x})^2}$$

i.e., Sum of the products of the deviations from means divided by the sum of squares of deviations from the mean x.

3.2.5 Safety interval (T-tol)

Safety intervals is the minimum number of days to be elapsed before the insecticide reaches the tolerance limit, was calculated using Hoskins formula (1961).

t tol = (log k_2 - log tol) Kl where

t tol = safety interval

k_2 = initial deposit (ppm)

tol = tolerance limit of the insecticide

k_1 = regression coefficient of the equation of RL_{50}.

3.3 PROCESSING PROCEDURES

The effectiveness of the following cooking procedures in removing the residues of monocrotophos and cypermethrin from okra

fruits was studied. For each processing procedure a representative sample of 25 g of okra fruits from the treated plots of each insecticide was used.

3.3.1 Washing

A representative sample of whole okra fruits were kept in a colander and left under tap water for two minutes and later the residues present in the fruits were estimated.

3.3.2 Washing and stir frying

After washing the whole okra fruits in running water as described above, they were cut into small pieces, fried in five ml groundnut oil for 3.0 minutes and residues were estimated.

3.3.3 Washing and boiling in tamarind juice

Okra fruits after washing were cut into small bits and boiled in 8 per cent tamarind juice (8 gm tamarind/100 ml water) for 10 min (time sufficient to evaporate all tamarind juice).

3.3.4 Washing and boiling in tamarind juice with turmeric

The okra fruits were washed and cut bits were boiled for 10 minutes in tamarind juice (8%) containing 25 mg of turmeric powder.

3.3.5 Washing in sodium bicarbonate water and stir frying

The okra fruits were soaked in sodium bicarbonate solution (75 mg/200 ml of water) for two minutes. Then the fruits

were cut into small pieces and fried in 5.0 ml groundnut oil for 3.0 minutes.

The residues of monocrotophos and cypermethrin were determined in the above processed okra fruits by following the procedure described earlier. From these values the per cent loss of residues by each cooking process was calculated.

3.4 TOXICITY STUDIES

3.4.1 Experimental subjects

Wistar strain adult female albino rats were obtained from National Institute of Nutrition (NIN).

3.4.2 Insecticides and mode of administration

Monocrotophos technical grade (72.2% purity) supplied by M/s NOCIL, Bombay and cypermethrin technical grade (92.0% purity) procured from Gharda Chemicals Limited, Bombay were used.

Monocrotophos and cypermethrin were dissolved in water and groundnut oil respectively. The pesticide was administered orally through intra gastric intubation using tuberculin syringe.

3.4.3 Acute toxicity study

Acute toxicity study was conducted to determine the LD 50 value i.e., the dose at which fifty per cent mortality of the experimental animals is observed.

Fifty rats were randomized into five groups consisting of ten rats each and technical grade monocrotophos was

administered orally at the rate of 7, 10, 12 or 15 mg/kg body weight respectively. Mortality was noted in all groups for 24 hours period.

The LD_{50} was calculated by graphic method (Finney, 1952).

In the case of cypermethrin dose related mortality was not observed and LD 50 value could not be computed. Therefore to decide the doses at which the experimental groups of rats have to be tested, a range finding test was carried out using Na^+K^+ATPase activity in whole brain. A dose range of 200, 500 and 800 mg/kg body weight was administered to four groups consisting five rats each. Control group received only groundnut oil.

3.4.4 Selection of doses for testing the effect on brain

The doses of 0.2, 0.1 and 0.05 mg monocrotophos/kg bw/day which corresponded to 1/5, 1/10, and 1/20 of LD_{50} of monocrophos were selected to test the effects on the brain.

Based on the range finding study 25, 50 and 75 mg cypermethrin/kg bw/day were chosen.

3.4.5 Schedule of feeding the insecticides

Female virgin rats weighing between 150 and 200 g were used. Three female rats were mated overnight with a normal male in a cage and the following morning the vaginal smears were examined for the presence of sperms. Findings spermatozoa in the

vaginal smear in the morning was considered as 0-day of pregnancy (Hariharan, 1980).

Each female pregnant rat was caged separately. The pregnant dam received the oral dose of insecticide from 6th to 15th day of pregnancy, (period of organogenesis, Rugh, 1968). The dose for each rat was calculated daily based on its body weight. The rats which served as controls received distilled water and groundnut oil for monocrotophos and cypermethrin groups respectively.

The dosing was resumed from the first day of delivery and continued till the pups were 21 days old (till weaning). Immediately after the birth, the pups were observed for gross malformations and their body weights and crown-rump length were recorded.

At the age of 21 days the pups were weighed, decapitated, the brains were dissected and weighed. The brains were stored under refrigeration. Subsequently, the brains were processed for estimation of acetyl choline esterase (AChE), Gama Amino butyric acid (GABA), Protein and DNA. In addition Na^+K^+ ATPase was also estimated in cypermethrin treated groups.

3.4.6 Acetylcholine esterase enzyme (Fleischer and Pope 1954)

3.4.6.1 Principle : The enzyme activity is indirectly measured by estimating the amount of acetylcholine remaining unhydrolysed at the end of the reaction.

The ester present in acetyl choline reacts with hydroxylamine to form acethydroxaminic acid.

$$(CH_3)^+ N - CH_2\ CH_2O - CO\ CH_3 + NH_2\ OH \longrightarrow$$
$$(CH_3)^+ N - CH_2\ CH_2OH + CH_3\ Co\text{-}NHoH$$
$$CH_3Co\text{-}NHOH + FeCl_3 \longrightarrow CH_3\ C(O\ldots FeCl_3)(NH - O)$$

The hydroxamic acid forms a soluble red purple complex with ferric ions in acid solution. The intensity of the colour is proportional to the concentration of Ach present.

3.4.6.2 Reagents

Phosphate buffer : 715 ml of M/15 disodium hydrogen phosphate (11.876 g of $Na_2HPO_4.H_2O$ per litre of glass distilled water) and 265 ml of M/15 potassium dihydrogen phosphate (9.078 g of $KH_2\ PO_4$ per litre of glass distilled water) were mixed together and pH of the solution was measured and adjusted to 7.2 with either M/15 $Na_2HPo_4.H_2O$ or KH_2PO_4.

Acetylcholine 0.004 M : A stock solution (0.04 M stable in refrigerator) was prepared by dissolving 0.7266 g of acetyl choline chloride in 100 ml of an Hcl solution of P^H 4.0. Before use this solution was diluted by 9 volumes of phosphate buffer of 7.2 pH.

Hydroxylamine Hydrochloride, 2 M: 13.9 g of NH_2OH HCL is dissolved in 100 ml of glass distilled water and kept in refrigerator.

Sodium hydroxide, 3.5 M : 14.0 g of NaOH was dissolved in 100 ml of glass distilled water.

Alkaline hydroxylamine solution : Equal volumes of the hydroxylamine solution and the NaOH were mixed immediately before use.

Hydrochloric acid : Concentrated HCl (sp. gravity 1.18) is diluted with 2 parts by volume of water.

Ferric chloride, 0.37 M : 10.0 g $Fecl_3$ 6 H_2O is dissolved in 100 ml of 0.1 N HCl.

0.1 N HCl : 0.117 ml of concentrated Hcl was diluted to 100 ml with glass distilled water.

TCA solution : 10.0 g of TCA was dissolved in 100 ml glass distilled water.

3.4.6.3 Procedure : To one half of the brain 50.0 ml of phosphate buffer (7.2 pH) was added and the tissue was homogenised.

For each sample two test tube marked test (T) and control (c) were taken. Into the tube marked as (T) 100 λl of the tissue homogenate was added. To both the tubes 1.0 ml of acetyl choline was added. Immediately the tubes were incubated for 10 minutes at 37°C in a water bath. The reaction was immediately stopped in both the tubes by adding alkaline hydroxylamine solution and shaking vigorously. Now to the tube marked as (c) 100 λl of homogenate was added. After not less than one

minute, 1.0 ml of 10% of TCA was added. 2.0 ml of HCl solution and 1.0 ml of $FeCl_3$ solution were then added and mixed by shaking. The mixture was then filtered through whatman no.40 filter paper. The amount of unreacted Ach in each tube was measured by reading optical density of the colour at 540 mm.

3.4.6.4 Calculation : The enzyme activity of each sample was calculated using the formula :

μ moles of Ach hydrolysed = 4 (1 - RT/Rc) where RT and RC are the reading of the test and control samples respectively. The enzyme activity was expressed in u moles of Ach hydrolysed by 1.0 g of brain at $37^{o}C$ in 10 minutes.

3.4.7 Sodium Potassium ATPase (Post and Sen, 1967)

3.4.7.1 Principle: Sodium-Potassium adenosine triphosphatase (Na^+ K^+ ATPase) transport Na^+ and K^+ against concentration gradient at the cost of ATP molecule liberating inorganic phosphate (Pi). The liberated Pi was estimated by Fiske and Subba Raw method (Oser, 1965).

3.4.7.2 Reagents

Enzyme preparation - One half of brain was taken in 50 ml cold glass distilled water and homogenised. This was placed in ice, till, further use.

Reaction mixture consists of 1 M Nacl (14.0 ml) 1 M Kcl (1.4 ml), 1 M $MgCl_2$ (0.3 ml), 0.5 M Tris HCL (10 ml) and 4.3 ml of glass

distilled water (store refrigerated). use 0.15 ml per 0.5 ml of assay medium.

30 mM. ATP : Dissolved 181.8 mg (disodium salt) in 6 ml water and adjusted pH to 7.4 with 0.5 M tris base. Made up to 10 ml with glass distilled water and Kept frozen in small convenient aliquots.

Ouabain : 10 mm, 14.57 mg in 2 ml. Stored frozen in amber coloured bottles.

0.02 mM EDTA : 0.378 g in 100 ml glass distilled water.

10% TCA in water

Reagents for Fiske and Subba Rao method for phosphate determination.

3.4.7.3 Procedure : ATP and ouabain were thawed and kept on ice. To two test tubes 0.15 ml of reaction mixture, 0.05 ml EDTA and 0.05 ml of enzyme were added. To one tube 0.05 ml 10 mm ouabain was added, while to the other an equal amount of glass distilled water was added. After pre incubation for 5 minutes reaction was initiated with the addition of 0.05 ml of 30mM ATP. The reaction was terminated after a period of 30 mts of incubation at 37°C in a water bath, by adding 0.5 ml of 10% cold TCA. After keeping on ice for 10 min, the tubes were centrifuged for 15 mts. Enzyme blank was run in a similar way but enzyme was added after the addition of TCA. After centrifugation the supernatant was transferred to separate tubes and the phosphorus

was estimated by Fiske and Subba rao method (Standard graph for phosphorus is given in Fig.3).

The enzyme activity was expressed as μ moles of Pi liberated/30 mts/g of brain tissue.

The difference in the activity in the absence and presence of ouabain is taken as $Na^+ K^+$ ATPase activity.

3.4.8 Gama amino butyric acid

Gama amino butyric acid in the rat brain was estimated by the method of Sadasivudu and Lajtha (1970).

3.4.8.1 Reagents

GABA standard solution - 20.62 mg of GABA was dissolved in 100 ml of distilled water

80% alcohol

Butanol : acetic acid : water (65:15:25) mixture

Ninhydrine (0.25%) in acetone with 1% pyridine

75% alcohol

75% alcohol containing 0.005% $CuSO_4$.

3.4.8.2 Procedure

3.4.8.2.1 Standard graph : Different aliquots of standard solution containing 10, 20, 30 and 40 ng of GABA were spotted on

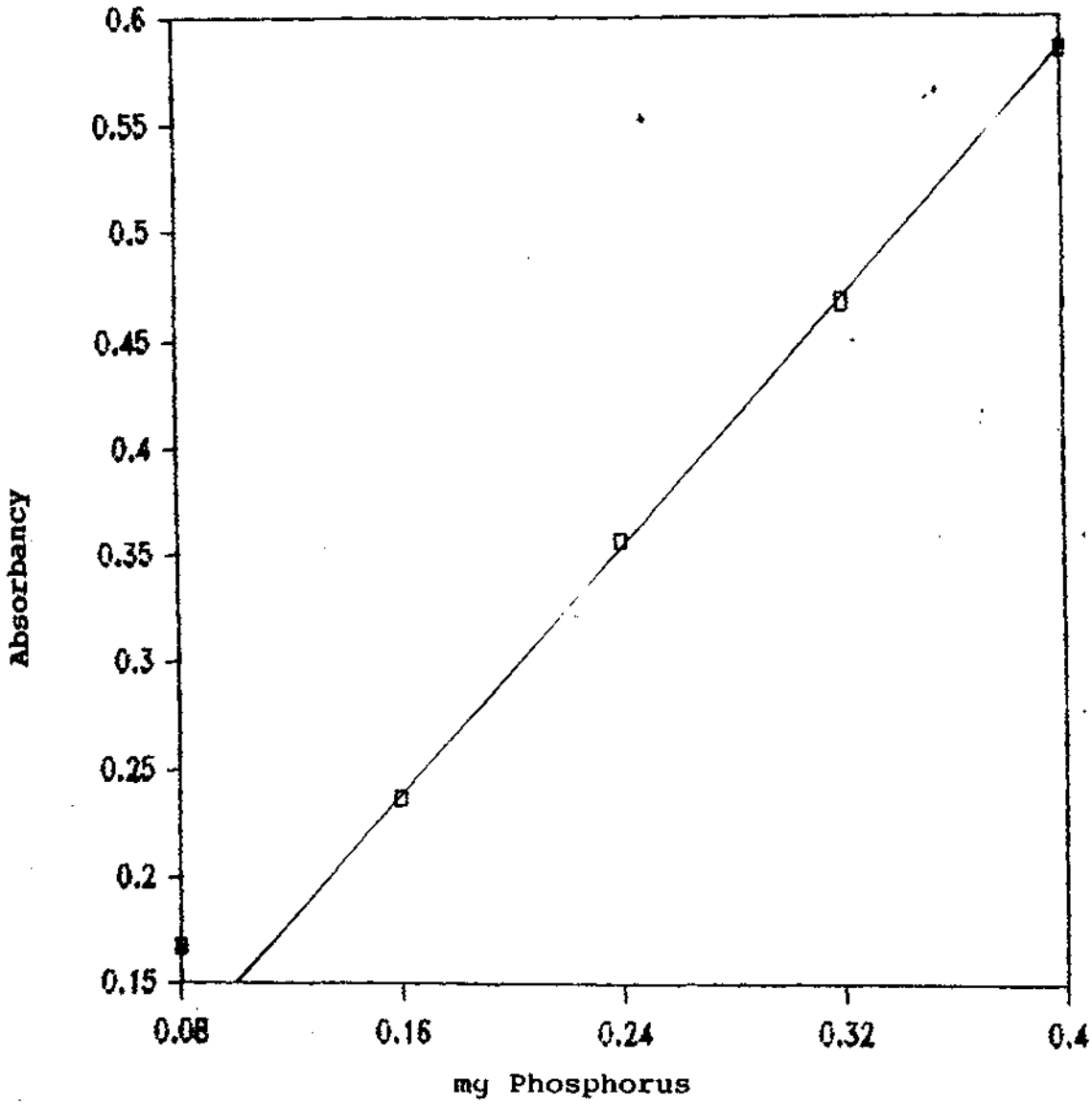


Fig.3 : STANDARD CURVE FOR PHOSPHORUS.

whatman No.1 filter paper and developed in Butanol : acetic acid : water solvent. The amino acid was then eluted in 3.0 ml of 75% alcohol containing 0.005% $Cuso_4$ after developing the colour with ninhydrine. The colour was read at 515 mm (Fig. 4).

3.4.8.2.2 Estimation in sample: Immediately after decapitation, one half brain was separated and put into ice cold 80% alcohol. To one half of the brain 25.0 ml of 80% alcohol was added and homogenised. 3.0 ml of the homogenate was centrifuged. The clear supernatant was carefully transferred to another test tube and evaporated to dryness at 70-80°C. The residue was dissolved in 250 ul of distilled water. The amino acid content (GABA) was determined by paper chromatography as described in standard graph.

3.4.8.3 Calculation : The amino acid content in the brain was calculated by extrapolating the sample reading on the standard curve. The value being equal to the amount of GABA in 25 ml of the extract. The amount present in 250 ml was then calculated which is equivalent to the amount present in 3.0 ml of the homogenate. Amino acid content was finally expressed as μ moles of amino acid per gm of Wet weight of brain.

3.4.9 DNA

3.4.9.1 Isolation of DNA (Schneider, 1945)

3.4.9.1.1 Principle: The procedure involves the isolation of DNA with hot TCA from the tissue.

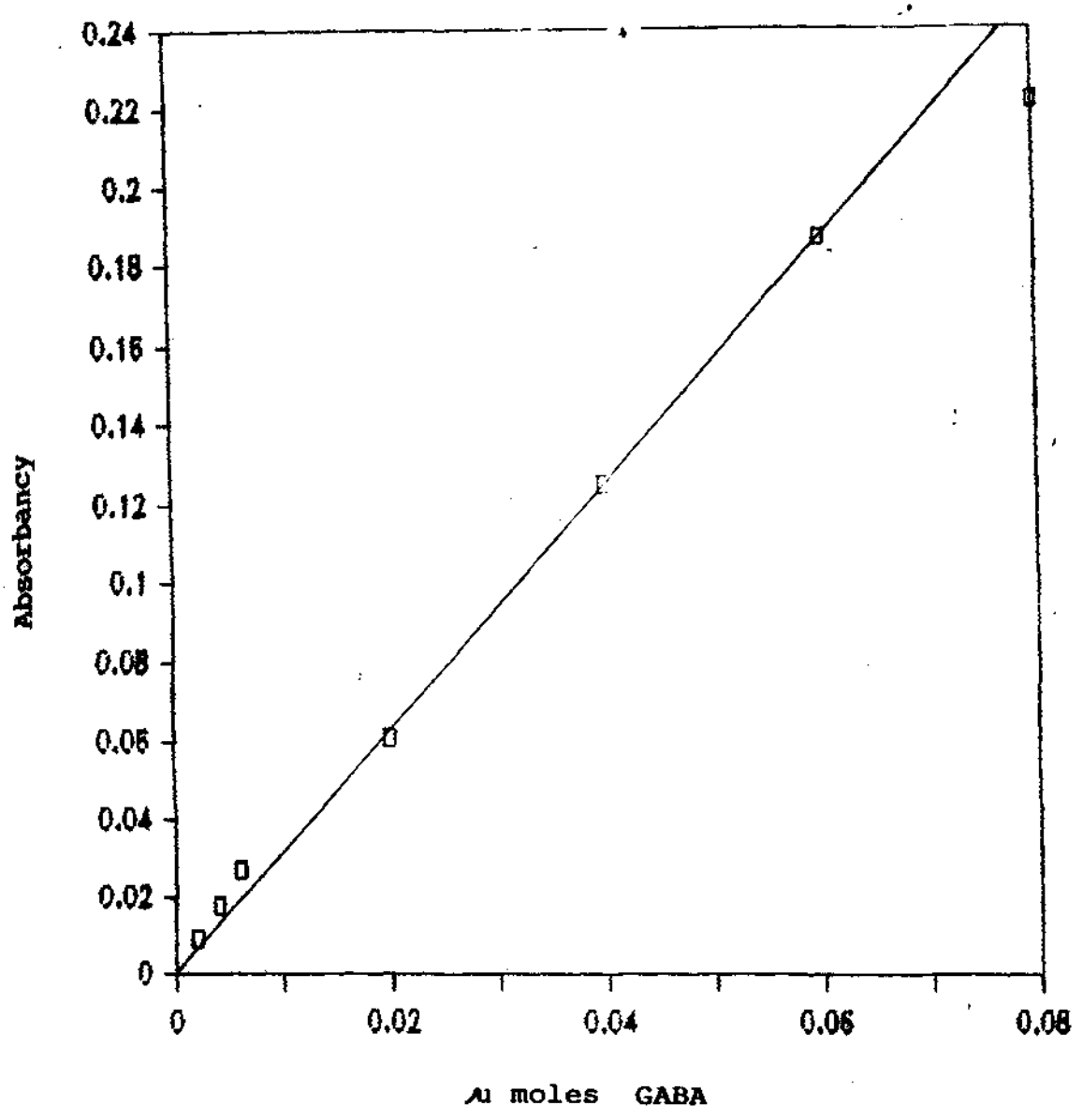


Fig.4 : STANDARD CURVE FOR GABA.

3.4.9.1.2 Reagents

TCA : 5% and 10% in glass distilled water

NaCl : 0.9% saline in glass distilled water

3.4.9.1.3 Procedure: To one half of the brain 10.0 ml of 0.9% saline was added and the tissue was homogenised. The homogenate was filtered through four layers of cheese cloth. To 5.0 ml of the filterate 5.0 ml of 10% TCA was added and then centrifuged, the supernatant was discarded and the residue was washed twice with 10% TCA followed by washing with 5.0 ml of ethanol twice. After which the residue was ether extracted twice to remove the phospholipids. The following acid extraction was done next.

To the residue 2.5 ml of 10% TCA was added and after centrifugation the supernatant was saved and stored in a separate test tube (a). 5.0 ml of 5% TCA was added to the residue from (a) and kept at 100°C for 15-20 min. After cooling the mixture it was centrifuged and the supernatant added to (a). 2.5 ml of 5.0% TCA was added to the final residue and heated as above. The final supernatant was saved and added to (a). From the total 10.0 ml of the clear acid extract, aliquots were used for DNA determination.

3.4.9.2 Estimation of DNA (Dische, 1955)

3.4.9.2.1 Principle: The deoxyribose moiety of DNA forms -hydroxy levulaldehyde in TCA solution. This reacts with diphenylamine to give a blue colour which can be read at 565 nm.

3.4.9.2.2 Reagents

Dische's Reagent: Dissolve 1.0 g of crystalline diphenylamine in 100 ml glacial acetic acid and 2.75 ml of concentrated H_2SO_4. The reagent is stored in the dark.

1 N Perchloric acid. 10.87 ml of perchloric acid was taken and the volume was made up to 100 ml.

5 mM NaOH : 0.02 g of NaOH was dissolved in distilled water and the volume was made upto 100 ml.

Standard DNA solution : A stock solution was prepared by dissolving 40.0 mg of calf thymus DNA and making up the volume to 100.0 ml with 5 mm NaOH. Working standards were prepared by mixing 10.0 ml of DNA stock solution with 1 perchloric acid and heating at 70°C for 15 mts.

3.4.9.2.3 Procedure: A standard curve was made by pipetting different aliquots of working standards of DNA solution containing 0.05, 0.1, 0.2 and 0.5 mg DNA into clean test tubes. Separately 1.0 ml of the acid extract was also taken into test tube. To this 2.0 ml of freshly prepared Dische's reagent was added and the total volume in each tube was made upto 5.0 ml with glass distilled water. The initial turbidity formed disappeared gradually. The tubes were covered with glass marbles and heated in a boiling water bath for 15 minutes. The solution was then cooled under tap water and the blue colour developed was measured at 565 nm in spectronic-20 against a blank treated in the same manner (Fig. 5).

