CHAPTER 3

EXPERIMENTAL RESULTS

3.1 Acute Oral Toxicity of Insecticide Combination in Rats

The test article was administered in a single dose to three groups of rats, each group comprised 5 male and 5 female at dose level of 325 (G2), 400 (G3) and 460 (G4) mg/kg body weight. Distilled water was used as a vehicle. A concurrent control group comprised 5 male and 5 female rats were dosed with vehicle only. Dose volume was calculated based on a factor 10 ml/kg body weight. All the animals were observed at hourly interval during first 3 hours post dosing and once daily thereafter for visible clinical signs and mortality. Animals were weighed on treatment day, on day 7 and 14. On day 15 all the surviving animals were sacrificed and subjected to necropsy.

Results observed were as follows:

3.1.1 Clinical Symptoms:

Reference Table: 1.2

Most of the animals in the entire test article treated groups (325, 400 and 460 mg/kg b. wt doses) showed toxic symptoms such as tremors, salivation, piloerection, lacrimation, ataxia, and lethargy. In addition, symptoms such as abdominal breathing, nasal discharge, cataract, clonic-convulsion were observed in few animals.

Surviving animals from 325 mg/kg b. wt (G2) and 400-mg/kg b. wt (G3) were recovered gradually from toxic symptoms by the end of first week.

3.1.2 Mortality:

Reference Table: 1.1

No mortalities were observed in control group. A total of 3 mortalities (1 male and 2 female) in 325mg/kg body weight (G2), 6 mortalities (4 male and 2 female) in 400 mg/kg body weight (G3) and 10 mortalities (5 male and 5 female) in 460 mg/kg body weight (G4) groups were observed.

3.1.3 LD_{50} Value

LD_{50} was calculated using Probit analysis-Finny's method and calculated LD_{50} was 374 mg/kg b.wt.

Fiducial limits:	Lower limit	319.201
	Upper limit	438.317

3.1.4 Gross Pathology

Animals from treated groups revealed mottled liver and congestion in kidney.

Acute Oral Toxicity of Combination Insecticide in Rats

Table-1.1

Summary of Mortalities

Dose (mg/kg b. wt)	No of Rats Dosed		Mortality		Combined Mortality (%)
	Male	Female	Male	Female	
0	5	5	0	0	0
325	5	5	1	2	30
400	5	5	4	2	60
460	5	5	5	5	100

LD_{50} was calculated Probit anlaysis - Finny's Method

Calculated LD_{50} was 374 mg/kg b.wt

Fiducial Limits : Lower limit 319.201
Upper limit 438.317

Acute Oral Toxicity of Combination Insecticide in Rats

Table-1.2

Summary of Clinical Symptoms

Symptoms	Dose (mg/kg body weight)							
	0		325		400		460	
	M	F	M	F	M	F	M	F
Tremors	0/5	0/5	1/5	5/5	3/5	4/5	2/5	5/5
Salivation	0/5	0/5	3/5	5/5	5/5	5/5	4/5	5/5
Lacrimation	0/5	0/5	1/5	0/5	4/5	5/5	1/5	4/5
Cataract	0/5	0/5	1/5	0/5	0/5	2/5	0/5	0/5
Nasal discharge	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5
Ptosis	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5
Piloerection	0/5	0/5	2/5	1/5	1/5	4/5	3/5	1/5
Abdominal Breathing	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5
Lethargic	0/5	0/5	1/5	3/5	1/5	5/5	4/5	3/5
Ataxia	0/5	0/5	0/5	0/5	5/5	5/5	4/5	5/5
Toe walking	0/5	0/5	0/5	0/5	0/5	0/5	4/5	0/5
Clonic convulsion	0/5	0/5	0/5	0/5	0/5	0/5	2/5	3/5

3.2 Acute Neurotoxicity Screening Battery Tests of Insecticide Combination in Rats

Animals were dosed with test substance at 25, 75 and 225 mg/kg body weight dose levels for low, mid and high dose groups respectively. Following dosing all the animals was observed frequently for signs of toxicity. Functional observational battery (FOB) tests were performed at 4th h post dosing (considered as peak time of effect) and on days 7 and 14. After 24 h of dosing blood samples were collected, serum was separated and serum cholinesterase was estimated. At the end of 14 days observation period all the animals were sacrificed.