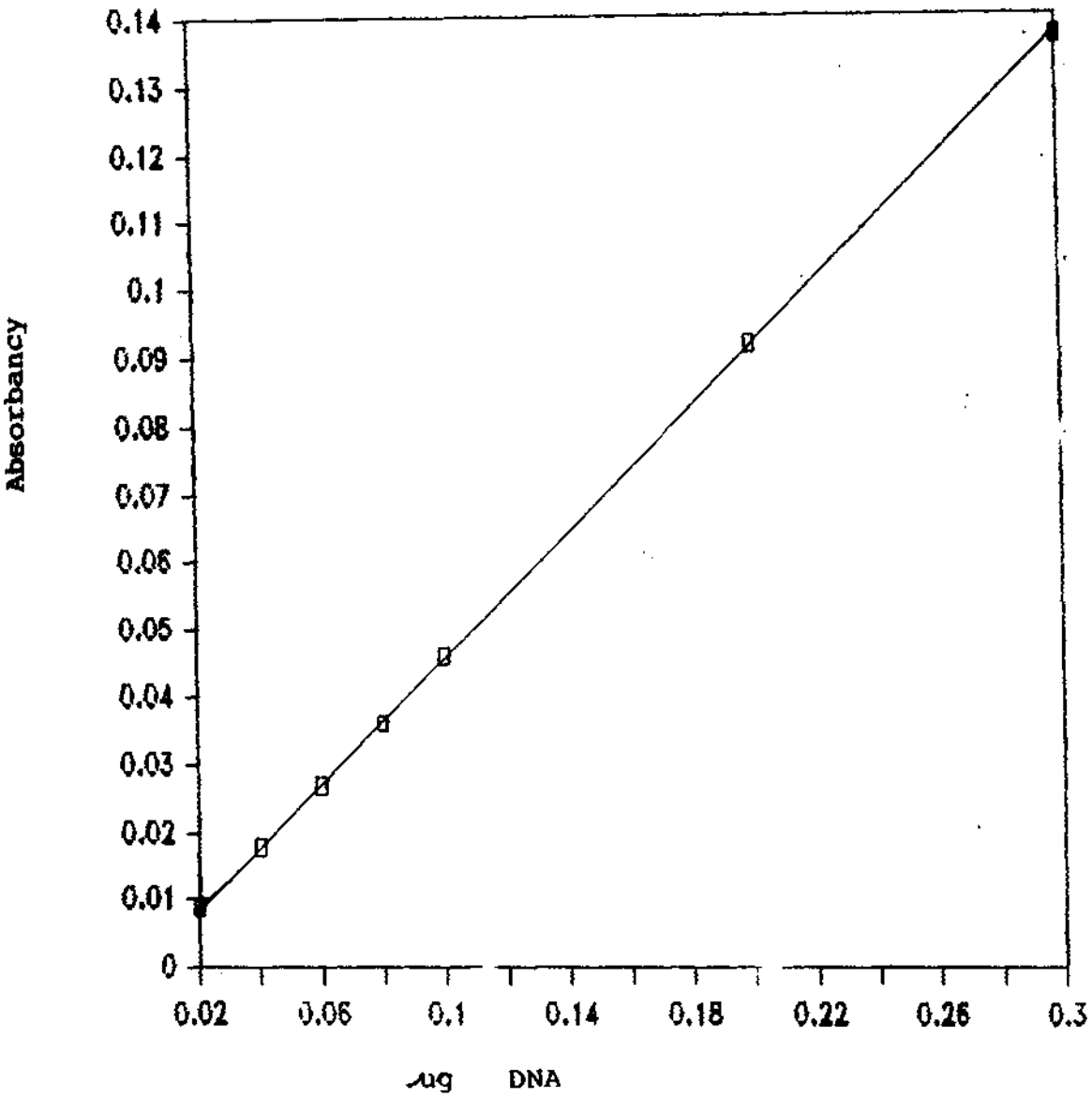


Fig.5 : STANDARD CURVE FOR DNA.

3.4.9.2.4 Calculation : From the standard curve the value of DNA was computed for 1.0 ml of extract used and finally calculated for the initial 1.0 ml of acid extract made, this being the amount present in 5.0 ml of the tissue extract. Final values were expressed as mg of DNA per gm of brain.

3.4.10 Protein

Protein was estimated by the method of Weichselbaum, 1946.

3.4.10.1 Principle: The - CoNH groups in the protein molecule react with copper sulphate in alkaline medium to give purple colour which is then read at 540 nm.

3.4.10.2 Reagents

Biuret Reagent : Dissolve 4.25 g potassium sodium tartarate ($KNaC_4H_4O_6.4H_2O$) 1.5 of cupric sulphate ($Cuso_4$ 5 H_2O) and 2.5 of potassium iodide in about 500 ml of distilled water. Dissolve 40 g of NaOH in the solution and make up the volume to 1.0 l.

Standard : The standard was prepared by dissolving 2.5 g of Bovine serum albumin and making up the volume to 100 ml with 0.9% saline.

3% NaOH

10% TCA

3.4.10.3 Procedure : To one half of the brain 10.0 ml of 0.9% saline was added and tissue was homogenised. From this 0.5 ml of

homogenate was taken and 0.5 ml of 10% TCA was added to it followed by centrifugation. The supernatant was carefully discarded and the residue was washed twice with acetone : ether (3:1) mixture followed by washing with ether. To the precipitate 10 ml of 3% NaOH was then added followed by addition of 5.0 ml of biuret reagent. The mixture was thoroughly mixed. After 30 minutes of addition of biuret reagent, readings were taken at 540 nm against a blank containing 0.2 ml glass distilled water, 1.0 ml 3% NaOH and 5.0 ml biuret reagent. The protein content from the aliquots of brain tissue was determined from a calibration curve and expressed as mg protein per gm of Wet weight of tissue.

3.4.10.4 Standard graph : Different aliquots of standard protein solution containing 0.5, 1, 2, 3, 4 and 5 mg of protein in 0.2 ml of normal saline were taken into clean test tubes. To each of these tubes was added 1.0 ml of 3% NaOH and 5.0 ml of biuret reagent. The purple colour developed was measured at 540 nm, against a reagent blank. A graph was plotted with the optical density against concentration of protein (Fig. 6).

3.5 STATISTICAL PROCEDURES

The statistical significance of neonatal mortality of pups at weaning was tested by SND test for proportions (Rao,1983). The data pertaining to biochemical changes in pup brains was analysed by one way analysis of variance as per Snedecor and Cochran (1967).

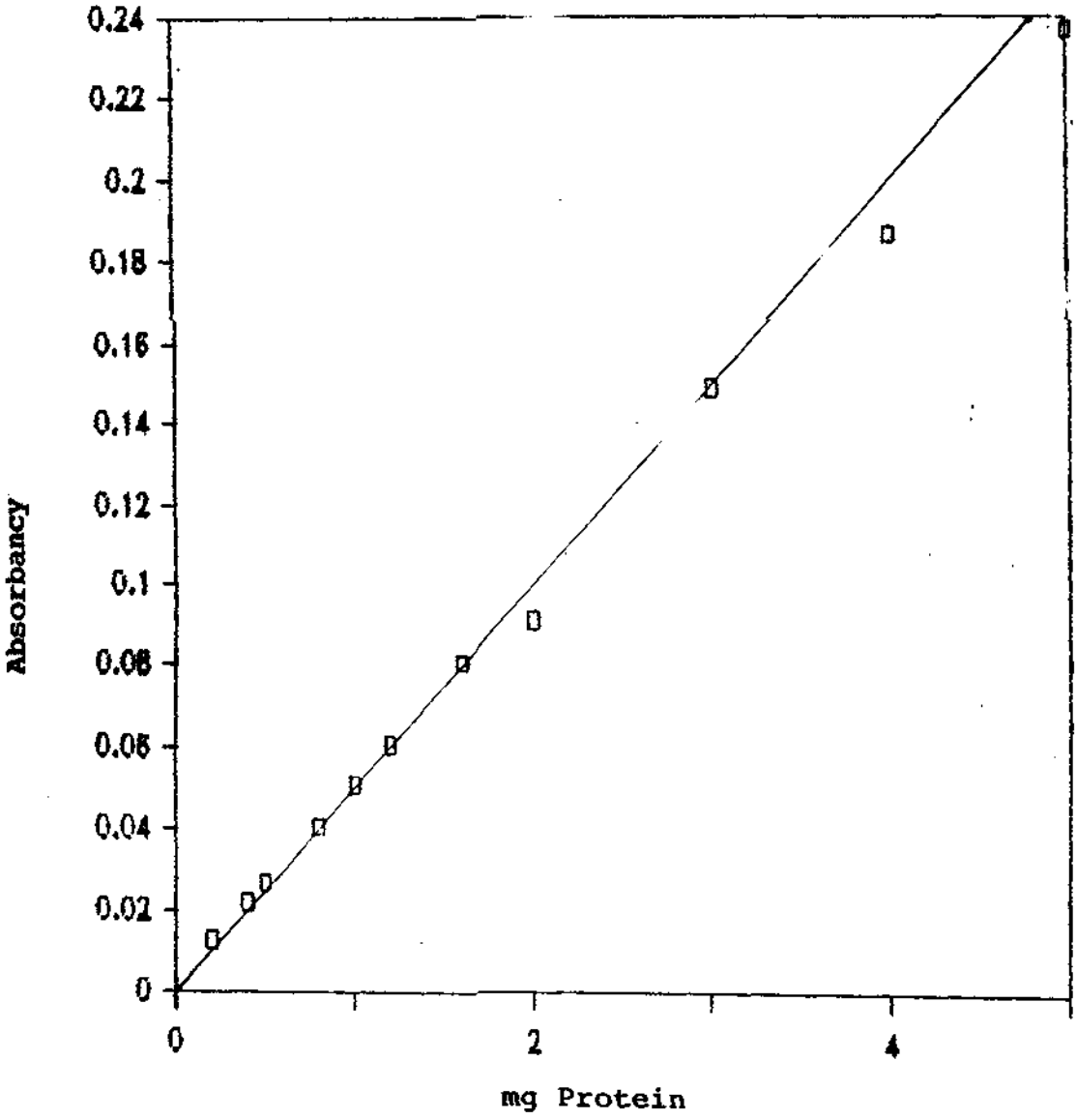


Fig.6 ; STANDARD CURVE FOR PROTEIN.

RESULTS

CHAPTER IV

RESULTS

The findings of the survey on pesticide usage on vegetable crops, dissipation of monocrotophos and cypermethrin residues on okra, effect of home processing on these residues and changes in certain biochemical parameters of brain in pups born to dams treated with monocrotophos and cypermethrin are detailed in this section.

4.1 SURVEY

Survey was conducted in three different regions of Andhra Pradesh viz., Guntur, Anakapalli and Ranga Reddy district categorised as high, medium and low pesticide consuming areas. The total number of farmers interviewed in Guntur, Anakapalli and Ranga Reddy district was 40, 42 and 40 respectively. Information regarding the following aspects was collected with the help of an interview schedule-

-source of information for farmers regarding pesticide and its dose to be used as well as method of application

-time interval given by the farmer between last insecticidal spray and harvest

-training received in the method of application and knowledge of expiry date

4.1.1 Use of pesticides on vegetable crops

The data regarding different sources of information on which a farmer depends to decide the pesticides to be used on vegetables is presented in Table 3.

Table 3: Frequency distribution of farmers regarding source of information on pesticides to be used on vegetables

Region	No. of farmers depending on					
	Self experience	Local farmers	Dealers	Advertisements	Official source	Radio
Guntur (n=42)*	9	25	19	1	5	1
Anakapalli (n=40)	7	18	14	-	8	-
R'R district (n=40)	11	18	23	-	-	-
Total (n=122)	27	61	56	1	13	1

* No. of farmers interviewed

A perusal of the data presented in Table 3 indicates that the farmers in all the regions depended mostly on local farmers, dealers and self experience, for information regarding the pesticide to be used for effective control of the pests on vegetables. Official sources, advertisements and radio appeared to play minor role.

The farmers relied mostly on their own experience for deciding the dose of the pesticide to be used (Table 4). The experiences of other farmers, advise of dealers were also considered to some extent in deciding the dose of pesticide to be sprayed on the crop.

Table 4: Frequency distribution of farmers' source of information on dose of pesticide

Region	No. of farmers depending for dosage of pesticide on				
	Self experience	Local farmers	Dealers	Container label	Official source
Guntur (n=42)*	26	20	12	5	1
Anakapalli (n=40)	10	7	21	2	11
R'R district (n=40)	16	13	11	-	1
Total (n=122)	52	40	44	7	13

* No.of farmers interviewed

The data shows that to a lesser extent farmers receive information from more than one source regarding dosage.

The frequency distribution of farmers on pesticide usage during different stages of plant growth is presented in Table 5. A perusal of this data indicates that all the farmers interviewed in Guntur and Anakapalli and most of the farmers in Ranga Reddy

(37) district used pesticides at least once on each vegetable crop during the season.

Table 5: Frequency distribution of farmers pesticide usage during different stages of plant growth

Stage of Plant growth	No. of farmers using pesticides	Average No. of pesticide applications	No. of farmers using more than recommended
Guntur (n=40)*			
Transplanting	9	1-3	8
Vegetative growth	35	1-4	21
Flowering	29	1-8	18
Fruiting	32	1-10	18
Anakapalli (n=42)*			
Transplanting	3	1	3
Vegetative growth	27	1-4	9
Flowering	17	1-3	6
Fruiting	34	1-25	15
R.R district (n=40)*			
Transplanting	8	2-3	6
Vegetative growth	14	1-10	6
Flowering	29	1-3	16
Fruiting	21	1-8	7

* No. of farmers interviewed.

The pesticides were less frequently used by farmers just before and after transplanting in all the districts. Majority of those who used, exceeded the recommended dose.

In Guntur, more than 30 farmers out of the 40 farmers interviewed used pesticides during vegetative growth, flowering and fruiting and more than 50 per cent of these exceeded the recommended dose and also number of applications of pesticides.

The number of farmers in Anakapalli using pesticides during vegetative growth, flowering and fruiting was 27, 17 and 34 respectively, of these 33-44 per cent exceeded the recommended dose of pesticides.

In Ranga Reddy district the number of farmers using pesticides during vegetative growth, flowering and fruiting was 14,29 and 21 respectively, of these 33 to 55 per cent exceeded the recommended dose of pesticides.

The data presented in Table 6 clearly indicates that in all the three districts surveyed, majority of the farmers harvest the vegetables between 1-2 days after the insecticidal spray to avoid maturation of the vegetable and loss of market value. Kovai (cucumber) is one vegetable where fruits are harvested a week after the insecticidal application in Guntur district, where this vegetable is grown in abundance.

The usual methods of applying pesticides were dusting or spraying, using hand operated or power sprayers. In Anakapalli a crude method of using twigs to sprinkle insecticide on plants and a locally made sprayer to spray insecticide are found to be used. The reasons given for not using a proper sprayer were non availability of sprayers in time for hire, and the frequent blockage

Table 6 : Frequency distribution of farmers ussing pesticides at harvest and the time interval between harvest and last insecticide spray on vegetable crops.

Vegetable	Region	No.of farmers		Interval between last spray and harvest (days)			
		Cultivating the vegetable	Using pesticide at harvest	1	1-2	3-5	6
Beans	Guntur	5*	5	-	1	-	4
	Anakapalli	2	2	-	2	-	-
	R.R.district	1	1	-	-	-	1
Bittergourd	Guntur	3	2	-	2	-	-
	R.R.district	5	4	-	-	3	1
Bottlegourd	Guntur	3	2	-	2	-	-
	Anakapalli	2	1	-	1	-	-
	R.R.district	1	1	-	-	-	1
Brinjal	Guntur	22	18	1	14	-	3
	Anakapalli	29	26	2	19	5	-
	R.R.district	16	10	-	-	6	4
Cabbage	Anakapalli	3	-	-	-	-	-
	R.R.district	4	2	-	-	2	-
Cauliflower	Guntur	10	10	1	6	3	1
Cowpea	Anakapalli	6	6	-	6	-	-
Kovai	Anakapalli	18	14	1	3	3	7
Okra	Guntur	7	5	-	5	-	-
	Anakapalli	16	13	1	11	1	-
	R.R.district	3	2	-	2	-	-
Ridge gourd	Guntur	8	5	-	5	-	-
	Anakapalli	10	5	-	5	-	-
	R.R.district	5	2	-	-	-	2
Snakegourd	Guntur	1	1	-	-	-	1
	R.R.district	1	1	-	-	-	1
Tomato	Guntur	4	3	-	2	1	-
	Anakapalli	16	13	-	8	4	1
	R.R.district	4	4	-	3	1	-
Amaranath	Anakapalli	1	1	-	1	-	-
Gogu	Anakapalli	1	1	-	1	-	-

* No.of farmers out of 40 in Guntur, 42 in Anakapalli and 40 in Rangareddy districts

of nozzle which takes a long time thus prolonging the spraying operation.

In Guntur hand operated (back pack) sprayers were used, when plants were young and power sprayers were used when the growth was dense. In Ranga Reddy district mostly hand operated sprayers were used. Table 7 gives the frequency distribution of farmers according to the source of information regarding method of application of pesticide.

Table 7: Frequency distribution of farmers according to source of information regarding method of application

Region	No. of farmers getting information from				
	Self experience	Local farmers	Dealers	Official source	Through demonstration
Guntur (n=42)*	2	34	5	5	4
Anakapalli (n=40)*	3	12	3	23	14
R.R district (n=40)*		38	2	-	2
Total (n=122)	5 (4) **	84 (69)	10 (8)	28 (23)	20 (16)

* No. of farmers interviewed.
** Figures in parenthesis indicate percentage.

A perusal of the data in Table 7 indicates that the influence of other farmers was high in selecting the method of application of pesticide. Very few farmers observed a demonstration of the spray operation before using different sprayers.

The awareness about the expiry date is summarised in Table 8.

Table 8: Frequency distribution of farmers regarding awareness of expiry date of pesticide

Region	No. of farmers aware of expiry date	No. of farmers aware but indifferent
Guntur (n=40)*	30 (71)**	1 (2.5)
Anakapalli (n=42)	10 (25)	-
R.R district (n=40)	23 (58)	1 (2.5)
Total (n=122)	63 (52)	2 (2)

* No. of farmers interviewed
** Figures in parenthesis indicate percentage.

The per cent of farmers aware of date of expiry was high in Guntur followed by Ranga Reddy District. In Anakapally only 25% of the farmers were aware of the date of expiry and mostly they do not know about it.

4.2 RESIDUES OF MONOCROTOPHOS AND CYPERMETHRIN IN OKRA

The residues of monocrotophos and cypermethrin in okra raised in experimental farm sprayed @ 0.25 kg (recommended) and 0.38 kg (higher dose, but frequenty used by farmers) ai/ha and 60 g (recommended) and 100 g ai/ha (higher) were determined at 0, 1, 3, 7, 10 and 15th day after the third spray. The residues were

observed upto 15 days in case of monocrotophos and 10 days for cypermethrin and hence the dissipation rates were studied till these periods. The intervals fixed for dissipation was mostly based on the rate of dissipation, i.e., the dissipation was faster immediately following the spray and slower later.

4.2.1 Residues of monocrotophos on Okra fruits

When 0.25 kg and 0.38 kg ai/ha of monocrotophos was sprayed on okra, an initial deposit (0 day) of 4.45 and 6.86 ppm was observed respectively. These initial deposits reduced by 84.5 and 85.3 per cent on 10th day of the spray. On 15th day after the spray the residues were below detectable level (Table 9).

Table 9: Residues of Monocrotophos on Okra

Day after spraying	Monocrotophos residues (ppm)			
	Applied at 0.25 kg ai/ha	% reduction	Applied at 0.38 kg ai/ha	% reduction
0	4.45	-	6.86	-
1	2.96	33.5	4.12	39.9
3	2.52	43.4	2.96	56.6
7	1.34	69.9	2.03	70.4
10	0.69	84.5	1.02	85.3
15	ND	ND	ND	ND

ND : Not detectable.
Tolerance limit = 0.2 ppm (FAO/WHO, 1973).

The residue levels were plotted against time to obtain exponential type of curves. When log value of ppm of residues were plotted against time (days) the curves showed straight line relationship (Fig.7) between the two variables, which help in quick visualisation of the loss of residues. The linearity was clearly seen in regression line computed with the help of regression analysis of log ppm residues and time. From the linear function, half life value of the insecticide and safety interval was inferred. However, these values were also calculated mathematically by using Hoskins formula (1961) and the half life values were found to be 4.03 and 4.14 days for 0.25 and 0.38 kg ai/ha dose of spray respectively (sample calculation in Appendix II).

The residues reached the levels equivalent to tolerance limit of 0.2 ppm set by FAO/WHO (1973) for vegetables in 17.5 and 19.9 days at recommended and high concentrations used in the present study respectively.

4.2.2 Residues of cypermethrin on okra fruits

The residues resulting from spraying cypermethrin at two different concentrations on okra are presented in Table 10.

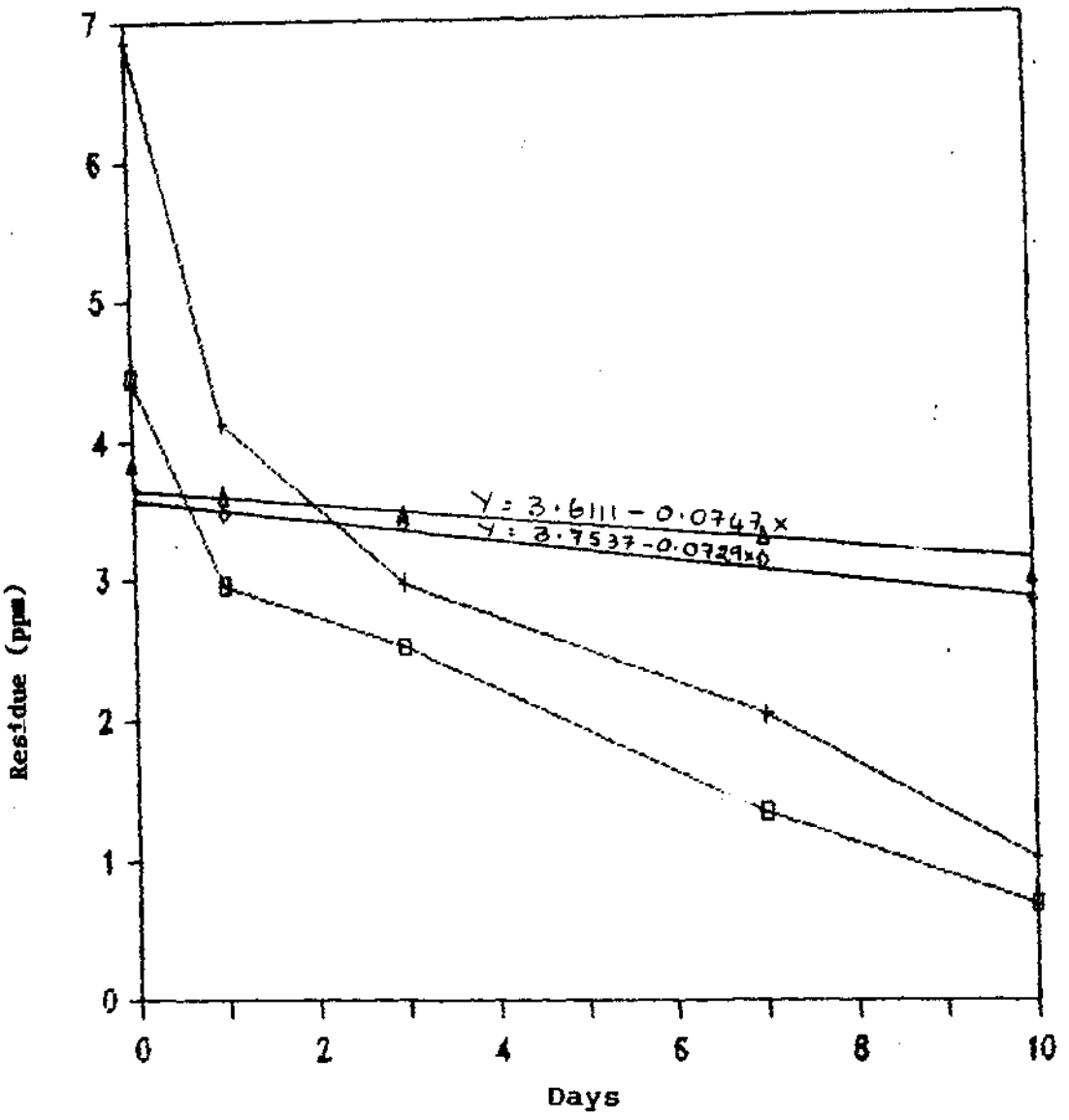


Fig.7 : DISSIPATION OF MONOCROTOPHOS ON OKRA.

Table 10: Residues of cypermethrin on okra

Days after spraying	Cypermethrin residues (ppm)			
	Applied at 60 g ai/ha	% reduction	Applied at 100 g ai/ha	% reduction
0	1.46	-	2.28	-
1	1.09	25.3	1.55	32.0
3	0.56	67.6	0.96	58.3
7	0.15	89.7	0.25	89.0
10	0.09	93.8	0.15	93.4

Tolerance level = 0.5 ppm (USHEW 1978 a,b)

The initial deposits of cypermethrin on okra were 1.46 ppm and 2.28 ppm when sprayed @ 60 g and 100 g ai/ha respectively. By 10th day after the spray about 93% of these deposits were lost at both the concentrations of spray.

As in the case of monocrotophos, residues were plotted against time and exponential type of curves were obtained (Fig.8). To obtain straight line relationship, the log values of ppm residues were plotted against time (days). The linearity was clearly seen by drawing a regression line computed with the help of regression analysis of log ppm residues and time. From this linear relationship, half life and safety intervals could be calculated. However, these values were also computed using Hoskins formulae (1961) and were found to be 2.41 days and 3.54 days respectively at 60 g ai/ha dose and 2.49 and 5.19 at 100 g ai/ha dose (sample calculation in Appendix II).

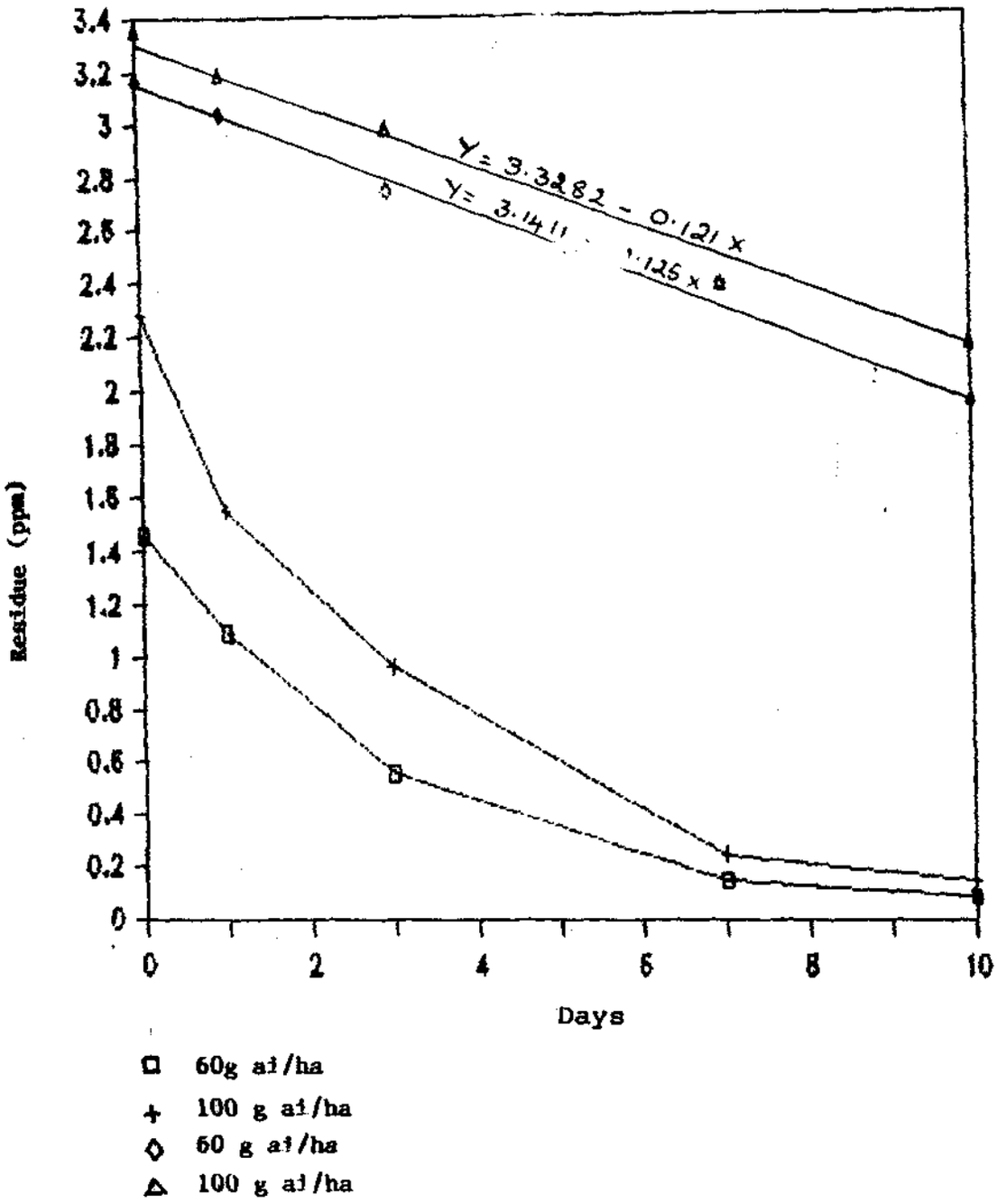


Fig.8 : DISSIPATION OF CYPERMETHRIN ON OKRA.

The effect of home processings such as washing in plain water, washing in alkaline water, stir frying, boiling in tamarind extract and boiling in tamarind extract containing turmeric was studied for the okra fruits treated with monocrotophos and cypermethrin and the results are presented in this section.

4.3.1 Monocrotophos

Okra harvested on 0, 1, 3, 7 and 10 days after spraying the field with monocrotophos @ 0.25 mg and 0.38 kg ai/ha, were subjected to different processing methods and the results are tabulated in Tables 11 and Table 12 and Figures 9 and 10.

A perusal of the data presented in Tables 11 and 12 indicate that washing in tap water could remove 58-60 per cent of the initial deposits of monocrotophos in both the doses. The per cent removal of deposit was slightly higher with processess like stir frying, boiling in tamarind extract with and without turmeric and washing in alkaline water followed by stir frying. It was also observed that the per cent removal of residues decreased with ageing of residues and even 10 days after spraying none of the cooking processes could reduce the residues below the tolerance limit of 0.2 ppm prescribed by FAO/WHO (1973) for vegetables. Irrespective of the doses applied the removal of the residues in all the processing procedures was almost similar.

Table 11 : Effect of method of processing on monocrotophos residues in okra sprayed @ 0.25 kg ai/ha.

Days after spraying	Monocrotophos residues in (ppm)					
	Unprocessed	Washed in running water	Washed and stir fried	Washed and boiled in tamarind extract		Washed in $NaHCO_3$ and stir fried.
				with turmeric	without turmeric	
0	4.45	1.87 (58.0)*	1.73 (61.1)	1.77 (60.2)	1.75 (60.7)	1.65 (62.0)
1	2.96	1.54 (48.0)	1.47 (50.3)	1.47 (50.3)	1.51 (48.9)	1.43 (51.0)
3	2.52	1.67 (33.7)	1.62 (35.7)	1.60 (36.5)	1.56 (38.1)	1.40 (44.0)
7	1.34	1.07 (20.2)	1.06 (20.9)	1.05 (21.6)	1.09 (18.7)	1.01 (24.0)
10	0.69	0.69 (-)	0.64 (7.3)	0.64 (7.3)	0.62 (10.0)	0.61 (11.6)

Tolerance limit = 0.2 ppm (FAO/WHO, 1973)

* Figures in parenthesis indicate percentage loss of residues

Table 12 : Effect of method of processing on monocrotophos residues in okra sprayed @ 0.38 kg ai/ha.

Days after spraying	Monocrotophos residues in (ppm)					
	Unprocessed	Washed in running water	Washed and stir fried	Washed and boiled in tamarind extract		Washed in $NaHCO_3$ and stir fried.
				with turmeric	without turmeric	
0	6.86	2.75 (59.9)*	2.46 (64.1)	2.66 (61.2)	2.64 (61.5)	2.34 (65.9)
1	4.12	2.10 (49.0)	2.06 (50.0)	2.14 (48.1)	2.10 (49.0)	2.01 (51.2)
3	2.98	2.08 (30.2)	2.08 (30.2)	1.94 (34.9)	1.92 (35.6)	1.93 (35.2)
7	2.03	1.52 (25.1)	1.05 (48.3)	1.51 (25.6)	1.51 (25.6)	1.29 (36.4)
10	1.01	0.92 (8.9)	0.88 (12.9)	0.93 (7.9)	0.93 (7.9)	0.82 (18.8)

Tolerance limit = 0.2 ppm (FAO/WHO, 1973)

* Figures in parenthesis indicate percentage loss of residues

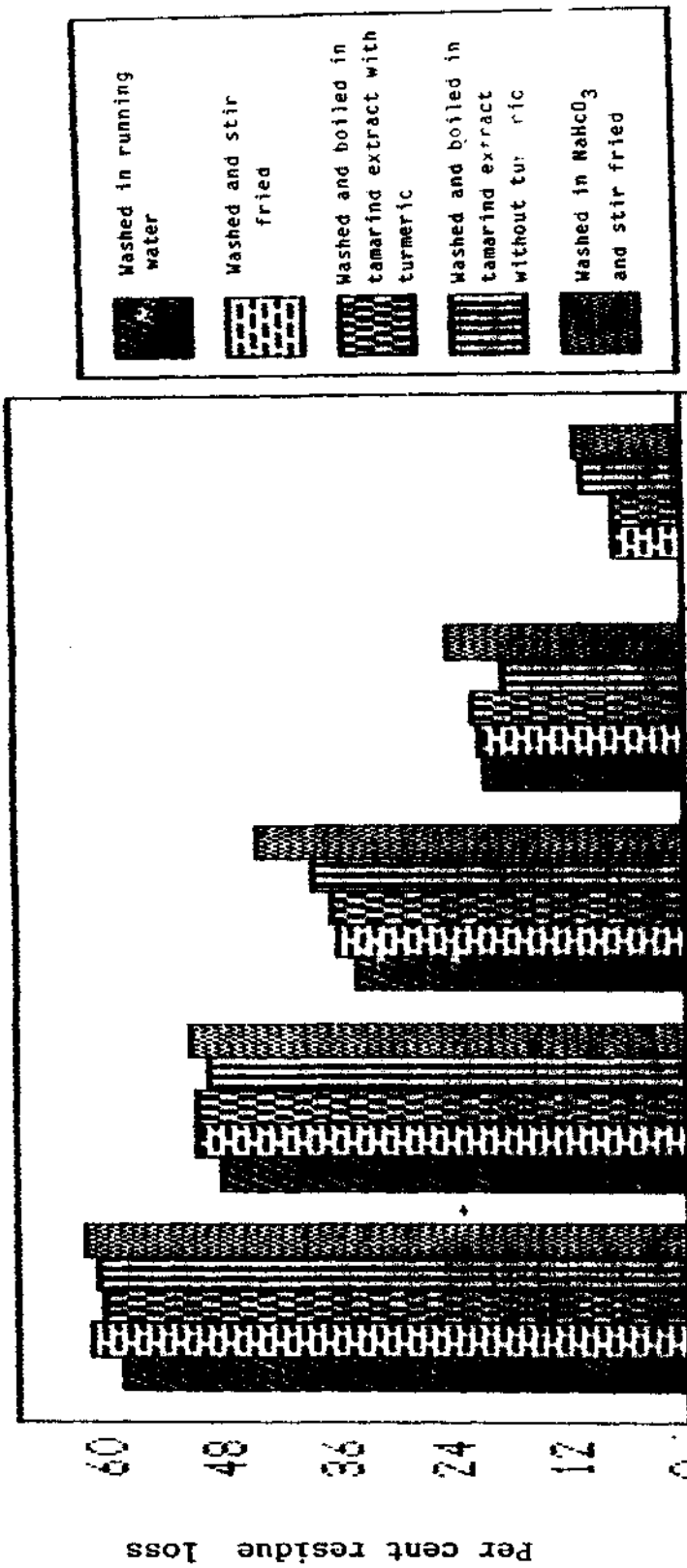


Fig.9 : PER CENT REMOVAL OF MONOCROTOPHOS RESIDUES DUE TO PROCESSING FROM OKRA SPRAYED @ 0.25 kg ai/ha.

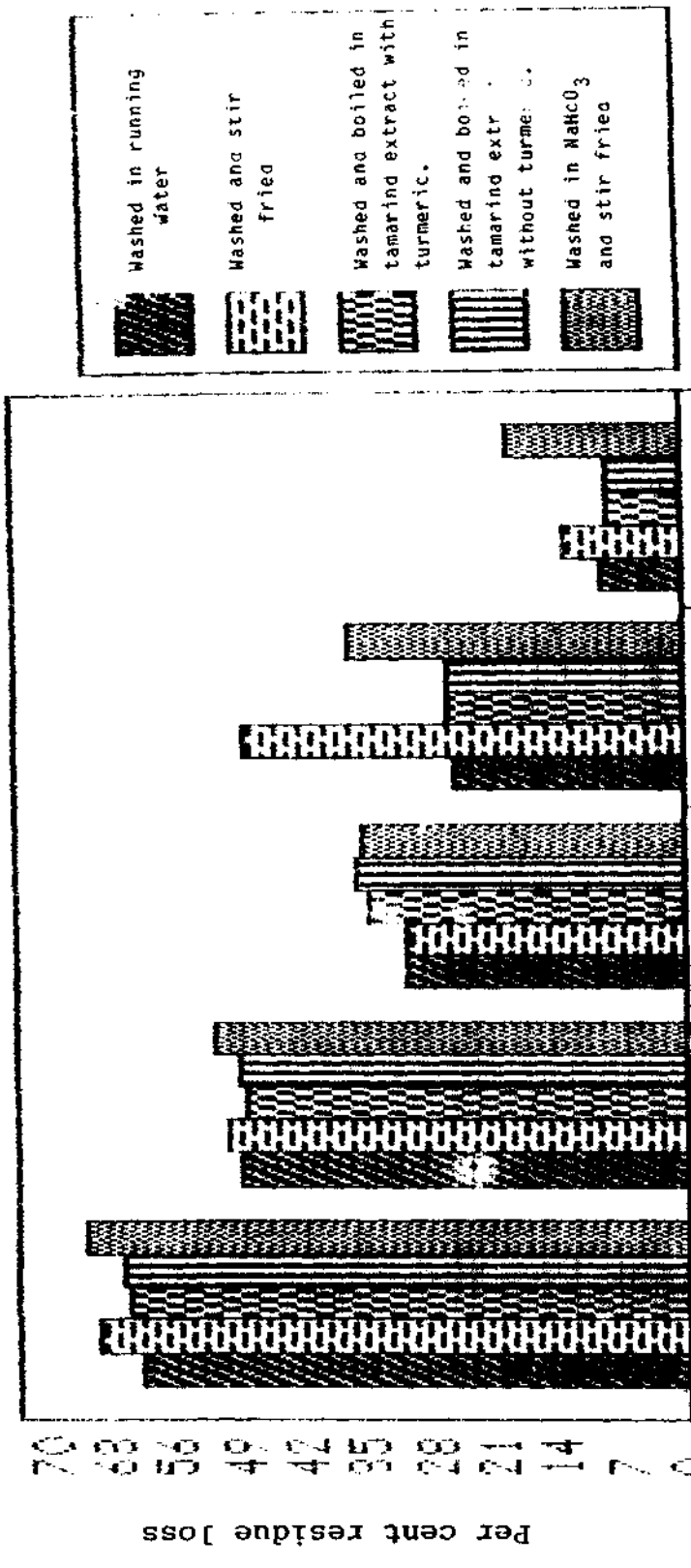


Fig.10 : PER CENT REMOVAL OF MONOCROTOPHOS RESIDUES DUE TO PROCESSING FROM OKRA SPRAYED @ 0.38 kg ai/ha.

4.3.2 Cypermethrin

Okra fruits harvested on 0, 1, 3, 7 and 10 days after spraying the fields with cypermethrin @ 60 and 100 g ai/ha were subjected to various cooking processes and the data on effectiveness of these processes in reducing the residues are presented in Tables 13 and 14 and Fig. 11 and 12.

Data presented indicate that washing okra fruits harvested on the same day of spraying, under running tap water could remove around 41-46 per cent of the residues. The per cent removal of the residues due to washing was less as the number of days between harvest and sprayng increased. On 7th day the residues were below tolerance limit after the fruits were washed, at both the concentrations. Washing the fruits in tap water or in sodium bicarbonate water followed by stir frying, could remove slightly high amount of residues. Boiling the washed fruits in tamarind extract with and without addition of turmeric, could remove nearly 75 to 79 per cent of residues. As in the case of washing, the per cent removal of residues was less with ageing of the residues, even with other processes.

4.4 TOXICITY STUDIES

The effect of oral feeding of adult rats with phos and cypermethrin during organogensis period of pregnancy and lactation on certain biochemical parameters of pup brain was studied. The biochemical parameters used were AChE, Na^+K^+ATPase (only for cypermethrin) GABA, DNA and protein.

Table 13 : Effect of method of processing on cypermethrin residues in okra sprayed @ 60 g ai/ha.

Days after spraying	Cypermethrin residues in (ppm)					
	Unprocessed	Washed in running water	Washed and stir fried	Washed and boiled in tamarind extract		Washed in $NaHCO_3$ and stir fried.
				with turmeric	without turmeric	
0	1.46	0.86 (41.1)*	0.59 (59.6)	0.36 (75.3)	0.32 (78.1)	0.49 (66.4)
1	1.09	0.67 (38.5)	0.43 (60.6)	0.29 (73.4)	0.35 (67.9)	0.45 (58.7)
3	0.56	0.44 (21.4)	0.24 (57.1)	0.15 (73.2)	0.2. (60.7)	0.27 (51.8)
7	0.15	0.12 (20.0)	0.0[illegible] (46.7)	0.06 (60.0)	0.05 (66.7)	0.07 (53.3)
10	0.09	0.07 (22.2)	0.04 (55.6)	ND (-)	0.02 (77.8)	0.03 (66.7)

Tolerance limit = 0.5 ppm (USHEW, 1978 a,b)

* Figures in parenthesis indicate percentage loss of residues

Table 14: Effect of method of processing on cypermethrin residues in okra sprayed @ 100 gm ai/ha.