Results observed are as follows:

3.2.1 Clinical Symptoms:

Reference Table: 2.1

Animals administered with insecticide combination (test article) at the dose rate of 75 and 225 mg/kg showed acute cholinergic symptoms *viz.*, tremors, salivation, writhing, hyperactivity to sound/touch, abnormal gait immediately after dosing and these symptoms were persisted for 8 hours. Gradually all the symptoms disappeared after 3 days of post treatment.

3.2.2 Body Weights

Reference Tables: 2.2 and 2.3

Group mean body weights of test article treated group (*i.e.*, 25, 75 and 225 mg/kg) animals during pre exposure, on the day of commencement of treatment, and on day 7 and 14 were comparable to control group.

3.2.3 Cholinesterase Estimation

Reference Tables: 2.4 and 2.5

Serum cholinesterase activity was significantly reduced (143.6 IU/L, 154.2 IU/L and 70.4 IU/L) in males treated with test article at 25, 75 and 225 mg/kg body weight dose level as compared to control group (246.2 IU/L).

Similar to males, cholinesterase activity was significantly reduced (146.8 IU/L and 55.8 IU/L) in females treated with test article at 75 and 225 mg/kg body weight dose level as compared to control group (289.8 IU/L).

3.2.4 Functional Observational Battery Tests

Results of neurobehavioural observations performed at various intervals during the experiment are explained below:

3.2.4.1 Pre-exposure:

Reference Table: 2.20

3.2.4.1.1 Home Cage Observations:

During pre-exposure observations, all the animals assigned to control and treatment groups revealed normal posture in the home cage and did not reveal any clonic or tonic movements.

3.2.4.1.2 Handling Observations:

Ease of removal from home cage and handling reactivity to observer was found very easy or easy for all the animals in the experiment irrespective of dose group. Palpebral closure was wide open for all the animals, eyes and skin was normal. None of the animals exhibited lacrimation, piloerection or salivation.

3.2.4.1.3 Open Field Observations:

Observations performed in open field before administration of test article revealed normal gait, mobility and high arousal in all the animals. None of the animals revealed clonic/tonic or stereotypic behaviour.

3.2.4.1.4 Sensory Reactivity Measurements:

Reference Table: 2.21

During pre-exposure observations, majority of animals from control and treatment groups responded energetically to approach, touch and click stimulus. Pupil response was normal in all animals. Majority of animals responded as flinch to tail pinch and revealed normal air righting reflex.

3.2.4.1.5 Forelimb and Hind limb Grip Strength

Reference Tables: 2.6, 2.7, 2.8 and 2.9

During pre exposure evaluations, all the animals across various groups revealed comparable fore limb and hind limb grip strength.

3.2.4.1.6 Motor Activity

Reference Tables: 2.12 and 2.13

During pre exposure evaluations, motor activity including total activity, ambulatory activity and stereotypic activity for animals from different groups found comparable.

3.2.4.2 Day 1 (At Peak Time of Effect):

Reference Table: 2.22

3.2.4.2.1 Home Cage Observations:

Animal from control group revealed normal postures and did not reveal any clonic or tonic convulsions. Whereas, all the males from 225 mg/kg dose group and females from 75 and 225 mg/kg dose groups exhibited vertical jumping, writhing, clonic and tonic convulsions. Severity of symptoms was more pronounced in females.

3.2.4.2.2 Handling Observations:

All the males from 0, 25 and 75mg/kg dose groups and all females from 0 and 25 mg/kg dose group revealed normal handling reactivity and absence of piloerection, lacrimation and salivation. Majority of males from 225 mg/kg dose group and majority of females from 75 and 225 mg/kg dose groups exhibited hyperactivity to handling, also showed slight to severe piloerection and salivation.

3.2.4.2.3 Open Field Observations:

Observations performed in open field revealed normal gait, mobility and high arousal in all the control and 25 mg/kg dose group animals of either sex. Whereas, few animals from 75 mg/kg dose group and all the animals from

225 mg/kg dose group exhibited slight to severely abnormal gait, slight to totally impaired mobility, very arousal level and mild clonic tremors of whole body and limbs.

3.2.4.2.4 Sensory Reactivity Measurements:

Reference Table: 2.23

Approach response was slight or energetic in almost all the animals from control and test article treated groups, whereas, majority of animals from 75 and 225 mg/kg body weight revealed exaggerated response to touch and click stimulus. No constriction of pupil was observed in majority of animals from 225 mg/kg body weight dose group. All the males from 225 mg/kg landed on side in air righting reflex test, whereas, 2 out of 5 females of the same dose group landed on side and rest of the females landed on back. Majority of males and females from 225 mg/kg body weight dose group did not respond to tail pinch and few responded slightly to tail pinch.

3.2.4.3 Day 7 and 14:

Reference Tables: 2.24, 2.26

3.2.4.3.1 Home Cage Observations:

On day 7 and 14 of experiment, all the animals from control and treatment groups revealed normal posture in the home cage and did not reveal any clonic or tonic movements.

3.2.4.3.2 Handling Observations:

Ease of removal from home cage and handling reactivity was very easy or easy for all the animals. Palpebral closure was wide open for all the animals, eyes and skin was normal. None of the animals exhibited lacrimation, piloerection or salivation.

3.2.4.3.3 Open Field Observations:

Observations performed in open field revealed normal gait, mobility and high arousal in all the animals. None of the animals revealed clonic/tonic or stereotypic behaviour.

3.2.4.3.4 Sensory Reactivity Measurements (Days 7 and 14):

Reference Tables: 2.25 and 2.27

Majority of animals from control and treatment groups responded energetically to approach, touch and click stimulus. Pupil response was normal in all animals. Majority of animals responded as flinch to tail pinch and revealed normal air righting reflex.

3.2.4.3.5 Forelimb Grip Strength

Reference Tables: 2.6 and 2.7

No statistically significant variation was observed in the forelimb grip strength of males and females from treatment groups as compared to respective controls.

3.2.4.3.6 Hind limb Grip strength

Reference Tables: 2.8 and 2.9

On day 1 of treatment *i.e.,* during peak time of effect, hind limb grip strength was significantly reduced in males (301.2 gram force) and females (296.6 gram force) treated at 225 mg/kg dose group as compared to control group values 327.4 gram force and 368.4 gram force respectively.

Females treated at 25 (296.6 gram force) and 75 (334.4 gram force) mg/kg dose level revealed statistically significant lower hind limb grip strength on day 7 post dosing as compared to control group values *viz.,* 421.4 gram force values.

On day 14 of post dosing hind limb grip strength in treated animals of either sex were comparable to control.

3.2.4.3.7 Hind limb Foot splay

Reference Tables: 2.10 and 2.11

Hind limb foot splay of both males (99.0 mm) and females (110.5 mm) measured on the day of treatment at peak time of effect revealed statistically significant increase in animals treated at 225 mg/kg body weight as compared to control values 73.8 mm and 76.3 mm respectively. Hind limb foot splays on pre exposure and on days 7 and 14 post doing were comparable to control group.

3.2.4.3.8 Motor Activity

Reference Tables: 2.16, 2.17, 2.18 and 2.19

Motor activity measured on the day of treatment at peak time of effect revealed significantly reduced total activity and ambulatory activity in animals treated at 225 mg/kg body weight as compared to control group. During pre exposure, on days 7 and 14 of post dosing, motor activity was comparable to respective control groups.

3.2.5 Gross Pathology

No treatment related gross pathological changes were observed at necropsy. Histopathology observations of brain, spinal cord, sciatic nerve and skeletal muscle did not reveal any treatment related changes.

3.2.6 Histopathology

Histopathology examinations performed on brain and spinal cord collected at the end of the 14 days of experiment from treatment group animals did not show any lesion and the observations were comparable to control group.