Days after spraying	Cypermethrin residues in (ppm)					
	Unprocessed	Washed in running water	Washed and stir fried	Washed and boiled in tamarind extract		Washed in $NaHCO_3$ and stir fried.
				with turmeric	without turmeric	
0	2.28	1.22 (46.5)*	0.73 (67.9)	0.48 (78.9)	0.47 (79.4)	0.69 (69.7)
1	1.55	0.89 (42.6)	0.61 (60.6)	0.44 (71.6)	0.48 (69.0)	0.63 (59.4)
3	0.95	0.58 (38.9)	0.48 (49.5)	0.39 (58.9)	0.43 (54.7)	0.49 (48.4)
7	0.25	0.12 (40.0)	0.115(54.0)	0.08 (68.0)	0.10 (60.0)	0.11 (56.0)
10	0.15	0.09 (40.0)	0.05 (66.7)	0.04 (73.3)	0.05 (66.7)	0.08 (46.7)

Tolerance limit * 0.5 ppm (USHEW, 1978 a,b)

* Figures in parenthesis indicate percentage loss of residues

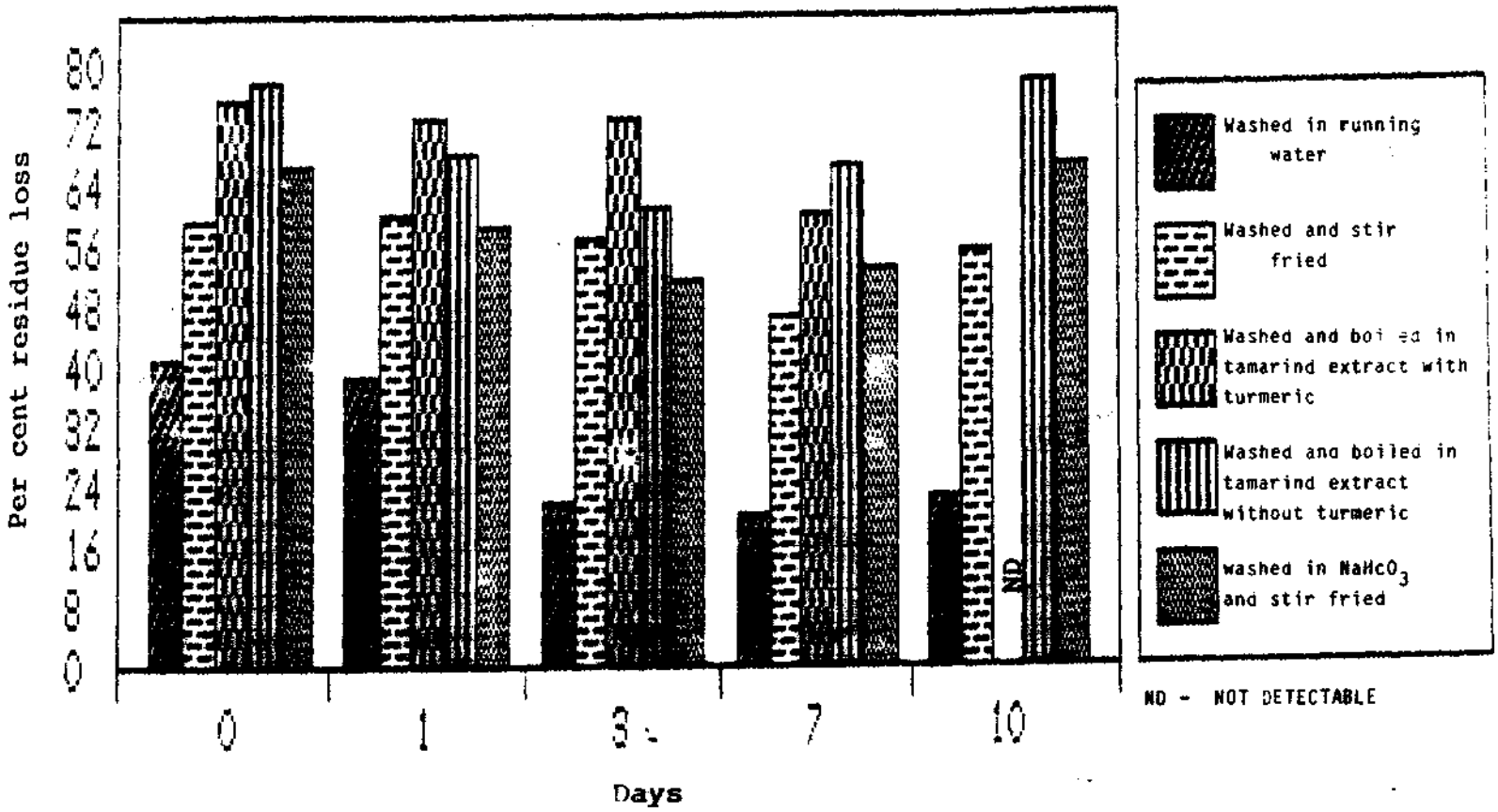


Fig. 11 : PER CENT REMOVAL OF CYPERMETHRIN RESIDUES DUE TO PROCESSING FROM OKRA SPRAYED @ 60 g ai/ha.

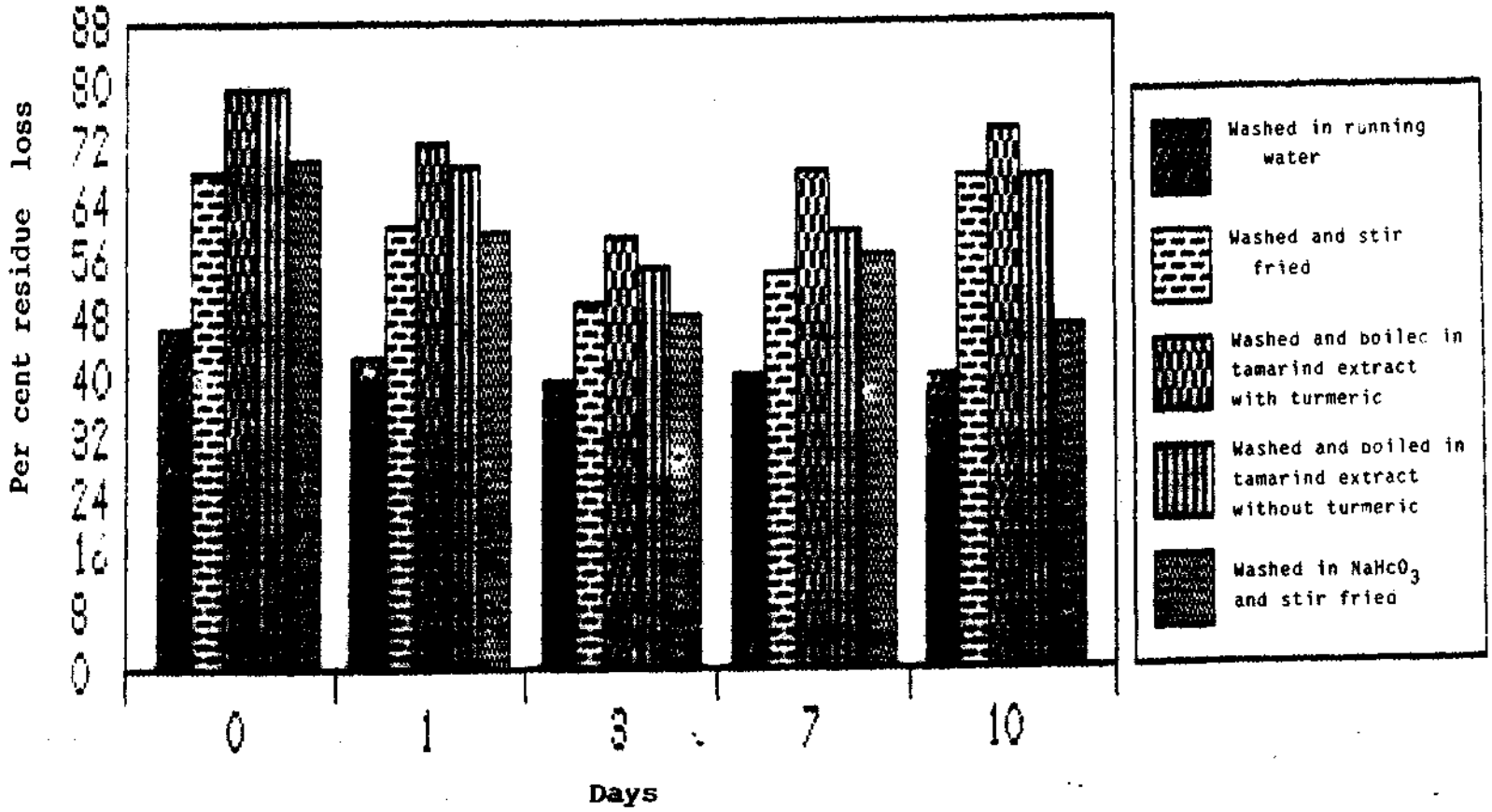


Fig.12 : **PER CENT REMOVAL OF CYPERMETHRIN RESIDUES DUE TO PROCESSING FROM OKRA SPRAYED @ 100 g ai/ha.**

4.4.1 Monocrotophos

4.4.1.1 Acute toxicity study

Clinical signs like lacrymation and salivation were observed even at 7 mg/kg body weight dose. At 10 mg/kg body weight and higher doses the other toxic symptoms such as paralysis of hind limbs, diarrhoea, excessive urination, convulsions, ataxia and prostration were also observed.

Table 15: Mortality of adult female rats due to acute dosing with monocrotophos

Dose(mg/kg body wt.)	No. of animals	Mortality No.	Mortality %	LD_{50}* (mg/kg body wt.)
Control	10	0	0^{a}	
7	9	1	11.1^{a}	
10	15	8	53.33^{b}	10
12	15	11	73.33^{bc}	
15	15	14	93.33^{cd}	

Per cent mortalities with different superscripts are significantly different.

$P < 0.01$

* Details of computing LD_{50} by probit analysis is given in Appendix III.

The mortality was significantly high ($P<0.01$) when the dose was increased from 7.0 to 10 mg/kg body weight and from 10 to 15 mg/kg body weight.

4.4.1.2 Teratogenicity testing

Adult female rats were given sub-acute doses i.e., 0.5, 1.0, 2.0 mg/kg body wt./day of monocrotophos during 6-15 days (both days inclusive) of pregnancy and from first to 21 days of parturition. In all the treated groups, except diarrhoea as indicated by soiled hind quarters, no other toxic symptom was observed in the dams through out the period.

4.4.1.2.1 Effect on pregnant dams

4.4.1.2.1.1 Body weight: The body weights of rats during pregnancy are presented in table 16 and Fig.13. The data shows that the weight gain during pregnancy in 2.0 mg/kw body wt./day treatment group was less ($P < 0.05$) than those of controls.

4.4.1.2.2 Effect on postnatal development: The effect of treating pregnant and lactating dams with monocrotophos on postnatal development viz., size, litter weight, neonatal deaths, survival of pups at 21 days age, weight gain of pups at 21 days of age are presented in Table 17.

Litter size: Litter size was not affected due to treatment of pregnant dams with monocrotophos at the doses tested in the present study.

Table 16 : Effect of monocrotophos on body weight of rats during pregnancy

Treatment mg/kg body wt/day	No. of rats	Mean weight (g) ± SD								Increase in weight (g)
		Days of pregnancy								
		0	3	6	9	12	15	18	21	
Control	11	214 ± 21.0	230 ± 21.0	235 ± 24.0	245 ± 23.0	254 ± 22.0	258 ± 23.0	275 ± 15.0	294 ± 13.0	80.0
0.5	13	225 ± 16.0	236 ± 16.0	240 ± 16.0	246 ± 15.0	253 ± 17.0	260 ± 16.0	278 ± 15.0	292 ± 21.0	67.0
1.0	12	217 ± 29.0	229 ± 22.0	235 ± 24.0	239 ± 22.0	247 ± 23.0	250 ± 22.0	269 ± 26.0	286 ± 26.0	69.0
2.0	14	224 ± 24.0	236 ± 20.0	239 ± 21.0	238 ± 17.0	236 ± 17.0	236 ± 15.0	260 ± 16.0	274 ± 15.0	50.0*

* $P < 0.05$

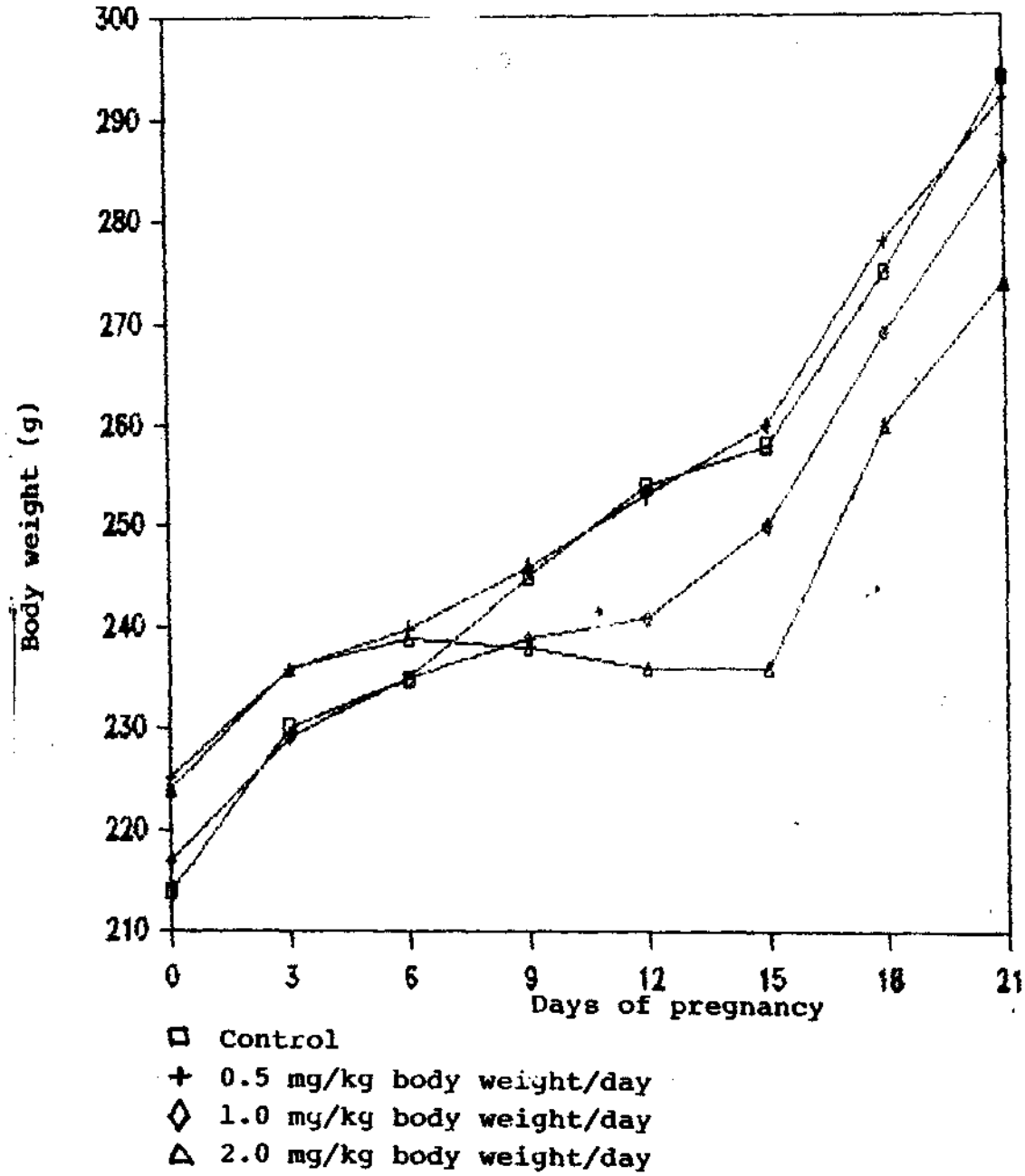


Fig.13 : BODY WEIGHTS OF MONOCROTOPHOS TREATED RATS DURING PREGNANCY.

Table 17 : Effect of Monocrotophos on neonatal and postnatal development of pups born to rats treated during organogenesis and post partum

Treatment mg/kg body wt.	No.of litters	At birth					At 21 days post partum				
		Mean litter size	Mean body wt.(g) ± SD	Mean crown-rump length (cm) ± SD	Neonatal deaths No.	Neonatal deaths %	Live pups No.	Live pups %	Mean body weight (g) ±SD	Dead pups No.	Dead pups %
0	11	8.1	6.45 ± 0.79	4.89 ± 0.4	5	5.61	60	67.4	23.4 ± 3.9	29	32.5
0.5	12	9.2	5.49* ± 0.73	4.69* ± 0.33	25	19.09**	50	45.5**	21.3 ± 5.8	60	54.6**
1.0	11	9.2	5.09* ± 0.54	4.53* ± 0.32	19	18.81**	50	49.5*	26.3 ± 5.6	51	50.5*
2.0	13	10.3	5.06* ± 0.46	4.61* ± 0.31	25	18.66**	33	24.6**	16.2** +3.6	101	75.4**

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

Birth weight and crown-rump length: As indicated in Table 17 the body weight and crown-rump length of the pups born to dams treated with 0.5, 1.0 and 2.0 mg monocrotophos mg/kg body wt/day were significantly ($P < 0.01$) decreased compared to pups born to controls.

Neonatal deaths: In all the treated groups the neonatal death rate was 20% which was not dose dependent.

Weights of pups at weaning: A perusal of data presented in Table 17 indicated that mean body weights of the new borns, born to dams treated with monocrotophos were decreased significantly. However, only the pups born to dams treated with 2.0 mg/kg body wt/day had significantly ($P<0.05$) lower body weights even at 21 days of age (weaning).

Pup mortality at weaning: The number of dead pups in all the treated groups at weaning was significantly ($P<0.01$) higher compared to those of control group.

Brain weights of pups at weaning: The brain weights of pups at 21 days of age are delineated in Table 18.

Table 18 : Effect of monocrotophos on brain weights of pups at weaning.

Group (mg/kg body wt./day)	Mean body wt. at (g) ± SD	mean brain wt. (g) ± SD	Brain wt. as % of body wt. ± SD
Control	23.40±3.9	1.21±0.09	4.82±0.69
0.5	21.25±5.8	1.17±0.13	6.19±2.2
1.0	26.29±5.6	1.26±0.12	4.99±0.8
2.0	16.20**±3.6	1.06**±0.10	6.90**±1.72

** - P<0.01

From table 18 it can be inferred that the monocrotophos at 2.0 mg/kg body wt/day reduced the brain weight significantly (P,0.01) as compared to control pups.

4.4.1.2.2 Effect on biochemical parameters of brain : The brains of 21 days old pups born to control and treated dams were assayed for AChE activity (Table 19) and, GABA, DNA and protein concentrations (Table 20).

Table 19: Brain AChE activity of pups born to monocrotophos treated dams.

Treatment (mg/kg body wt./day)	AChE activity[1] ± SD	AChE % inhibition
Control	241.50 ± 35.51	-
0.5	143.08** ± 12.86	40.75
1.0	94.17** ± 10.57	61.01
2.0	63.26** ± 28.69	73.81

1 µ moles ACh hydrolysed by 1 g of brain tissue at 37°C in 10 min.
** - $P < 0.01$

The AChE activity was significantly decreased ($P<0.01$) at all the doses tested (Fig.14) and the per cent inhibition of the enzyme activity showed dose dependent increase.

The brains of pups born to dams treated with 1.0 and 2.0 mg monocrotophos showed significantly low GABA levels when compared to controls.

The DNA content of brains was significantly low at all the doses tested in the present study. But protein levels were affected only at medium and high doses of monocrotophos. The protein/DNA ratio though increased in all the treatment groups as compared to controls, this increase was only significant at 1.0 mg kg bw/day dose. The number of cells of total brain was calculated (Winnick, 1976) and it was found that the cell number was significantly affected due to monocrotophos treatment at all the three doses.

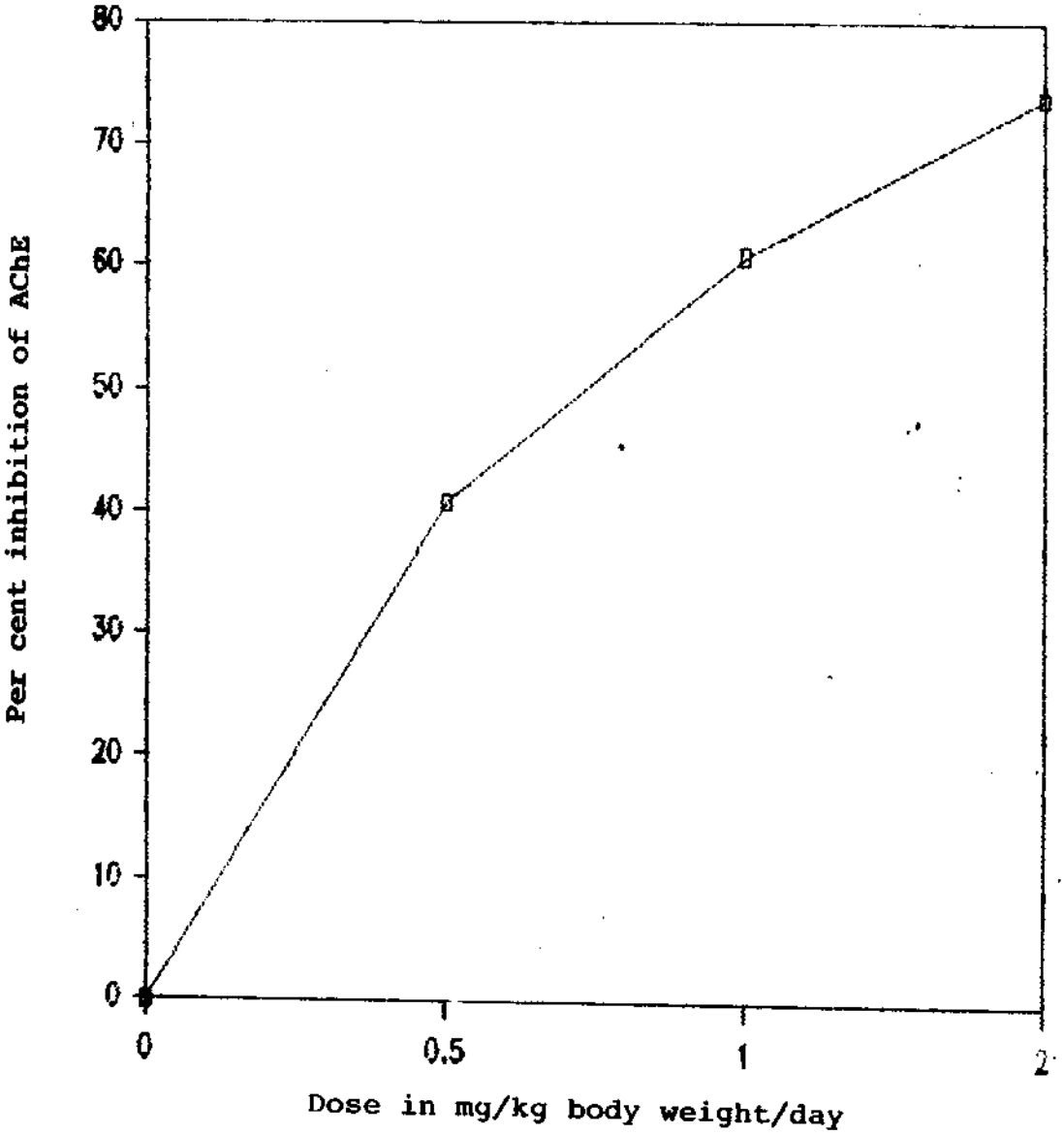


Fig. 14 : INHIBITION OF AChE ACTIVITY IN MONOCROTOPHOS TREATED RATS.

Table 20 : GABA, DNA and protein levels in brain of 21 days old pups born to monocrotophos treated dams

Treatment (mg/kg/day)	GABA (mole/g tissue) + SD	DNA (mg/g brain) + SD	Protein (mg/g brain) + SD	Protein / DNA + SD	No.of cells in brain + (x 10^7) + SD
Control	2.09 ±0.69	4.11 ±0.98	144.8 ±23.52	36.66 ±8.7	83.55 ±19.41
0.5	1.79 ±0.53	3.27* ±0.9	132.9 ±10.53	45.72 ±18.5	63.7 ±16.39
1.0	1.22** ±0.26	2.17** ±0.82	122.1* ±27.92	67.74** ±32.45	44.5** ±16.34
2.0	0.97** ±0.13	1.43** ±0.29	71.69** ±15.79	52.60 ± 12.82	24.0** ±5.44

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

+ Winick 1976.