Acute Neurotoxicity Screening Battery Tests in Rats

Table 2.1

Summary of Clinical Symptoms

Clinical signs / Symptoms	Dose (mg/kg body weight)							
	0		25		75		225	
	M	F	M	F	M	F	M	F
Abnormal gait	0/5	0/5	0/5	1/5	1/5	4/5	0/5	0/5
Ataxia	0/5	0/5	0/5	0/5	3/5	0/5	5/5	0/5
Tremor	0/5	0/5	0/5	0/5	2/5	2/5	5/5	5/5
Piloerection	0/5	0/5	0/5	0/5	1/5	1/5	0/5	0/5
Lacrimation	0/5	0/5	0/5	0/5	0/5	0/5	5/5	2/5
Salivation	0/5	0/5	0/5	0/5	0/5	3/5	5/5	5/5
Paralysis	0/5	0/5	0/5	0/5	0/5	0/5	4/5	5/5
Convulsion	0/5	0/5	0/5	0/5	0/5	2/5	5/5	5/5
Hyperactivity	0/5	0/5	0/5	0/5	0/5	0/5	3/5	5/5
Hyperesthesia	0/5	0/5	0/5	0/5	0/5	0/5	4/5	5/5
Abdominal breathing	0/5	0/5	0/5	0/5	0/5	0/5	0/5	5/5
Retropulsion	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5

Acute Neurotoxicity Screening Battery Tests in Rats

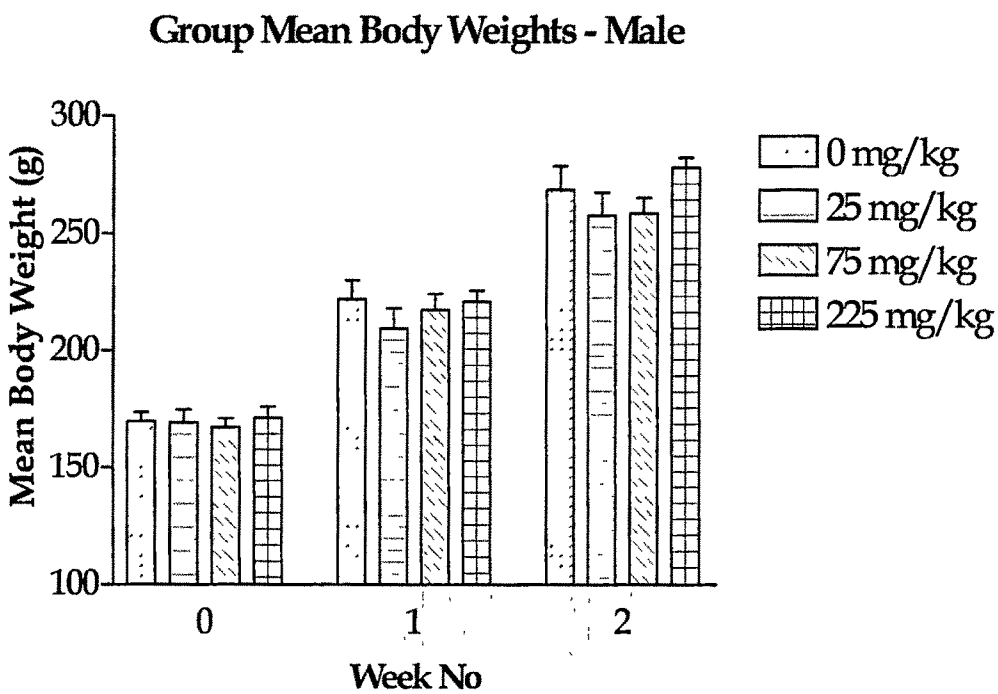
Table 2.2

Effect of Insecticide Combination on Body Weights (g)-Male

Week N°	Dose (mg/kg body weight)			
	0 (N=5)	25 (N=5)	75 (N=5)	225 (N=5)
0	169.8± 4.02	169.2± 5.54	167.4± 3.75	171.4± 4.63
1	222.0± 8.28	209.4± 8.65	217.4± 6.94	221.0± 4.76
2	268.8± 9.71	257.8± 9.84	258.8± 6.38	278.0± 4.27

Note: Values are expressed as Mean ± SE

Figure 1.1



Acute Neurotoxicity Screening Battery Tests in Rats

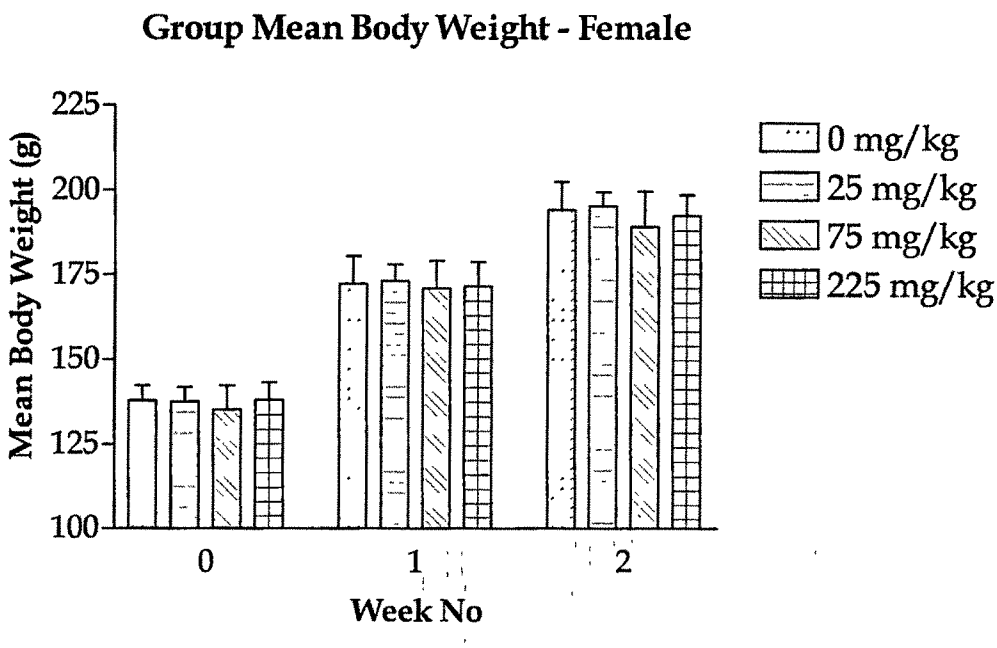
Table 2.3

Effect of Insecticide Combination on Body Weights (g)-Female

Week N°	Dose (mg/kg body weight)			
	0 (N=5)	25 (N=5)	75 (N=5)	225 (N=5)
0	137.8± 4.44	137.6 ± 4.24	135.2 ± 7.00	138.0 ± 5.22
1	172.4± 8.16	173.2 ± 4.87	171.0 ± 8.24	171.6 ± 7.20
2	194.2 ± 8.37	195.4 ± 3.98	189.2 ± 10.58	192.4 ± 6.17