4.4.2 Cypermethrin

4.4.2.1 Range finding test : Initially an acute toxicity study was conducted to find the LD_{50}, but even after several trials, dose related mortality could not be obtained. Therefore a range finding test was conducted, using $Na^+ K^+$ ATPase as indicator of toxicity (Table 21) since 200 mg kg body weight also affected the $Na^+ K^+$ ATPase levels significantly, 100, 300 and 500 mg kg bw/day were decided as the doses to be given during period of organogenesis and lactation. However, at 100 mg/kg body wt./day itself the pregnant dams developed toxic symptoms on 9th day of pregnancy (i.e., on 3rd day of dosing). Therefore, the doses were altered to 25, 50 and 75 mg/kg body weight.

Table 21 : $Na^+ K^+$ ATPase levels in rat brain at different doses of cypermethrin.

Dose (mg/kg body wt./day)	No. of animals	$Na^+ K^+$ ATPase (mg P/g tissue) ± SD
Control	4	$6.81^a \pm 1.12$
200	5	$5.45^b \pm 0.34$
400	5	$4.92^b \pm 0.42$
800	3	$1.47^c \pm 0.34$

$P < 0.05$; means with different supercripts are significantly different.

4.4.2.2 Teratogenicity testing : Adult female pregnant rats were administered with cypermethrin at the dose rate of 25,50 and 75 mg/kg body wt./day during 6-15 days (both days inclusive) of preganancy and from first to 21st day of parturition.

4.4.2.2.1 Effect on pregnant dams

4.4.2.2.1.1 Body weight: The body weight of pregnant dams recorded every 3 days is presented in Table 22.

Unlike in the case of monocrotophos, administration of cypermethrin to pregnant dams did not affect the body weight during pregnancy (Fig.15).

4.4.2.2.2 Effect on postnatal development : The effect of cypermethrin on pregnant and lactating dams on litter size, weight, crown-rump length, pup mortality, weights of pups at 21 days of age are recorded in table 23.

Litter size: A perusal of results presented in Table 23 would reveal that the litter size was affected at 50 mg and 75 mg/kg body wt./day dose.

Birth weight and crown-rump length: A highly significant ($P < 0.05$) decrease in both the weight and crown-rump length at 50 and 75 mg/kg body wt./day was observed.

Neonatal death rate: Cypermethrin did not increase the neonatal death rate significantly at the doses tested in the present study.

Table 22 : Effect of sub acute doses of cypermethrin on body weight of rats during pregnancy

Treatment mg/kg body wt/day	No. of rats	Body weight (g) ±SD								Increase in body wt (g)
		Day of pregnancy								
		0	3	6	9	12	15	18	21	
Control	10	183 ±15	196 ±20	201 ±18	208 ±21	215 ±22	227 ±19	241 ±29	272 ±23	89
25	9	193 ±9	204 ±11	215 ±11	217 ±7	225 ±7	235 ±8	264 ±13	290 ±12	97
50	9	182 ±14	193 ±15	203 ±14	211 16	217 ±16	227 ±18	246 ±19	266 ±18	84
75	9	183 ±19	195 ±18	196 ±18	204 ±20	217 ±26	222 ±25	254 ±27	269 ±32	86

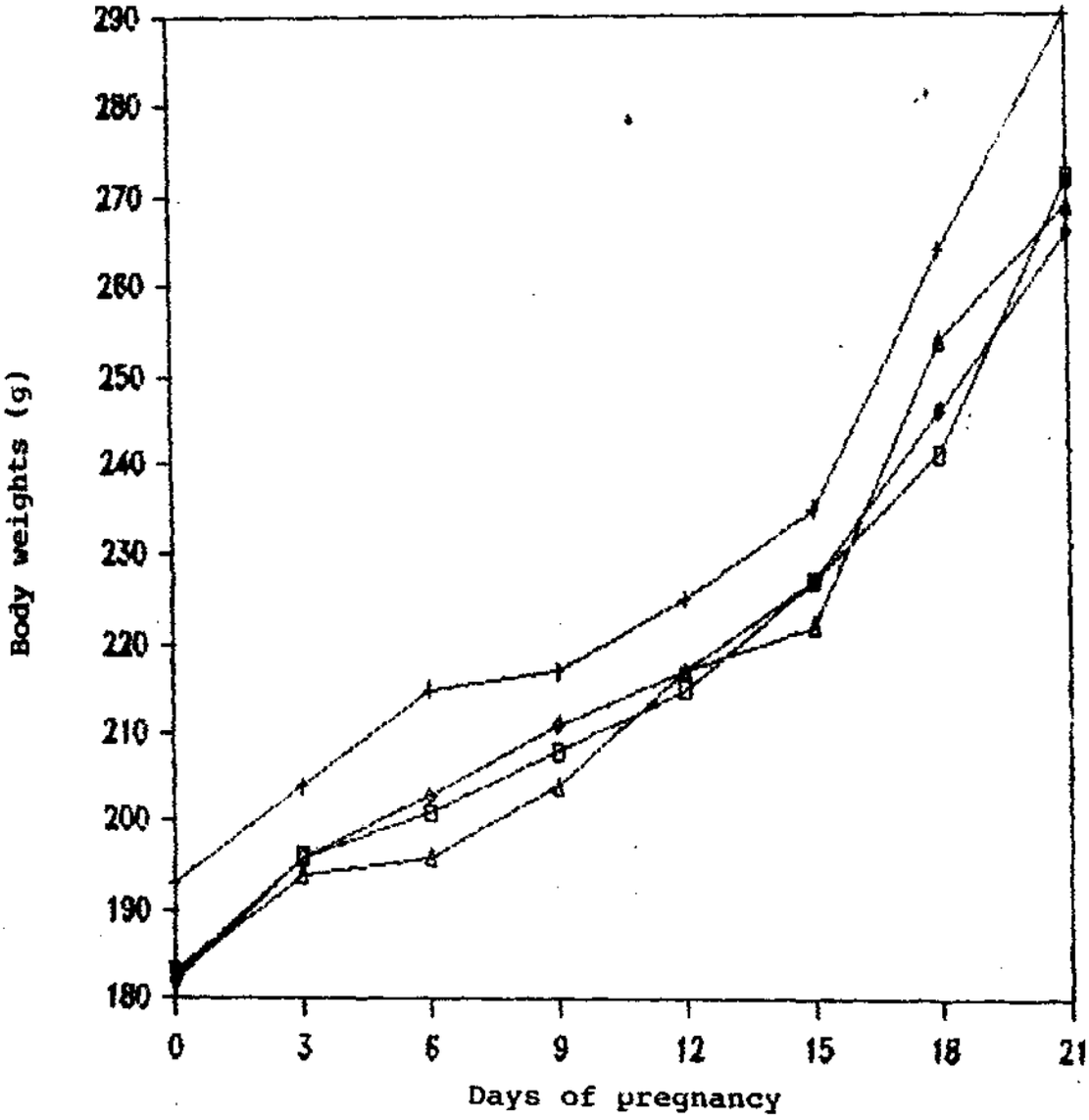


Fig.15 : BODY WEIGHTS OF CYPERMETHRIN TREATED RATS DURING PREGNANCY.

Table 23 : Effect of Cypermethrin on neonatal and postnal development of pups born to rats treated during organogenesis and post partum

Treatment mg/kg body wt.	No. of litters	At birth: Mean litter size	At birth: Mean body weight (g) ±SD	At birth: Mean crown rump (cm) ±SD	At birth: Neonatal deaths No	At birth: Neonatal deaths %	At 21 days post partum: Live pups No.	At 21 days post partum: Live pups %	At 21 days post partum: Mean body weight (g) ±SD	At 21 days post partum: Dead pups No	At 21 days post partum: Dead pups %	At 21 days post partum: Gross abnormalities
0	6	9.	5.18 ±0.49	4.71 ±0.31	6	10.9	43	78.18	23.5 ±6.2	12	21.8	-
25	9	9.9	5.25 ±0.61	4.76 ±0.34	4	4.49	71	79.78	16.9** ±3.2	18	20.2	-
50	9	8	5.47** ±0.61	4.95** ±0.35	12	16.67	36	50.0**	19.1** ±2.6	36	50.0*	-
75	9	8.1	5.61** ±0.62	4.97** ±0.35	14	19.18	35	47.9**	22.1 ±4.9	38	52.1*	Mal formed tails in 3 pups

* $P < 0.05$; ** $P < 0.01$.

Mortality at weaning: The mortality of pups was significantly high ($P < 0.05$) in 50 and 75 mg/kg body wt./day treatment group as compared to controls.

Weight of pups at weaning: The body weight was significantly ($P < 0.01$) affected at 25 and 50 mg/kg body wt./day, compared to control. Pups in 75 mg/kg body wt treatment group showed a non significant decrease in body weight.

Gross abnormalities: In the 75 mg/kg body wt./day group abnormal tail formation was observed in three pups. The tails were short and rounded unlike controls where the tails were long and tapering.

Brain weight at weaning: At 21 days pups were dissected and brain weight was recorded (Table 24), AChE and $Na^+ K^+$ ATPase activities were estimated. GABA, DNA and protein concentrations in brain were measured.

Table 24: Effect of cypermethrin on brain weight of pups at weaning

Group	Mean body weight	mean brain weight	Brain weight as per cent of body wt.
Control	23.4 ± 6.16	1.412 ± 0.14	6.30 ± 1.62
25.0	16.9** ± 3.22	1.335* ± 0.14	7.79** ± 1.32
50.0	19.0** ± 2.64	1.294* ± 0.11	6.88 ± 1.03
75.0	22.1 ± 4.91	1.394 ± 0.18	6.42 ± 1.21

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

The brain weights expressed as per cent body weight showed an increase on cypermethrin treatment. with a highly significant increase at 25 mg/kg body wt./day.

4.4.2.2.2.1 Effect on biochemical parameters of brain

The AChE and $Na^+ K^+$ ATPase activities are presented in Table 25 and GABA, DNA and protein concentrations in the brain are given in Table 26.

Table 25: Brain AChE and $Na^+ K^+$ ATPase activities in pups born to cypermethrin treated dams

Treatment mg/kg bw/day	AChE activity[1]	Na^+K^+ATPase activity
Control	142.3 ± 26.7	2.41 ± 0.65
25	109.7** ± 28.9	1.19** ± 0.35
50	77.84** ± 28.3	1.89** ± 0.50
75	72.06** ± 21.2	1.01** ± 0.48

1. u moles of ACh hydrolysed by 1 g of brain tissue at 37^oC in 10 minutes

** - $P < 0.01$

Dose related decrease in brain AChE activity was noticed

($P<0.05$) in treated groups. The per cent inhibition (Fig.16) of the enzyme activity increased as the dose increased.

Na^+K^+ATPase activity was significantly ($P<0.01$) decreased in all the treated groups compared to controls.

DNA and protein concentration were significantly decreased ($P < 0.01$) in cypermethrin treated groups. Consequently the protein to DNA ratio increased significantly in all the treated groups. The cell number in the brain was significantly ($P < 0.01$) lowered at all the doses.

Table 26: GABA, DNA and protein content of brain of pups born to cypermethrin treated dams

Treatment mg/kg body wt.	GABA u moles g brain	DNA mg/g brain	Protein mg/g brain	Protein / DNA	No. of cells in brain x 10^7
Control	2.66 ± 0.83	3.94 ± 1.19	137.2 ± 16.2	32.99 ± 12.28	97.0 ± 21.63
25.0	1.85** ± 0.42	2.38** ± 0.39	101.0** ± 5.6	44.71* ± 8.56	45.6** ± 8.27
50.0	1.45** ± 0.29	2.24** ± 0.28	91.1** ± 8.8	47.9* ± 10.0	45.69** ± 5.71
75.0	0.92** ± 0.36	1.99** ± 0.33	91.39** ± 5.7	39.33* ± 6.12	40.0** ± 11.1

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

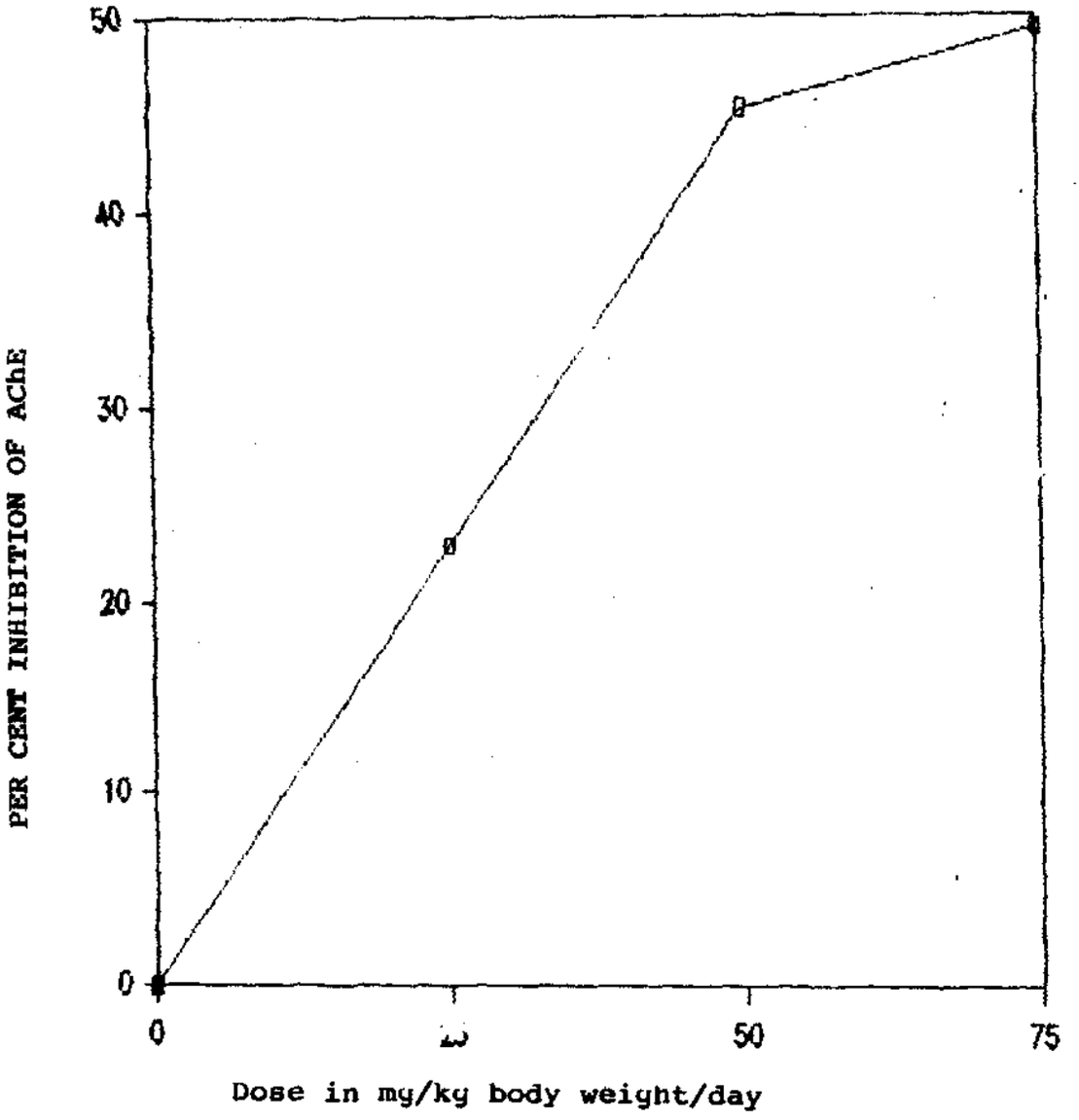


Fig.16 : PER CENT INHIBITION OF BRAIN AChE ACTIVITY IN CYPERMETHRIN TREATED RATS.

DISCUSSION

CHAPTER V

DISCUSSION

5.1 SURVEY

An appraisal of the results (Tables 3 to 8) of survey conducted to elicit information regarding pest control practices for vegetable crops revealed that farmers relied mostly on local farmers, dealers and their own experience in selection of pesticides, doses to be used, frequency and method of application. This indicates that there is a need for information on pesticides use, to be given to the farmer.

It was found that farmers could not think it unwise to increase the pesticide dose and frequency of application in terms of both hazard to humans health and consequence of development of pest resistance. They were of the opinion that a dose that cannot kill pests cannot do any harm to humans.

Fifty per cent of the farmers were unaware of the date of expiry and those who were aware, occassionally bought them since they were available at a lesser price. Srivastava and Patel (1969) are of the opinion that most of the farmers being illiterate cannot read the expiry date written in English on the label and are cheated by dealers in this regard.

The time gap between the insecticidal spray and harvest of vegetables is an important factor in deciding the ultimate amounts of pesticide residues reaching the consumer. But unfor-

tunately the farmers were unable to follow the recommendations in this regard, as they considered that it would result in overripening of the vegetable crops, thus decreasing the market value.

5.2 DISSIPATION OF INSECTICIDES

5.2.1 Monocrotophos

Monocrotophos is one of the commonly used organophosphate insecticides, on vegetables. This insecticide was applied on okra crop at two different doses, viz., 0.25 kg a i/ha (recommended dose) and 0.38 kg ai/ha (higher dose), selected on the basis of farmers use. The deposits of residues on '0' day were 4.45 and 6.86 ppm respectively (Table 9). The initial deposits appear to be higher. Dixit et al. (1981) have also reported such higher initial deposits of 3.8 and 6.25 ppm when monocrotophos was sprayed at 0.03 and 0.05 per cent. However, Krishnaiah and Prasad (1978) even at a very high dose of 0.5 kg ai/ha observed a very low initial deposits ranging from 0.23 to 0.49 ppm in three different sets of experiments over three seasons at Bangalore. Reports of low initial deposits on brinjal, chilli, onion (Awasthi, 1986; Nandihalli and Thondadarya, 1986; Srinivasan and Lingappa, 1986) and also high initial deposits on coriander, chickpea, green chillies (Jain et al., 1987; Singh and Gupta, 1981; Patil and Dethe, 1984; Narkhede et al., 1977) are not uncommon. The low initial deposits reported by Krishnaiah and Prasad (1978) on okra and also our present investigations are higher than the prescribed tolerance limit of 0.2 ppm (FAO/WHO, 1973). In view of these findings these vegetables should not be

consumed on the same day of insecticidal spray. The reasons for such a wide variations in the initial deposits are not clearly known. This may be due to amount of spray deposit received by the fruits, use of different spraying equipment, and spraying conditions.

The rate of dissipation for monocrotophos i.e., the per cent loss of pesticide was 33 and 39; 43 and 56; 70 and 70; and 84 and 85 by 1,3,7 and 10 days at 0.25 and 0.38 kg ai/ha respectively (Table 9). It can be seen that the residues dissipated at a slightly faster rate at the higher dose compared to recommended dose of pesticide application. The half life values (calculated after Hoskins, 1961) were 4.03 and 4.14 days and safety intervals were 17.5 and 19.9 days respectively for recommended and higher doses used in the present study.

The half life and safety intervals for monocrotophos found in the present study are higher than the values 2.6 - 4.8 days, half life; 14.4 - 17.1 days - safety interval reported by Dixit et al. (1981), and half life - 1.63 to 2.3 days and safety interval of 4 days reported by Krishnaiah and Prasad (1978). In the light of 0.2 ppm tolerance limits prescribed and on the basis of our data a 14 to 17 day gap between spraying of monocrotophos and harvesting appears to be safe for consuming okra fruits. The residue levels were higher than the tolerance limit even upto 10 days and at below detectable level at 15 days after application. Extensive data are available for the deple-

tion of monocrotophos on field crops.(West lake et al., 1970; Aharonson and Kesnick, 1971) indicate slower dissipation rate on fruits than on cereals. The systemic properties of monocrotophos permit the absorption and retension of residues on fruits and hence slower dissipation.

5.2.2 Cypermethrin

Cypermethrin a synthetic pyrethroid insecticide, when sprayed at two different concentrations viz., 60 g ai/ha (recommended dose) and 100g ai/ha (higher dose) on okra crop, the initial deposits were found to be 1.46 and 2.28 ppm (Table 10). The initial deposits were slightly higher compared to 1.1 and 1.3 ppm reported by Rai et al. (1980) with the 40 and 60 g ai/ha rates of application respectively. Lower initial deposits (0.93 mg/ kg) was also observed on brinjal fruits sprayed with cypermethrin (Awasthi, 1986). Eventhough the initial deposits are less, they are still above the tolerance level of 0.5 ppm (USHEW, 1978 a,b).

The dissipation of cypermethrin resulted in 25.3 and 32.0; 67.6 and 58.3; 89.7 and 89.0; and 93.8 and 93.4 per cent less by 1,3,7 and 10 days at 60 and 100 g ai/ha respectively (Table 10). Unlike with monocrotophos, the rate of dissipation did not differ for the two doses after the first day. The half life's were 2.4 and 2.5 days and safety periods were 3.5 and 5.2 days for 60 and 100 g ai/ha doses respectively. In brinjal fruits Awasthi (1986) observed the fall of cypermethrin residues to 53%

by second harvest 85.3% by third harvest giving half life value of 1.46 days. The rate of decrease of pyrethroid residues from plant material under normal conditions was influenced essentially by fruit growth resulting in the dilution of residues (Ebling, 1963; Awasthi and Anand, 1983) by evaporation and photodegradation (Elliott, 1980). The non polar and non systemic properties of synthetic pyrethroids do not permit the absorption and movement of the residues from the site of application and they remained localised on the surface of the fruit exposed to the processes of degradation. Hence the residues of cypermethrin should be removed through the decontamination process.

A large body of information on the levels of residues in crop commodities where cypermethrin has been used was available (FAO/WHO, 1980, 1985c) and proposed maximum residue levels ranged from 0.05 to 2.0 mg/kg . In addition to these data, a limited information on residues have been published by Lauren and Henzel (1977), Braun et al. (1982), Frank et al. (1982) and Awasthi and Anand (1983). Based on these maximum residue levels prescribed,when the okra fields are sprayed with cypermethrin, 3 to 5 days gap is necessary before harvesting.

5.3 EFFECT OF HOME PROCESSING ON RESIDUE LEVELS

All vegetables are generally cooked before consumption. Preparatory steps like blanching, steaming, peeling before cooking, medium of cooking and other ingredients used in cooking varies with vegetables. Effect of common methods of cooking

followed in Andhra Pradesh for okra were used to test the effect of processing on residues. As washing is the first step, the effect of washing on residual insecticide was determined and was found to remove monocrotophos deposits to an extent of 58-60% at both the doses (Tables 11 and 12). Our results are in conformity with the findings of Krishnaian and Prasad (1978) who reported a reduction of 61% residue by washing. Since the compound is readily soluble in water, surface residues are also likely to be reduced considerably by washing process. Such conclusions have been found to apply in practice. For example, Fahey et al. (1971) showed that a cold water wash removed 36 to 72 per cent of monocrotophos from field treated tomatoes.