Note: Values are expressed as Mean ± SE

Figure 1.2



Acute Neurotoxicity Screening Battery Tests in Rats

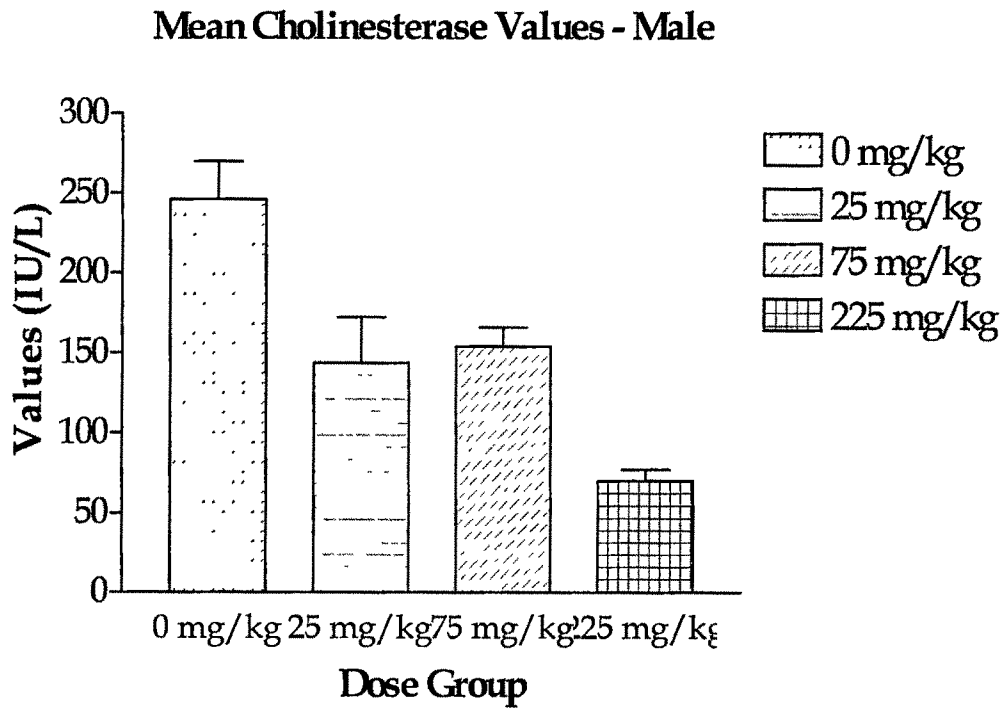
Table 2.4

Effect of Insecticide Combination on Cholinesterase (IU/L) Values - Male

Period	Dose (mg/kg body weight)			
	0 (N=5)	25 (N=5)	75 (N=5)	225 (N=5)
24th Hour	246.2 ± 23.7	143.6± 28.76*↓	154.2 ± 11.91*↓	70.4 ± 7.29*↓

Note: Values are expressed as Mean ± SE,
*↓ = Significantly lower than control (P ≤ 0.05)