When okra after washing was stir fried or boiled in tamarind extract with or without turmeric the removal of deposits did not show any appreciable increase (61-64%). Washing in alkaline water followed by stir frying (62-66%) was not any more advantageous than simple washing in tap water followed by stir frying (61-64%) in terms of decontamination. Monocrotophos is thermally unstable, decomposing readily at 75°C and above (Brown et al., 1966) and thermal decomposition is likely to play a major role in loss of residues. Since the compound is also readily soluble in water and most of the residues were removed during washing by hydrolysis, the difference in per cent removal of deposits between washing and stir frying and boiling was not appreciable. The per cent removal of residues was observed to decrease with increase in time gap between insecticidal spray and

harvest (Fig.8 and 9). Similar observations were made by Krishnaiah and Prasad (1978). Monocrotophos being a systemic insecticide may migrate into the tissues and get firmly bound to the tissues, thus making the process of dislodging them and destruction difficult with increasing [illegible].

As with monocrotophos, the effect of commonly used cooking processes on loss of residues from okra was studied for cypermethrin (Tables 14 and 15). Washing okra fruits (after one hour of spray) in tap water could remove 41-47% residues only, which is lesser than the removal of monocrotophos residues. This may be because of lesser amount of initial residues present in case of cypermethrin. Awasthi (1986) also reported only 30.11 to 19.2% loss of residues in brinjal fruits through washing. When washed fruits were stir fried it resulted in a further 19-21% reduction, increasing the total residues loss to 60-68 per cent. The process of washing in alkaline medium before stir frying did not appreciably increase the loss of residues (66-70%) as with monocrotophos residues. Boiling in tamarind extract removed nearly 78-79 per cent of the initial residues. The chemical constituents such as tartaric acid in the presence of high temperatures may have caused this higher loss of residues. When turmeric was added to tamarind extract, the extent of residue reduction was similar to that when only tamarind extract was used, on '0' day of spray. However, on subsequent days the removal of residues was slightly higher when turmeric was used. Rai et al. (1980) have reported a loss of 70-80% when three day

old okra fruits were washed and boiled. The residue amounts were well below the tolerance limit of 0.5 ppm (USHEW , 1978 a,b), based on which they recommended a waiting period of 3 days. A similar effect (as with monocrotophos) of decreasing efficacy in removal of residues was found on ageing of the residues. The decreasing effect of these treatments in reducing the residues on ageing may be due to reduced availability of the free form of the pesticides as the pesticides molecules become physically bound or conjugated with chemical constituents of the fruit skin and become more resistant to the decontamination process even with alkaline medium. Synthetic pyrethroids are susceptible to alkaline hydrolytic breakdown, but are stable under acidic conditions (Elfott et al., 1978) and therefore washing the fruits in sodium bicarbonate solution enhanced the degradation pattern of the residues, compared to plain water washing.

Decontamination of the insecticidal residues through various processes is essential, since decontamination through the dilution of residues during fruit growth and physico-chemical degradation may not always be sufficient to prevent unacceptable levels of pesticide remaining on fruit intended for human consumption, particularly under present conditions in India where there is no regular monitoring of market sampels of fruit and vegetables. It is therefore advisable to utilise a suitable decontamination process, such as those described here, on a regular basis as a precaution aimed at reducing the possible hazards of pesticide residues.

5.4 TOXICITY STUDIES

5.4.1 Monocrotophos

Considerable amount of literature has been reported on LD_{50} of monocrotophos ranging from 12 to 21 mg/kg body wt (Agrochemicals Hand Book, 1984; Eto, 1976; Janardhan, 1981). FAO/WHO (1973) reported an oral LD_{50} of 20 mg/kg body wt for female rats. In the present study the LD_{50} for monocrotophos in female adult rats was found to be 10 mg/kg body wt.

In the present study monocrotophos treated rats had shown signs of diarrhoea during pregnancy and lactation. Similar observations were reported with monocrotophos (Janardhan 1981) and DDVP (Given et al. 1980) in adult rats. Such gastro-intestinal manifestations usually appear due to local anticholinesterase action in the gastro-intestinal tract. This may be one of the reasons for decreased body weight gain during pregnancy as observed in the present study (Fig.13 and Table 16).

The litter size was not affected in any of the treated groups. Ramsey et al. (1969) immersed rats in a 1% solution of an organo phosphate insecticide during different days of gestation and found that litter size and survival of offspring until weaning were unaffected. A similar effect was observed in pigs with oral feeding of DDVP (Collins et al., 1971). It is apparent from the present findings and also the findings of other workers that the litter size is not affected by the ingested sub-acute doses of organophosphorus insecticides. However, Janardhan et al.

(1984) found reduced litter size in the rats treated with higher dose of (2.4 mg/kg body wt) monocrotophos.

The body weight and crown-rump length at birth were significantly ($P < 0.05$) less compared to those of control group. Growth retardation is a manifestation of abnormal development (Maclaren and Michie, 1960). It is contended (Budreau and Singh, 1973) that the lower birth weight was due to availability of nutrients to the fetus being affected due to inhibition of esterases by the insecticides. Perrota and Lewis (1968) have shown the presence of esterases in human, guinea pig and rat placenta. The neonatal deaths were significantly high ($P < 0.01$) in all the treated groups (Table 17). Similar results were reported in rats with malathion (Kalow and Morton, 1961) and methyl parathionn and DFP (Fish, 1966) and monocrotophos (Janardhan et al., 1984).

Neonatal deaths have been attributed to diminished secretary function of the mammary gland, which interferes with nursing (Barnes and Denz, 1951) or the chemical could be excreted in milk without being detoxified in the body, thus exerting toxic effects on the infant (Newman and Gross, 1963; and Areis et al., 1964). It is also possible that the chemical could be excreted in milk and render it unpalatable to the infant (FDA, 1970).

Crown-rump length is considered as one of measures of growth parameters in rats, other mammals and also chicks monocrotophos significantly affected the crown rump length

adversely and recent available evidence (Kalakumar, 1988) also clearly indicated significant decrease in the crown-rump length. Growth retardation is a manifestation of abnormal development and is stated to be the most sensitive parameter to judge the embryo toxicity. The growth retardation may be due to placental insufficiency resulting in decreased rate of DNA synthesis during intra uterine period.

In the present study the mean weight from birth to weaning was found to be reduced in 2.0 mg/kg body wt/day treatment group (Table 17). Eisenlord and Loquram (1965 and 1966) in a three generation study found a similar effect with monocrotophos at 12 and 30 ppm in diet. Menzer and Casida (1965) found that monocrotophos is excreted in milk when goats were orally fed with it. In the light of above observation it could be possible that a similar excretion might have occurred in rat milk, exerting adverse effects on the young one or rendering it unpalatable to the young one (FDA, 1970).

A dose of 20 mg/kg body wt during the period of organogenesis of pregnancy and lactation, reduced the brain weights of pups at 21 days of age (Table 18). Similar decrease in brain weights in day-old chicks with monocrotophos was reported (Kalakumar, 1988). The decrease in the brain cell numbers (Table 20) might reflect the decreased brain weights.

In the present study the reduction in brain weight was accompanied by a reduction in both DNA and protein content, with

an increase in protein/DNA ratio. Similar effects were observed with methylazoxy ethanol (MAM) when rats were exposed. A prenatal exposure of rats to MAM reduced brain growth in the first three days, regaining the normal growth after 3 days, but reduction in DNA persisted throughout maturity of animal. Apholate was found to interfere with nucleic acid and protein synthesis in male albino rats (Haqqui and Adami, 1979). The vulnerable period of development extends nearly over the whole perinatal period. Particularly the brain growth spurt reaches its maximum at the end of the first week in the rat. This corresponds to the late fetal phase of human brain development (Benesova et al., 1984). Therefore the whole period of organogenesis, and post natal development till weaning was chosen in rats for sub-acute treatment with monocrotophos. Alteration of protein synthesis in growing organs may produce a reduction of DNA synthesis, and measurement of DNA synthesis is the biochemical basis of cell proliferation. Since any brain growth deficit may be caused either by a reduction of cell numbers or by diminution of average cell size, the DNA content and protein DNA ratio respectively were estimated in weaning rats. The perusal of data (Table 20) suggests that inhibition of neonatal protein synthesis adversely affected permanently the growth of the brain.

The effect on rate of synthesis of DNA seems to be more than that of protein as indicated by increases in protein/DNA ratio, which is a measure of increase in cell (Table 20) size.

Exposure of dams during pregnancy and lactation to monocrotophos decreased the brain AChE activity ($P<0.05$) in pups. Hoffman and Silco (1984) observed a similar inhibition of brain ChE activity in hatchlings from mallard eggs when exposed to topical application of EPN on day 3 of development. Dose-dependent decrease in brain ChE activity in day old chicks was reported (Kalakumar, 1988). When chick embryos were exposed to monocrotophos during embryogenesis in vovo. Similar reduction in brain ChE activity has been reported in Japanese quails with monocrotophos (Shellenberger, 1966), rats with cyolane (Hussein and Abd-El Massih, 1977) and monocrotophos (Janardhan et al., 1984), chicks (Naidu et al., 1978) with dichlorovos.

GABA is the most powerful inhibitory transmitter (system) in the mammalian brain which plays a major role in functioning of nervous system. The GABA concentration in the brain is an indicator of the functional state of GABA receptors. In the present study the GABA levels were significantly lowered in groups treated with 1.0 mg/kg bw and 2 mg/kg bw (Table 20). A similar phenomenon has been noted by Ali and Hasan (1977), with dichlorvos in adult rats. Roberts and Kuriyan (1968) have reported that neonatal cerebral GABA levels are generally low and increase with age. In rat GABA and glutamic acid in brain increase dramatically during first 21-30 days of life. Vernadakis and Woodbury (1962) also have pin pointed a progressive increase in GABA and other amino acids in cortical grey matter of rat brain from birth to 25th day. Therefore decrease in brain GABA

levels in the present study is yet another indication of the disturbances of inhibitory brain mechanisms consequent to perinatal brain maldevelopment (Benesova et al., 1984).

5.4.2 Cypermethrin

In the present study the LD_{50} of cypermethrin could not be arrived at, since the mortalities were not correlating with the dose of the chemical. LD_{50} is known to be affected by concentration, vehicle, temperature, age and strain of the experimental subjects (Coombs et al., 1976). The toxic symptoms viz., splayed gait, tip-toe walk, with occassional tremors and convulsions leading to prostration and death in severe cases of poisoning observed in the present study on acute dosing were similar to those observed by Coombs et al. (1976).

The results of the sub acute dosing of pregnant dams during the period of organogenesis indicate that the body weight gain in treated groups were similar to that of the controls (Table 22). In contrast Tesh et al. (1978) observed a slight but significant reduction in maternal body weight gain at 35 and 70 mg/kg bw cypermethrin. This difference could be due to difference in strain of rats used.

The average litter size was reduced by 50 and 75 mg/kg bw (Table 23). These results are in accordance with those of Ahmed and Gupta (1988), who reported that at lower doses (15 and 30 mg/kg bw) cypermethrin did not affect the litter size.

The neonatal deaths in treated groups were not signifi-antly different from those of controls. A similar result at 15 nd 30 mg/kg body wt doses was reported by Ahmed and Gupta 1988).

In the present study the mean litter weight and crown-ump length at birth were significantly less in 50 and 75 mg/kg ody wt/day treatment groups. Hend et al. (1978), in a three eneration study found a similar decrease in total litter weight nd size at 500 mg/kg diet (25 mg/kg body wt) in F1b generation.

The survival of pups at weaning was significantly less t the higher doses of 50 and 75 mg/kg body wt, as compared to ontrols. However the body weight gain showed an unsual trend ith significantly low body weight at 25 and 50 mg/kg body wt and o effect at 75 mg/kg body wt. This may be due to high neonatal eath rate at 75 mg/kg dose compared to 25 and 50 mg/kg body t/day groups. This could be the result of the fact that less umber of pups were nursed by the dam during post-natal period. Ahmed and Gupta (1988) also recorded a significant reduction in weight gain at 4, 14 and 21 days of lactation. At 30 mg/kg the lactation index was reported to be significantly reduced by Croucher et al. (1985) and Swaine and Saplets (1980) have reported that cypermethrin residues were present in milk when cows were fed cypermethrin in feed, even at as low levels as 2, 5 and 10 mg/kg feed. The low body weight gain of pups in the present study may be due to either unpalatability/insufficiency of milk or the adverse effects of the chemical in the growth process.

The results of the present study revealed decreased brain weights in all treated groups (Table 24) with significant decrease only at 25 and 50 mg/kg bw/day groups. But, the significant reduction in brain weight was recorded only in the group treated with 25 mg/kg bw/day. This discrepancy could not be explained and could be an artifact. DNA and protein content of brains were significantly reduced in all the treated groups (Table 27). The protein to DNA ratio showed a significant increase in all the treated groups. This suggests that the effect on cell proliferation as a consequence of decreased DNA synthesis is much more compared to the effect on cell size due to a decreased protein synthesis. Protein metabolism was shown to be affected in branchial tissue of fish (Reddy and Basha Mohideen, 1986). Wherein, the total, structural and soluble proteins were shown to be significantly decreased with the increase in catobolic enzymes. Therefore it could be construed that the decrease in protein content could probably be due to microzonal stimulating effect of cypermethrin, resulting in increased metabolising enzymes.

The AChE as well as Na^+K^+ ATPase enzyme activities (Table 25) were found to be inhibited due to cypermethrin. The AChE has been known to be inhibited to a lesser degree due to cypermethrin as compared to monocrotophos. Since the organophosphates are specific cholinesterase inhibitors, the enzyme activity is a measure of toxicity of organophosphate pesticides. Similarly the Na^+K^+ ATPase activity is a measure of toxicity due

to pyrethroids. The primary target site of cypermethrin and of pyrethroid insecticides in general is the sodium channel, causing a long-lasting prolongation of the normally transient increase in sodium permeability during excitation, resulting in long lasting train of repetitive impulses. The decreases in Na^+K^+ ATPase activity was significant at all the doses tested, but this decrease was not dose-related .

A dose-related decrease in the brain GABA (Table 26) levels in all the treated rats point to disturbances of inhibitory brain mechanisms consequent to perinatal maldevelopment of brain.

In conclusion it can be stated that pesticides are invariably used as one of the most effective pest control measures on all most all the food crops. When used on vegetable crops they have a special significance in that they tend to leave higher residues by the time they are consumed as compared to other crops such as cereals, millets or pulses. This is because of non adherence to recommended crop protection schedules such as use of appropriate pesticides in their right concentration, number of applications and safety intervals. As a step towards reducing the residue content before consumption different pretreatments before cooking and different cooking procedures have been reported in literature.

Dissipation studies on two most commonly used pesticides ie., monocrotophos and cypermethrin have revealed that since

safety intervals range from 16 to 19 days for monocrotophos and 3 to 5 days for cypermethrin, the former pesticide should be used at least 20 days before harvest and the latter can be used 5 days before harvest, so that as the safety intervals can be maintained without loosing the economic viability of the vegetables due to over ripening. Of the different cooking processess studied in an endeavour to seek most effective methods for further reducing the residue levels, washing okra fruits prior to cooking was most effective in case of monocrotophos residues, but cypermethrin residues could be removed to a higher levels by washing the fruits and stir frying or boiling in tamarind extract with or without adding turmeric.

The toxicological studies on the offspring of the dams exposed to both monocrotophos and cypermethrin during pregnancy and lactation indicate that the brain parameters such as AChE, $Na^{+}K^{+}$ ATpase, GABA, protein and DNA show slight to significant changes depending on the dose to which the animals were exposed pointing to maldevelopment of brain in young ones. The urban population may or may not be exposed to such levels of these pesticides, since even if the recommended safety intervals are not adhered to, the transit time may cause further degradation of residues, specially where safety intervals are less as with cypermethrin. But the rural masses stand greater chances of receiving vegetable crops with higher residues as the produce reaches them without much delay after harvest. Moreover the exposure to pesticides is not only through consuming foods

containing residues but also during agricultural operations. It is a common practice for women to participate in agricultural operations and though they may not be actually involved in spray operation are exposed to these sprays either by inhalation or dermal rout. If this kind of exposure, coupled with consumption of foods containing high residues occurs during pregnancy or lactation, may forebode complications in fetal development and development of new born. Thus there is a great need for proper education of those involved in agriculture regarding health hazards and precausions to be taken.

Since in India the food habits and agricultural practices differ from region to region, it is desirable to assess the levels of various pesticides present in composite diets of diffesrent regions with emphasis on rural areas and study the toxic effects in terms of physical and mental well being of the population.

SUMMARY

CHAPTER VI

SUMMARY

The investigations carried out both under field as well as in the laboratory includes (i) survey on the pesticide use in three vegetable growing areas of Andhra Pradesh representing high, medium and low pesticide consuming area, (ii) dissipation of monocrotophos and cypermethrin on Okra and effectiveness of various cooking proceaures in reducing the residue levels, and (iii) teratogenic effects of monocroptophos and cypermethrin through monitoring of relavent bio-chemical parameters in albino rats.

The survey under taken in three different pesticides using areas of Andhra Pradesh viz., Guntur, (high pesticides consuming area), Anakapalli (medium pesticides consuming area). and Ranga Reddy District (low pesticides consuming area) selecting around 40 farmers as subjects from each area, clearly indicated dinstinct differences in the pesticides usage. In guntur region most of the farmers apply higher than the recommended doses and number of applications of pesticides on vegetable crops, whereas at Anakapalli and Ranga Reddy District regions mostly farmers use less than the recommended doses, except few farmers who exceed the recommended doses.

In all the three regions, safety intervals between application and harvest of the produce were not followed by the farmers. The source of information for the pesticide to be used

and dose to be applied was mostly the dealers of the pesticides and experienced farmers but not the official sources and advertisements. Few farmers are aware of the expiry date of the pesticide at Anakapalli and Ranga Reddy regions. However at Guntur region most of the farmers were aware about expiry date of pesticide.

The dissipation studies on Okra under field conditions for monocrotophos applied at 0.25 kg. a.i/ha (recommended dose), 0.38 kg. a.i/ha (higher dose) and cypermethrin 60 and 100 g a.i/ha (recommended and higher dose respectively) recorded initial deposits of 4.45 and 6.86 ppm for monocrotophos and 1.46 and 2.28 ppm for cypermithrin on Okra fruits.

Half life periods for monocrotophos were 4.03 and 4.14 days and for cypermethrin 2.41 and 3.54 days. The t.tol periods were 17-19 days for monocrotophos and 2.5 to 5 days for cypermethrin.

Among the processing procedures tried viz., washing in running water, washing and stir frying, washing and boiling in tamarind extract with and without turmeric and washing in sodium bicarbonate water followed by stir frying; washing in running water removed 58 to 60% of initial monocrotophos residues. The other processing procedures removed 60-65% initial monocrophos deposits. In the subsequent days (1,2,7 and 10 days) the effect of processing procedures in removing the residues was more or

less similar like initial deposits, however the percentage removed was less.

Boiling with tamarind extract (with or without turmeric) proved to be best in removing the initial deposits of cypermithrin (75 to 79%) followed by washing in water and stir frying (66 to 70%) and washing in running water (41 to 47%). The extent of the removal of residues appears similar and followed the same order of the processing procedures during the subsequent days after application.

In teratogenicity studies, monocrotophos was administered at the dose rate of 0, 0.5, 1.0 and 2 mg/kg body wt day to pregnant rats during the period of organogenesis and postnatal development.

Monocrotophos significantly affected the body weight in the rats during pregnancy at 2 mg/kg body wt./day. Similarly mean reduction in birth weight, mean crown rump length, and increase in neonatal deaths were significant at all the doses (0.5, 1 and 2) of monocrotophos. Effect on the litter size by the pesticide was not apparent. Per cent survival of pups was significantly affected at all doses and mean litter weight at weaning was affected only at higher dose.

Monocrotophos also resulted in the decrease in brain weights, AChE activity and DNA in all the doses. Decrease in GABA and protein concentration in the brain was observed at

higher doses (1 and 2 mg kg body wt./day) indicating maldevelopment of brain during post natal period.

For cypermethrin the dose range of 0, 25, 50 and 75 mg/kg body wt./day chosen for teratogenicity testing did not affect the weight gain of dams during pregnancy. Similarly litter size and per cent neonatal deaths were not affected. Significant reduction in mean litter weight and mean crown-rump length and per cent servival of pups of 25 days age was observed at 50 and 75 mg/kg body wt./day. Mean litter weight was significantly affected at 25 and 50 mg/kg body wt./day with abnormal tail formation (only at higher dose).

Effects of cypermethrin on the decrease in brain weights, AChE activity and DNA were similar to monocrotophos. Protein, GABA in brain decreased significantly at all doses. Protein/DNA ratio significantly increased at all doses. Sodium and potassium ATP are significantly decreased at 50 and 75 mg/kg body wt./day indicating the teratogenic potential of both the pesticides.

...

LITERATURE CITED

LITERATURE CITED

Abbott W S 1925 A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18:265-267.

Agnihotri N P, Dewan R S and Dixit A K 1974a Residues of insecticides in food commodities from Delhi I. vegetables. Indian Journal of Entomology 36:160-162.

Agnihotri N P, Dewan R S, Jain H K and Pandey S Y 1974b Residues of insecticides in food commodities from Delhi II. High fat content of food materials. Indian Journal of Entomology 36: 203-208.

Agnihotri N P, Jain H K, Rai S and Gajbhiye V T 1980 Relative toxicity and residues of synthetic pyrethroids on cabbage and cauliflower. Indian Journal of Entomology 42: 717-722.

Agnihotri N P, Gajbhiye V T, Jain H K, Srivastava K P and Sinha S N 1983 Residues of synthetic pyrethroids insecticides on Capsicum frutescens (Linn.) (chilli), Abelmoschus esculentus (Moench) (Okra) and Allium cepa (Linn.) (Onion). Journal of Entomological Research 7: 10-14.

Agnihotrudu V and Mithyantha M S 1978 Organophosphhate insecticides in pesticide residues Rallis India Limited, Bangalore pp.22.

Ahmed N and Gupta P K 1988 Reproduction (one generation) toxicity of cypermethrin in rats. Journal of Environmental Biology 9: 149-156.

Aharonson N and Resnick C H 1971 The dissipation of some new organophosphorus compounds in vegetable crops. Abstract 2nd International Congress on Pesticide Chemistry, Israel pp.118.

Ali S F and Hasan M 1977 Effect of organophosphate insecticide dichlorvos on the amino acid content of different regions of the rat brain and spinal cord. Indian Journal of Experimental Biology 15:759-761.

All India Coordinated Research Project 1990 Bulletin pesticide contamination. Pesticide residue scheme. Andhra Pradesh Agricultural University pp.23.

Anderson A, Anderson O, Akerblom M 1982 Pesticide residues in fruits, berries, vegetables, root vegetables, potatoes, mushroom, juices and marmalades on the Swedish markets, January 1978 - December 1980. Var. foda supplementary 34: 186-216 and Food Science and Technology Abstracts 15: 12 J 1961, 1983.

Aries I M, Garther L M, Deifter S and Furman M 1964 Prolonged neonatal unconjugated hyperbilirubinemia associated with breast feeding and asteroid pregnandiol, maternal milk that inhibits glucuronide formation in vitro, Journal of Clinical Investigation 43:2037-2047.

Awasthi M D and Anand L 1983 Dissipation and persistence of synthetic pyrethroids on fruits of Okra. Journal of Entomological Research 7: 55-59.

Awasthi M D 1984 Fate of Quinolphos and monocrotophos residues on bitter gourd fruits. Journal of Food Science and Technology 21:113-115.

Awasthi M D 1985a A rapid and efficient extraction - clean up method for GLC determination of synthetic pyrethroid residues in fruits and vegetables. Journal of Food Science and Technology 22:4-6.

Awasthi M D 1985b Persistence pattern and safety evaluation of synthetic pyrethroids on egg plant. Indian Journal of Agricultural Science 55:600-603.

Awasthi M D 1986 Chemical treatments for the decontamination of Brinjal fruit from residues of synthetic pyrethroids. Pesticide Science 17:89-92.

Awasthi M D, Handa S K, Dikshit A K and Bhattacharjee N S 1978 Dissipation of metasystox and monocrotophos in or on soybean crop. Indian Journal of Agricultural Science 48: 245-247.

Awasthi M D, Kalra A N and Dewan R S 1977 Fate of monocrotophos and carbaryl on cowpea. Indian Journal of Plant protection 5:55.

Bagle B G 1987 Studies on persistence of phorate and monocrotophos on soybean and okra. Ph.D. Thesis Abstracts XV(3): 206, no. 235 1989.

Baker P G and Bottomley P 1982 Determination of residues of synthetic pyrethroids in fruits and vegetables by gas liquid and high performance liquid chromatography. Analyst 107:206-212.

Barnes J M and Denz F A 1951 The chronic toxicity of p-nitrophenyl diethyl thiophosphate (E.605). A long term feeding experiment with rats. Journal of Hygiene (London) 49: 430.

Bayer S M and McMurray W C 1967 The metabolism of amino acids in developing rat brain. Journal of Neurochemistry 14: 695-706.

Benesova O, Petova J and Pavlik A 1984 Brain development and drugs. Avecenum, Czechoslovak Medical Press, Prague.

Bennet E L, Rosenzweig M R, Krech D, Ohlander A and Morimoto H 1962 Cholinesterase activity and protein content of rat brain. Journal of Neurochemistry 6:210-218.

Bhatt V H 1985 Pesticide hazards in animal. Journal of Health Sciences XI: 116-122.

Bhunya S P and Behera B C 1988 Mutagenicity assay of an organophosphate pesticide, monocrotophos in mammalian in vivo test system. Cytologia 53: 801-807.

Bindra O S and Kalra R L 1973 A view of work done in India on pesticide residues. Presented at symposium : Progress and problems in pesticide residue analysis PAU, Ludhiana.

Braun H E, Frank R and Miller L A 1966 Residues of cypermethrin in milk from cows wearing impregnated ear tags. Bulletin of Environmental Toxicology 35: 61-64.

Braun H E and Stanek J 1962 Application of the AOAC multiresidue method to determination of synthetic pyrethroid residues in celery and animal products. Journal of the Association of Official Analytical Chemists 65: 685-689.

Buckwell A C 1981 A two year feeding study in dogs on W L 43467, Sittingborne, Shell Research (SBGR 81.126).

Buckwell A C and Butterworth S T G 1977 Toxicity studies on the pyrethroid insecticide W L 43467, a 13 week feeding study in dogs. Sittingborne, Shell Research (TLGR .0127.77).

Budreau C H and Singh R P 1973 Effect of fenthion and dimethoate on reproduction in the mouse. Toxicology Applied pharmacology 26: 29-38.

Chakrabarthy P and Banerjee V 1988 Effect of sublethal toxicity of three organophosphorus pesticides on the peripheral hemogram of the fish. Channa punctatus. Environmental Ecology 6: 151-158.

Chanda R, Wood Ward D J and Griffith S 1973 Cerebellar development in the rat after early postnatal damage by MAM. DNA, RNA and protein during recovery. Journal of Neurochemistry 21:547.

Chengli, Miller W T and Jiang J 1989 Pulmonary edema due to ingestion of organophosphate insecticide. American Journal of Rodentology 152: 265-266.

Civen H, Leeb J E, Wishnow R M, Wolfsen A and Morin R J 1980 Effect of low level administration of dichlorvos on adreno corticotrophic hormone secretion, adrenal cholesterol ester and steroid metabolism. Biochemical Pharmacology 29 : 635-641.

Collins J A, Schooley N A and Singh V K 1971 The effect of dietary dichlorvos on swine reproduction and viability of their offspring. Toxicology and Applied Pharmacology 19 : 377.

Coombs A D, Carter B I, Hend R W, Butterworth S G and Buckwell A C 1976 Toxicity studies on the insecticide WL 43467. Summary of results of preliminary experiments, Sittingbourne, Shell Research (TLGR 0104.76).

Crawford M J 1977 The metabolism of WL 43467 in mammals. The fate of a single oral dose (14_C cyclopropyl) WL 43467 in the rat. Sittingbourne, Shell Research (TLGR. 0004-77).

Crawford M J, Croucher A and Hutson D H 1981a Metabolism of cis- and trans cypermethrin in rats. Balance and tissue retention study. Journal of Agriculture and Food Chemistry 29 : 130-135.

Crawford M J, Croucher A and Hutson D H 1981 b The metabolism of the pyrethroid insecticide cypermethrin in rats : excreted metabolites. Pesticide Science 12 : 399-411.

Croucher A, Hutson D H and Stoydin G 1985 Excretion and residues of the pyrethroid insecticide cypermethrin in lactating cows. Pesticide Science 16 : 287-301.

Dahiya B and Chauhan R 1982 Organochlorine insecticides residues in vegetable samples from Hissar market. Indian Journal of Agricultural Science 52(8): 533-535.

Dahl J L and Hokin L E 1974 The sodium potassium adenosine triphosphatase. Annual Review of Biochemistry 43:327-355.

Davies K L, Berger P A, Hollister L E and Barchas J D 1978 Cholinergic involvement in mental disorders. Life Science 22: 1865.

Davis J M, Janowsky D, Tamminga C and Smith R C 1978 Cholinergic mechanisms in schizophrenia, mania and depression in cholinergic mechanisms and psychopharmacology (ed. Jenden D J), Plenum Press, New York pp.805.

Declume C, Cambon C and Derache R 1978 The effects on newborn rats of repeated carbaryl administration during gestation. Toxicology Letter AMST 3 : 191.

Desi I, Varga L, Dobronyi I and Szklenarik G 1985 Immunotoxicological investigation of the effects of a pesticide. Cypermethrin. Archives of Toxicology Supplementary 8 : 305-309.

Deutsch J A 1973 The cholinergic synapse and the site of memory In The physiological basis of memory (ed. J A Deutsch), Academic Press, New York pp.59.

Dische Z 1955 Color reactions of nucleic acid components. In "The nucleic acids" Vol.I. (Eds. Ernin chargaff and J N Davidson) Academic Press Inc., Pub. New York.

Dix K M 1978 Toxicity of WL 43467 : Teratological studies in rabbits given WL 43467 orally, Sittingbourne, Shell Research (TLGR 0010 : 78).

Dixit A K, Awasthi M D, Handa S K and Verma S 1981 Persistence of monocrotophos residues on Abelmoschus esculentus. Indian Journal of Entomology 43 : 450-452.

Eadsforth C V and Baldwin M K 1983 Human dose excretion studies with the pyrethroid insecticide. Cypermethrin. Xenobiotica 13 : 67-72.

Ebling W 1963 Dissipation, degradation, persistence and effectiveness of pesticides. Residue Reviews 3:35-163.

Edward C A, Veeresh G K and Kruegar H R 1978 Pesticide residues in the environment in India. University of Agricultural Sciences, Bangalore, Technological Series No.32.

Eisenlord G and Loquram G S 1965 Results of short route reproduction study of rats fed diets containing S D 9129 insecticide over three generations. Data from the Hine laboratory submitted by the shell chemical company (unpublished).

Eisenlord G and Loquram G S 1966 Results of long route reproduction study of rats fed diets containing S D 9129 insecticide over three generations. Data from the Hine laboratory Submitted by the Shell Chemical Company (unpublished).

Elliott M 1980 Established pyrethroids Pesticide Science 11:119-163.

Elliott M, James N F and Potter C 1978 The future of pyrethroids in insect control. Annual Review of Entomology 23:443-469.

Eto M 1976 Individual pesticides P.286 In Organophosphorus Pesticides Organic and Biological Chemistry. Pub. CRC Press.

Fahey J E, Gould G E and Nelson P E 1969 Removal of Gardona and Azodrin from vegetable crops / commercial preparative methods. Journal of Agriculture and Food Chemistry 17: 1204-1206.

Fahey J E, Nelson P E and Gould G E 1971 Removal of Azodrin residues from tomatoes by commercial preparative methods. Journal of Agriculture and Food Chemistry 19:61-62.

FAO/WHO 1973 Report on the 1972 Joint FAO/WHO on peaticide residues in food. FAO Agricultural studies No: 90, P.25.

FAO/WHO 1975 Evaluation of some pesticide residues in Food AGI/974/M/11, WHO Pesticide residues series No.4.

FAO/WHO 1980, 1979 Evaluations of some pesticide residues in food, Rome, Food and Agriculture Organisation of the United Nations (FAO Plant Production and Protection Paper No.20, Suppl.).

FAO/WHO 1982 Pesticide residue in food : 1982 evaluations FAO Plant Production and Protection Paper No.49 : vii.

FAO/WHO 1982 b, 1981 Evaluations of some pesticide residues in food, Rome Food and Agriculture Organisation of the United Nations (FAO Plant Production and Protection Paper No.42).

FAO/WHO 1983, 1982 Evaluations Evaluations of some pesticide residues in food, Rome, Food and Agriculture Organisation of the United Nations (FAO Plant Production and Protection Paper No.49).

FAO/WHO 1985, 1984 Evaluations of some pesticide residues in food, Rome Food and Agriculture Organisation of the United Nations (FAO Plant Production and Protection Paper No.67).

Finney D J 1952 Probit analysis, Cambridge University Press, Cambridge pp.318.

Fish S A 1966 Organophosphate cholinesterase inhibitors and foetal development. American Journal of Obsterics and Gynacology 96: 1148.

Gupta D S 1980 Residue analysis of insecticides. Haryana Agricultural University, Hissar, Haryana.

Gupta P K 1985 Pesticide pollution vs Animal health. 72nd Indian Science Congress, 2nd - 8th January pp.121-122.

Gupta P K 1986 Pesticides in the Indian Environment Ist ed. Interprint, New Delhi.

Gupta P K 1989 Established effects of pesticide residues In Pesticide residues in food. Pub. Consumer Education Research Centre, Ahmedabad pp.114-127.

Gupta S C 1979 Insecticide residues in the market samples of vegetables from Ludhiana. M.Sc. Thesis, Punjab Agricultural University, Ludhiana.

Gupta R E, Rech R H, Lovell K L, Melsh F and Thomburg J E 1985 Brain cholinergic, behavioural and morphological development in rats exposed in vitro to methyl parathion. Toxicology and Applied Pharmacology 77: 405-415.

Haber B, Kuriyama K and Roberts E 1970a Mitochondrial localization of new L-glutamic acid dicarboxylase in mouse and human brain. Brain Research 22: 105-112.

Handa S K 1989 Pesticide residues in food, Retrospect and prospect. In pesticide residues in food Consumer Education and Research Centre pp.260-266.

Hariharan S 1980 The Laboratory Rat. In LAIS Centre News, Laboratory Animal Information Service Centre, ICRM pp.1-26.

Hart T B, Spinks C A, Harlow R C and Suthers J R 1982 A study of the exposure and health of Indian workers spraying, Ambush and Cymbush on cotton using high volume hand held spray applicators, Fernhurst, Imperial Chemical Industries, Plant Protection Division (Report TMF 1841-B).

Haqqui M T and Adhami M 1979 Effect of apholate (ENT 26316 : NSC 26812) on Nucleic acids, total protein content and acidity. Indian Journal of Experimental Biology 17: 448.

Fleisher J H and Pope E J 1954 Calorimetric method determination of red blood carpuscles cholinesterase activity in whole blood. Archives of Industrial Hygiene and Occupational Medicine 9:323.

Flickinger E L, White W H and Mitchell C A 1984 Monocrotophos and dichrotophos residues in birds as a result of misuse of organophosphates in metagorda county, Texas. Journal of Association of Official Analytical Chemists 67: 827-828.

Fonnum F, Storn - Mathisen J and Walberg F 1970 Glutamate decarboxylase in inhibitory neurons. A study of the enzyme in Purkinje cells axons and buotous in the cat. Brain Research 20: 259-275.

Food and Drug Administration Advisory Committee on protocols for safety evaluations 1970 Toxicology Applied Pharmacology 16: 264-296.

Forsyth C S and Chambers J E 1989 Activation and degradation of the phosphorothionate insecticides parathion and EPN by rat brain. Biochemistry and Pharmacology 38: 1597-1603.

Frank R, Braun M E, Ritcey G, Meewen F L and Sirons G J 1982 Pesticide residues in onions and carrots grown on organic soils, Ontario, 1975-80. Journal of Economic Entomology 75: 560-565.

Gaines T B 1969 Acute toxicity of pesticides. Toxicology and Applied Pharmacology 14: 515-534.

Gershon S and Shaw F M 1961 Psychiatric sequelae of chronic exposure to organophosphorus insecticides. Lancet 1: 1371.

Getz M E and Watts R R 1964 Application of 4-(P-nitrobenzyl) pyridine as a rapid quantitative reagent for roganophosphate pesticides. Journal of Association of Analytical Chemists 47: 1094-1096.

Glaister J R, Gare C W, Marsat G J, Phillips C and Pratt I 1977 90-day feeding study in rats, Fernhurst, Imperial Chemical Industries, Central Toxicology Laboratory 383 (unpublished ICI report).

Haber B, Kuriyama K and Roberts E 1970b An anion stimulated L-glutamic acid decarboxylase in non-neural tissues, occurence and subcellular localization in mouse kidney and developing chick embryo brain. Biochemical Pharmacology 19 : 119-1136.

Haber B, Kuriyana K and Roberts E 1970c L-Glutamic acid decarboxylase : a new type in glial cells and human brain gliomas. Science 168 : 598-599.

He F, Sun J, Han K, Wu Y, Yao P, Wang S, Liu L 1988 Effects of pyrethroid insecticides on subjects engaged in packaging pyrethroids. British Journal of Industrial Medicine 45 : 548.

He F, Wang S, Liu L, Chen S, Zhang Z and Suu J 1989 Clinical manifestations and diagnosis of acute pyrthroid poisoning. Archives of Toxicology 63 : 54-58.

Hend R W and Butterworth S T G 1976 Toxicity studies on the insecticide WL 43467 a three month feeding study in rats, Sittingbourne Shell Research (TLGR 0027-76) (unpublished).

Hend R W, Hendy R and Fleming D J 1978 Toxicity studies on the insecticide WL 43467 : a three generation reproduction study in rats, Sittingbourne, Shell Research (TLGR 0188-78).

Hoffman D J and Silco L 1984 Neurotoxic and teratogenic effects of an organophosphate insecticide phenyl phosphonothioic acid-O-ethyl-O (4-nitrophenyl) ester on mallard development. Toxicology and Applied Pharmacology 73 : 284.

Hoskins W M 1961 Mathematical treatment of the rate of loss of pesticide residues. FAO Plant Protection Bulletin 9:163-168.

Hussein M F and Abd-El-Massih V F 1977 Thesis submitted for M.Sc. degree, Cairo University.

Hussein M F, Ahmed N A and Ismail H 1972 The effect of reserpine on the levels of some amino acids in the central nervous system of two mammalian animals (guinea pig and Psymmomys obesue). Bulletin of the Faculty of Science, Cairo University 45 : 223-238.

Hussein M F, Ahmed N A and Rawi S M 1985a Effect of the organophosphorus insecticide cyolane on inhibitory glycine and alanine in rat brain. Proceedings of Zoological Society 8 : 139-184.

Hussein M F, Ahmed N A and Rawi S M 1985b Physiological and biochemical induced changes in glutamic acid and aspartic acid in rat brain by the organophosphorus insecticide cyolane. Bulletin of Science, Cairo University, 193:94-113.

Hussein M F, Ahmed N A and Rawi S M 1986 The alterations of rat brain GABA and glutamine induced by the organophosphorus compound cyolane. Comparative Biochemistry and Physiology 84c : 165-170.

Indira M and Kurup P A 1980 Effect of black gram fibre on toxic effects of organophosphorus insecticides. Indian Journal of Experimental Biology 18 : 1523-1524.

Jain H K, Pandey S Y, Agnihotri N P and Dewan R S 1974 Rapid estimation of organophosphorous insecticides. Indian Journal of Entomology 36 : 145-148.

Jain P C, Sharma A and Bhatnagar K N 1987 Residues of phosphamidon and monocrotophos on Coriander. Journal of Food Science and Technology 24 : 49-51.

Janardhan A 1981 Toxicological and Teratological studies of MCP and Methyl benzimidazole carbamate. Ph.D. thesis, Osmania University, Hyderabad.

Janardhan A, Sisodia P and Pentiah P 1984 Teratogenicity of monocrotophos in rats and rabbits. Indian Journal of Pharmacology 15 : 293-302.

Jouvet M 1975 Cholinergic mechanisms and sleep In Cholihergic mechanisms (ed. G. Vaser), Raven Press, New York p. 455.

Jusic A, Jurenic D and Milic S 1980 Electromyographical neuromuscular synapse testing and neurological findings in workers exposed to organophosphorous pesticides. Archives of Environmental Health 35 : 168-175.

Kalakumar B O 1988 Effect of monocrotophos on development of brain in chick. M.V.Sc. Thesis, Andhra Pradesh Agricultural University.

Kalow W and Marton A 1961 Second generation toxicity of malathion in rats. Nature 192 : 464-465.

Kalra R L and Chawla R P 1981 Indian Pesticide Industry : Facts and Figures (ed. David Bv.), Vishwas Publications, Bombay pp.

Kalra R L and Chawla R P 1983 Studies on pesticide residues and monitoring of pesticide pollution. Final Technical Report PL480. Project Department of Entomology, Punjab Agricultural University, Ludhiana, Punjab.

Kennedy G L Jr, John P, Frawley J . and Calandra J C 1973 Multigeneration reproductive effects of three pesticides in rats. Toxicology and Applied Pharmacology 25 : 589-596.

Kim J H, Kim M H 1989 Determination of residual pesticides in bean sprouts. Korean Journal of Food Science and Technology and Food Science and Technology Abstracts 21, 2, 224-228.

Knox J M, Tucker S B, Flannigan S A 1984 Parasthesia from cutaneous exposure to a synthetic pyrethroid insecticide. Archives of Dermatology 120 : 744-746.

Kolomodin-Hedman B, Swesson A, Akerblom M 1982 Occupational exposure to some pyrethroids (Permethrin and Fenvalerate). Archives of Toxicology 50 : 27-33.

Krishnaiah K and Prasad V G 1978 Note on the persistence of monocrotophos residues in Okra. Indian Journal of Agricultural Science 48 : 56-58.

Krishna Murthi C R 1984 Pesticide residues in food and biological tissue. Indian National Science Academy, New Delhi pp. 18-27.

Le Quesne P M, Maxwell I C 1980 Transient facial sensory symptoms following exposure to synthetic pyrethroids : a clinical and electrophysiological assessment. Neurotoxicology 2 : 1-11.

Lindsay S, Banham P B, Chart I S, Chalmers D T, Godley M J and Taylor K 1982 Cypermethrin : life-time feeding study in mice, Alderley Park, Imperial Chemical Industries Report No.(TL/P 687).

Majmudar S K 1973 In Progress and problems in pesticide residue analysis Ed. Bindra O S and Kalra R L. Punjab Agricultural University, Indian Council of Agricultural Research pp.10-32.

Matsumol H, Manjaspatz and Laquer G I. 1972 Quantitative changes with age in the DNA content of methylazoxy methanol (MAM) induced Microencephalate in rat brain. Journal of Neurochemistry 19 : 297.

McAusland H E, Butterworth S T G and Hunt P F 1978 Toxicity studies on the insecticide W L 43467, a two year feeding study in rats, Sittingbourne, Shell Research (TLGR, 0189.78).

Maclaren A and Michie D 1960 Control of prenatal growth in mammals. Nature (London) 187, 363-365.

Meinjel K 1978 Anticholinesterase agents and axial teratogenesis in quail embryos. Wilhelm Rouxo's. Archives of Developmental Biology 185 : 209.

Menzer R E and Casida J E 1965 Nature of Toxic metabolites formed in mammals, insects and plants from 3-(Dimethoxy phosphinyloxy)-N, N-dimethyl-cis-crotonomide and its N-methyl analog. Journal of Agriculture and Food Chemistry 13: 102-112.

Moss D E and Deutsch J A 1975 Review of cholinergic mechanisms and memory In cholinergic mechanisms (ed. P G Woser), Raven Press, New York pp. 483.

Kumar S and Panth S C 1985 Renal pathology in fish (Puntius conchonius Ham.) following exposure to acutely lethal and sublethal concentrations of monocrotophos. Bulletin of Environmental Contamination and Toxicology 35 : 228-223.

Kurokawa M, Kato M and Machiyama Y 1961 Choline acetylase activity in a convulsive strain of mouse. Biochemistry Biphysiology Acta 50 : 385-386.

Kurokawa M, Machiyama Y and Kato M 1963 Distribution of acetylcholine in the brain during various states of activity. Journal of Neurochemistry 10 : 341-348.

Kortz P J 1977 Behavioral and biochemical effects of the carbonate insecticide : MOBAM. Pharmacology and Biochemical Behaviour 6 : 303-310.

Ladomery L G 1986 Work of the codex alimentarius commission in the control of pesticide residues in food. In Control of pesticide applications and residues in Food. A guide and directory 1986. (eds. Hofsten BV and Ekstrom G), Swedish Science Press, p. 25.

Lakshminarayana V and Menon P K 1969 Determination of organochlorine insecticide residues in market vegetables by thin layer chromatography using a multiband plate for clean up. Journal of Food Science and Technology 6 : 272-274.

Lakshminarayana V and Krishna Menon P K 1975 Screening of Hyderabad market samples of food stuffs for organochlorine insecticide residues. Indian Journal of Plant Protection 3:4-19.

Lal R, Rao V V S N and Moorthy V K 1989 Pesticide residues in food and overview of studies undertaken in India. In Pesticide residues in food. Pub. Consumer Education and Research Centre, Ahmedabad p.99-105.

Lauren D R and Henzel R F 1977 Residual lives of two synthetic pyrethroid insecticides applied to pasture. In Proceedings of the 30th New Zealand Weed and Pest Control Conference pp. 207-210.

Murthy M K and Reddy G P V 1978 Proceedings of symposium on pesticide residues in the environment, India, UAS Bangalore p.146-149.

Marei A E M, Russo L O and Casida J E 1982 Analysis and persistence of permethrin, cypermethrin, Deltamethrin and Fenvalerate in the fat and brain of treated rats. Journal of Agriculture and Food Chemistry 30 : 558-562.

Narayama T, Maki T, Kan K and Iida M 1986 Survey of pesticide residues in vegetables and fruit 1984-86. Annual report of Tokyo Metropolitan Research laboratory of Public Health 37, 173-183 Food Science and Technology Abstracts 1987, 19, 9J6.

Naidu N V, Reddy K S, Janardhan A and Murthy M K 1978 Toxicological investigation of dichlorovos in chicks. Indian Journal of Pharmacology 10:323-326.

Nandihalli B S and Thondadarya T S 1986 Studies on the residues of different insecticide cum-acaricides used in the control of chilli (Capsicum annuum L) leaf curl. Pesticides 20:48-50

Narkhede S S, Quadros F, Anvika D G and Borle M N 1977 Estimation of monocrotophos on chilli. Pesticides 11:34

Natekar M G 1987 Chemical control of brinjal pest complex Thesis abstract 1989 XV: No. 234.

Neal M J and Iversen L L 1969 Subcellular distribution of endogenous and 3[H]-γ amino butyric acid in rat cerebral cortex. Journal of Neurochemistry 16 : 1245-1252.

Newman A J and Gross S 1963 Hyperbilirubinemia in breast fed infants. Pediatrics 32: 995-1001.

Noronha A B C 1978 Studies on pesticide residues in food M.Sc., Thesis, University of Bombay.

Oja S S 1967 Studies on Protein Metabolism in developing rat brain. Annales Academiae Scientiarum Fennicae (Medica) 131 : 7-81.

Oser B L 1965 Determination of Phosphorus p.113. In Physiologocal Chemistry, ed. Oser B L 14 Edn.

Ottevanger C F 1976 An epidemiological and toxicological study of occupational exposure to organophosphorus pesticide. Thesis, Phoenix, Potterdam.

Pant S C 1982 Toxic and histopathologic input of some pesticides, heavy metallic salts and pH on a teleost, Puntius conchonius Ham, Ph.D Thesis, Kumaun University India.

Patil N G Dethe M D 1984 Residues of monocroptophos and phosphamidon in green chillies. Pesticides 18: 19-21.

Perrota C A and Lewis P R 1968 A histochemical safety of placental esterases in the guinea pig and in the human Journal of Anatomy 92:110-117.

Pitts F N and Quick C 1967 Brain succinate semial dehyde dehydrogenase II. Changes in the developing rat brain. Journal of Neurochemistry 14 : 561-570.

Post R L and Sen A K 1967 Sodium and potassium stimulated ATPase. pp.762. Methods in Enzymology. Vol.X. eds. in chief. Sidney P. Colowick and Nathan O. Kaplan ed. by Ronald W. Estabrook and Maynard E. Pullman. A P New York and London.

Potter D and McAusland H E 1980 Toxicity studies on the insecticide W L 43467 : a study of liver microsomal enzyme activity in rats fed W L 43467 for 2 years. Sittingborne, shell research (TLGR 80.057).

Prevention of food adulteration act 1976 Extract from the Gazette of India. Part II. Section 3, Sub Section (i). Appearing on page No.(S) 1468-1471 dated 29-5-1976. Ministry of Health and Family Planning (Department of Health). New Delhi, the 15th May, 1976 p.47-51.

Rao G N and Ramasubbaiah K 1985 Dissipation of Insecticides in green and ripe fruits of tomato. Andhra Agricultural Journal 32:238-241.

Rao G N 1983 Chapter 10 Tests of hypothesis In statistics for Agricultural sciences. Oxford and IBH Publishing Co. New Delhi pp.79.

Raj S, Jain H K and Agnihotri N P 1980 Bioefficacy and dissipation of synthetic pyrethroids in okra. Indian Journal of Entomology 42:657-660.

Reddy P M and Bashamohideen M D 1988 Toxic impact of fenvalerate on the protein metabolism in the Branchial Tissue of a fish Cyprinus carpio current science 57: 211-212.

Reiter L Talens G and Wooley D 1973 Acute and Subacute parathion treatment effects on cholinesterase activities and learning in mice. Toxicology and Applied Pharmacology 25 : 582.

Ritter E J, Scott W J and Wilson J G 1971 Teratogenesis and inhibition of DNA synthesis induced in rat embryos by Cytosine arabinoside. Teratology 4:7.

Roberts P J 1974 The release of amino acids with proposed neurotransmitter function from the cuneate and gracile nuclei of the rat in vivo. Brain Research 67 : 419.

Roberts E and Kuriyama K 1968 Biochemical-Physiological correlations in studies of the r-amino buytric acid system. Brain Research 8: 1-35.

Roberts E and Matthysse S 1970 Neurochemistry : At the crossroads of Neurobiology. In Annual Review of Biochemistry, Vol.39 eds. E.S.Snell, P.D.Boyer, A Meister, R.L.Sinheimer. Annual Reviews, Inc., Palo AHO p.777-820.

Roberts E and Simonsen D G 1963 Some properties of L-glutanic decarboxylase in mouse brain. Biochemistry and Pharmocology 12:113-134.

Rose G P and Dewar A J 1978 Toxicity Studies on the insecticide W L 43467: the effect of age on the neurotoxicity of W L 43467 to rats, sittingbourne, Shell Research. (TLGR, 0039. 78)

Rugh R 1968 The mouse its reproduction and development. Burgess, Minneapolis, Minnesota.

Ramsey T S, Cabell D A and Bond J 1969 Effect of an organophosphorous systemic insecticide on reproductive performance in rats. American Journal of Veterinary Research 30: 2209-2214.

Ruplal V V, Narayana Rao S and Krishnamurthy V Pesticide residues in food, an overview of studies undertaken in India. In pesticide residues in Food pub Consumer Education Research Centre 1989 pp.99

Sadasivudu B and Lajtha A 1970 Metabolism of amino acids in incubated slices of mouse brain. Journal of Neurochemistry 17:1299-1311.

Salagami Co and De Robertis E 1965 Subcellular distribution of the enzymes of the glutamic acid, glutamine and r-anunobutyric acid cycles in rats. Journal of Neurochemistry 12 : 287-309.

Saleh M A, Ibrahim N A, Soliman N Z and E l, Sheiny M K 1986 Persistence and distribution of cypermethrin, Deltamethrin and Fenvalerate in laying chickens. Journal of Agriculture and Food Chemistry 34 895-898.

Salvadar R A and Albers R W 1959 The distribution of glutamic-r-amino butyric transaminase in the nervous system of the rhesus monkey. Journal of Biological chemistry 234 : 922-925..

Schom C B, Walker N F and Abbott U K 1972 Azodrin and its effects on four avian species. Poultry Science 51:1860.

Schom C B, Abbot U K and Walker N E 1979 Adult and embryo responses to organophosphate pesticide : Azodrin Poultry Science 58 : 60-66.

Schom C B, Abbott U K 1977 Temporal morphological and genetic response of Avian embryos to Azodrin, an organophosphate insecticide. Teratology 15:81-87.

Schneider W C 1945 Phosphorus compounds in animal tissue. I. Extraction and estimation of deoxypentose nucliec acid. Journal of Biological Chemistry 161:293.

Scott W J, Ritter E J and Wilson J G 1971 DNA synthesis inhibition and cell death associated with hydroxy urea teratogenesis in rat embryo Developmental Biology 26:306.

Shanbag R 1989 An investigational Report of possible pesticidal toxicity in pesticide residues in food. Pub. CERC Ahmedabad p.291-303.

Shellenberger T E 1966 ChE inhibition and toxicological evaluations of two organophosphate pesticides in Japanese quail. Toxicology and Applied Pharmacology 8:22.

Singh A K and Gupta D S 1981 Dissipation of monocrotophos in/on Chickpea crop. Indian Journal of Entomology 43 : 373-377.

Smith T M, Stratton G W 1986 Effects of synthetic pyrethroid insecticides on nontarget organisms. Residue Reviews 97:93-120.

Snedecor G W and Cochran W G 1976 Chapter 10 one-way classifications. Analysis of Variance. Statistical methods 6th Edn. Oxford and IBH Publishing Company, Calcutta pp.258.

Srinivasan S and Lingappa S 1986 Dissipation of monocrotophos residues in/on onion crop. Pesticides 20 : 31-33.

Srivastava U K and Patel N T 1989 An overview of pesticides industry in India. In pesticides residues in food Consumer education and Research centre. Ahmedabad, India pp.12-33

Strombrog 1986 Reproductive toxicity of monocrotophos to Bob white quail. Poultry Science 65: 51-57.

Suthers J R and Marlow R G 1981 A Study of the exposure and health of Indian workers spraying Ripcord on cotton over five consecutive days using mist blower and knapsack applicators. The Hague, Shell International Research Mij. (Tox 81-003).

Suzuki M, Yamamoto Y and Watanabe T 1976, Organochlorine insecticide residues in vegetables of the Kitakyushu district, Japan. Pesticide Monitoring Journal 10, 2, 35-40 and Biological Abstracts 1977- 63 4 Abs No. 23916.

Swaine H and Sapiets A 1980 Residue levels of the major metabolites of the insecticide in the milk and tissues of dairy cows fed on a diet containing cypermethrin at 50 mg/kg, Fernhurst, Imperial chemical Industries (unpublished report No.RJ/0198-B).

Talekar N S 1977 Gas liquid chromatographic determination of -Cyano-o-phenoxy tenzyl -isopropyl-4-chlorophenyl acetate. Journal of the association of official analytical chemists 60:908-910.

Taylor J E El Fakahany E and Richelson E 1979 Long term regulation of muscarinic acetylcholine receptors on cultured nerve cells. Life Science 25 : 2181.

Tejada A W, Magallona E D, Lokan-ilaw E B 1983 Pesticide residues in vegetables application of the modified approach to organophosphate insecticide residues in spring bean. Phillipine Agriculturist 66, 4, 405-416 and Food Science and Technology Abstracts 17, 8, 8J117, 1985.

Tesh J M, Tesh S A and Davies W 1978 W L 43467 : Effects upon the propgress and outcome of pregnancy in rat, stock, Life Science Research (LSR Report No 78/SHL 2/364).

The Agrochemicals Handbood, Royal Society of Chemistry 1984 The University of Nottingham. NG7 2RD England. Insecticide/ Acaricide monocrotophos A 284/June 84.

Tucker S B and Flannigan S A 1983 Cutaneous effects from occupational exposure to fenvalerate.Archives of Toxicology 54 : 195-202.

USDHEW, Washington 1978a Agricultural chemicals News and Report No.104 p.13. United States Department of Health, Education and Welfare, Washington D C.

USDHEW, Washington 1978b Agricultural chemicals News and Report No.107 p.8. United States Department of Health, Education and Welfare, Washington D C.

US EPA 1984 Cypermethrin tolerances for residues in or on raw agricultural commodities. Final rule. Part III Federal Regulations 49 (117) : 24865-24872.

Van Woert M H, 1976 Parkinson's Disease, Tardive Dyskinenia and Muntingtons chorea in Biology of Cholinergic function (ed Am Gold berg I Harrin) Raven Press, New York pp.583.

Van Sittert N J, Eadsforth C V and Bragt P C 1985 Human and dose excretion study with ripcord, the Hague, Shell International Petroleum Maatschappy, (HSE. 85.008).

Veeravel R and Bhaskaran P 1979 Biological longevity of certain insecticide spray deposits on egg plant (Solanum melongina). As evidenced by aphid bioassay. Pesticides 13:44-45.

Verma A N 1980 Review of the Work done on pesticide residues in Haryana. Paper presented at meeting of the the ICAR expert conmmittee to review the pesticide residues problems in the country and formulate a need based programme to be implemented. Held in Ludhiana on April 14-15, 1980.

Verberk M M and Salle M J 1977 Effects on nervous function in voluntary ingesting of mevinphos for one month. Toxicology applied pharmacology 42:351-358.

Vernadakis A and Woodbury D M 1962 Electrolyte and amino acid changes in rat brain during maturation American Journal of Physiology 203 : 748-752.

Vijayakumar D and Janardhan A 1988 Mutagenicity of monocrotophos in mice. Bulletin of Environmental contamination and Toxicology 41 : 189-194.

Weichselbaum T E 1946 An accurate and rapid method for the determination of protein in small amounts of blood, serum and plasma. American Journal of Clinical Pathology 16:40.

Weiss B L, Foster F G and Kupfer D J 1976 Cholinergic involvement in neuropsychiatric syndromes In Biology of Cholinergic function (ed Am Goldberg and I. Harris) Raven Press, New York pp.603.

Westlake W E, Gunther F A and Jeppson L R 1970 Persistance of azodrin residues on and in valencin oranges and in laboratory processed citrus pulp cattle feed. Journal of Agricultural and Food Chemistry 18:864.

Whittam R 1964 The interdependence of metabolism and active transport In: The cellular functions of membrane transport ed. J F Hoffman p.139-154, New York, Prentice Hall, Englewood, Cliffs, New Jersey.

Winick M 1976 Chapter 2 Normal Cellular Growth of the Brain. In Malnutrition and Brain development. ed. Winick M and Williams R R, New York, Oxford University Press, London Toronto p.35-62.

WHO 1984 The WHO recommended classification of pesticides by hazard Guidelines to classification 1984-1985, Geneva, World health organisation (unpublished report) VBC/84.2.

WHO 1986 Organophoshorus Insecticides A General Introduction Environmental health criteria Geneva pp.48.

APPENDICES

APPENDIX I

Interview schedule for information regarding insecticide usage on vegetable crops

Name of the vegetable : Date :

Season :

Name of the farmer :

Village :

District :

1. Area under cultivation of the crop :

2. Who recommends the insecticides to be used. :

3. Source of information regarding :

   a) The dose to be used :

      1. From label on the container :

      2. From other farmers :

      3. From agricultural officers :

      4. Any other :

   b) The method of application :

      1. From the dealer :

      2. From the farmers :

      3. From Agricultural officer :

      4. Any other :

4. Did you receive any training in the method of application? Yes/No If yes, state from whom training was received ? :

APPENDIX II

Calculation of RL_{50} and T.tol. for monocrotophos residues

Days	Residue in ppm x	Log ppm y	x^2	xy
0	4.45	3.6484	0	0
1	2.96	3.4713	1	3.4713
3	2.52	3.4014	9	10.2042
7	1.34	3.1271	49	21.8897
10	0.69	2.8388	100	28.3880
15	ND			
21		16.4870	159	63.9532

$$\bar{x} = \frac{\Sigma x}{n} = \frac{21}{5} \quad 4.2$$

$$y = \frac{\Sigma y}{n} = \frac{16.4870}{5} = 3.2974$$

$$\text{Correction factor (CF)} = \frac{(\Sigma x)^2}{n} = \frac{(21)^2}{5} = 88.2$$

$$\Sigma x = \Sigma x^2 - CF = 159 - 88.2 = 70.8$$

$$\Sigma xy = \Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n} = 63.9532 - \frac{(21)(16.4870)}{5}$$

$$= -5.2922$$

$$b = \frac{\Sigma xy}{\Sigma x} = \frac{-5.2922}{70.8} = -0.0747$$

$$\bar{y} = a + b\bar{x}$$

$$3.2974 = a - [(0.0747)(4.2)]$$

$$a = 3.6111$$

$$T.tol = \frac{\log K_2 - \log tol}{K_1} \quad \text{where}$$

$\log K_2 = a$; $K_1 = b$ and

tol = prescribed tolerance limit

$$= \frac{3.6111 - 2.3010}{-0.0747} = 17.5382$$

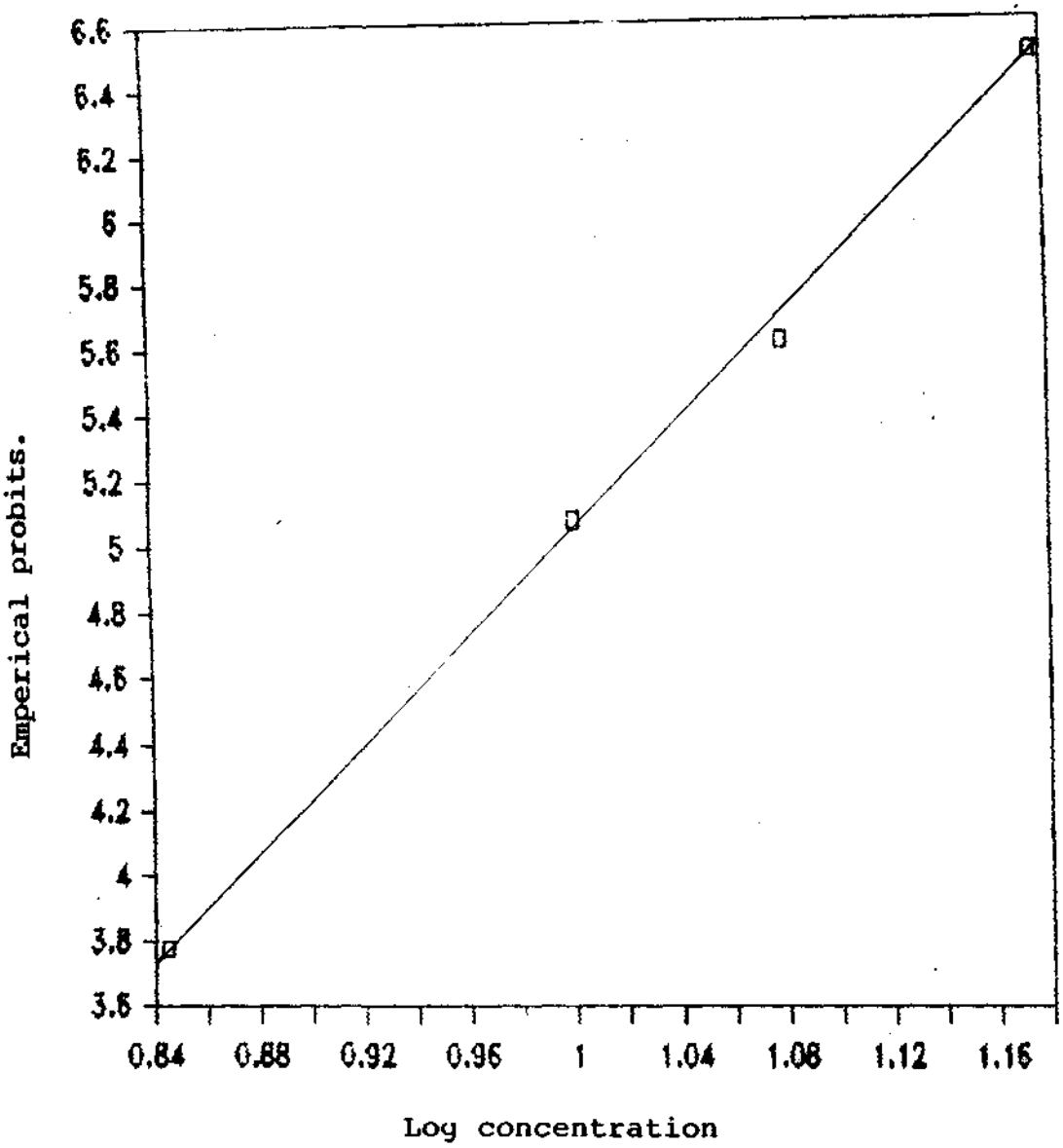
RL_{50} or half life $= \frac{\log 2}{K_1}$ where $K_1 = b$

$$= \frac{0.3010}{0.0747} = 4.03$$

...

Appendix 3 : Calculation of LD50 (Monocrotophos)

Dose mg/kg	x log concentration	n Sample size	Mortality	% mortality	Net percent	emperical probits	expected probits	working probits (y)	W	nw	nwx	nwy	nwx2	nwxy	nwy2
-	0.8415	9	1	11.11	11.11	3.78	3.6	3.774	0.37	3.33	2.8142	12.5674	2.3783	10.6208	47.4294
10	1.0000	15	8	53.33	53.33	5.08	5.1	5.075	0.63	9..45	9.5445	47.9588	9.6399	46.4383	243.3907
12	1.0792	15	11	73.33	73.33	5.62	5.7	5.610	0.56	8.4	9.0653	47.124	9.7833	50.8563	264.3656
15	1.1761	15	14	93.33	93.33	6.50	6.5	6.475	0.27	4.05	4.7632	26.2238	5.6020	30.8417	169.7988
										25.23	26.1871	133.874	27.4035	140.7571	724.9845



GRAPH FOR EXPECTED PROBITS.

$$\bar{X} = \frac{\Sigma nwx}{\Sigma nw} = 1.0379; \quad \bar{Y} = \frac{\Sigma nwy}{\Sigma nw} = 5.3061; \quad a = \frac{(\Sigma nwx)^2}{\Sigma nw} = 27.1805; \quad b = \frac{(\Sigma nwx)(\Sigma nwy)}{\Sigma nw} = 138.9525$$

$$C = \frac{(\Sigma nwy)^2}{\Sigma nw} = 710.3547;$$

Col A = 27.4035	Col B 140.7571	Col C 724.9845
- a = 27.1805	-b 138.9525	-c 710.3547
Σxx = 0.2230	Σxy = 1.8046	Σyy = 14.6298

$$b = \frac{\Sigma xy}{\Sigma xx} = 8.0924$$

$$y = \bar{y} + b\,(x - \bar{x})$$

$$LD_{50} = 5 = 5.3061 + 8.0924\,(x - 1.0379)$$

$$x = 1.000074$$

Antilog of 1.000074 = 10.0 mg/kg body weight

Fiducial limits = $x \pm t \times SE(m)$

$$V(m) = \frac{1}{b^2}\left(\frac{1}{nw} + \frac{(x-\bar{x})^2}{\Sigma xx}\right)$$

$$= \frac{1}{(8.0924)^2}\left(\frac{1}{25.23} + \frac{1.00074 - 1.0379}{0.2230}\right)$$

$$= 0.01527\,(0.0396 + 60.0064)$$

$$= 0.0007024$$

$$SE(m) = \sqrt{Vm} = 0.0265$$

Fiducial limits = $1.000074 \pm (1.96 \times 0.0265)$

$= 1.00074 \pm 0.05194$