Figure- 1.3



Acute Neurotoxicity Screening Battery Tests in Rats

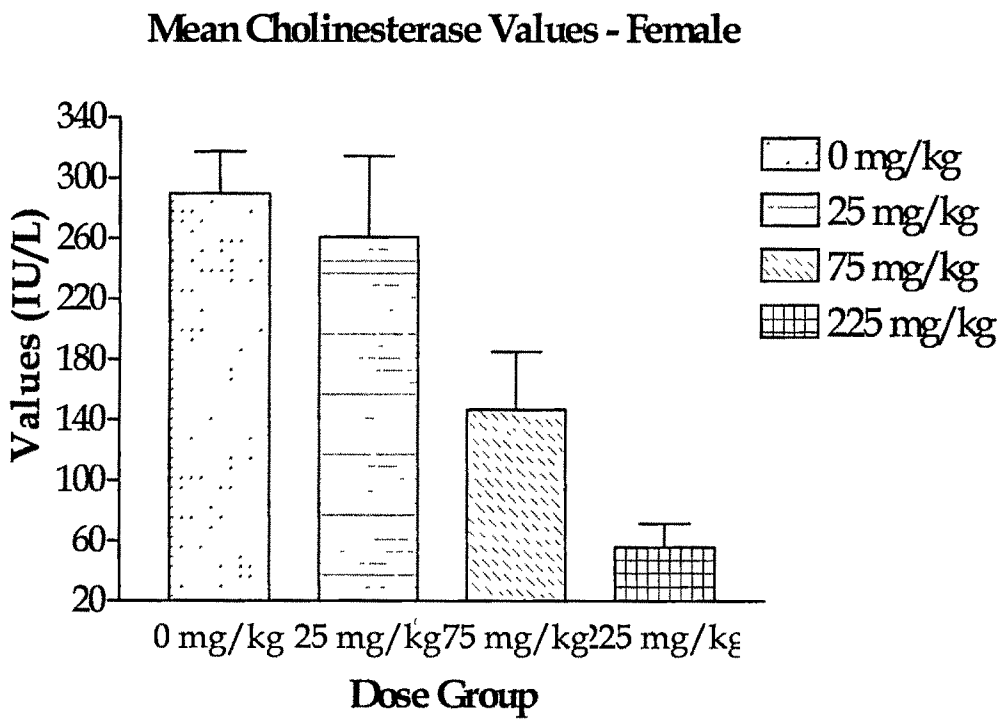
Table 2.5

Effect of Insecticide Combination on Cholinesterase (IU/L) Values - Female

Period	Dose (mg/kg body weight)			
	0 (N=5)	25 (N=5)	75 (N=5)	225 (N=5)
24th Hour	289.8 ± 27.78	261 ± 53.54	146.8 ± 38.5*↓	55.8 ± 15.5*↓

Note: Values are expressed as Mean ± SE
*↓ = Significantly lower than control (P ≤ 0.05)

Figure 1.4



Acute Neurotoxicity Screening Battery Tests in Rats

Table 2.6

Effect of Insecticide Combination on Forelimb Grip Strength (g)-Male

Period	Dose (mg/kg body weight)			
	0 (N=5)	25 (N=5)	75 (N=5)	225 (N=5)
Pre-exposure	744.2 ± 40.18	726.8 ± 34.39	666.8 ± 69.23	741.4 ± 34.46
Day 1	598.8 ± 60.99	609.6 ± 44.92	724.0 ± 37.04	675.2 ± 60.63
Day 7	705.2 ± 12.53	734.4 ± 36.11	773.6 ± 61.43	863.6 ± 53.99
Day 14	732.6 ± 67.65	694.6 ± 51.06	805.0 ± 47.15	844.4 ± 63.91

Note: Values are expressed as Mean ± SE

Figure 1.5

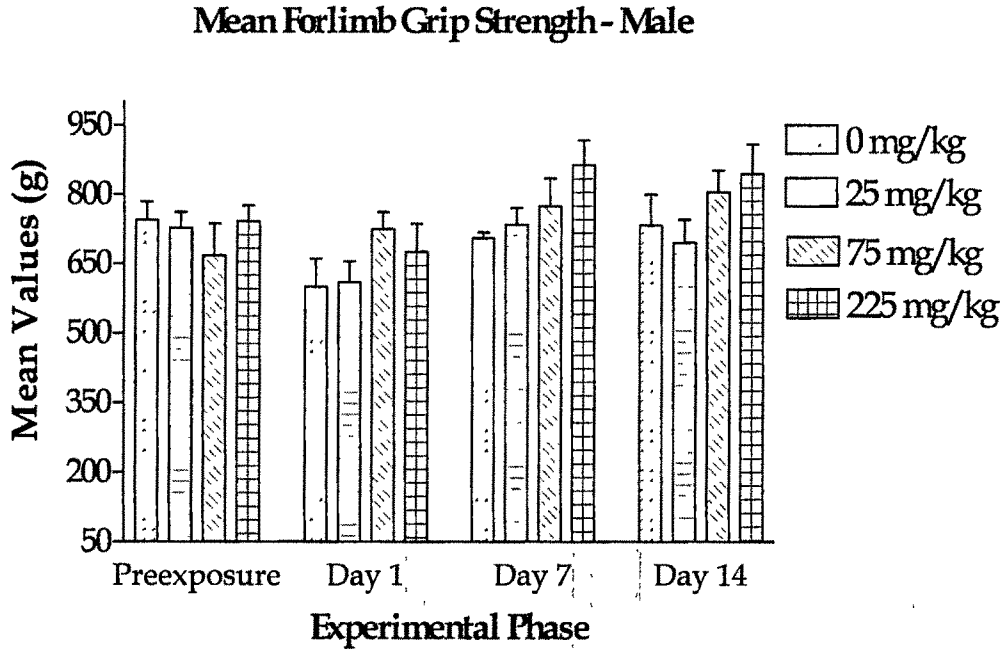


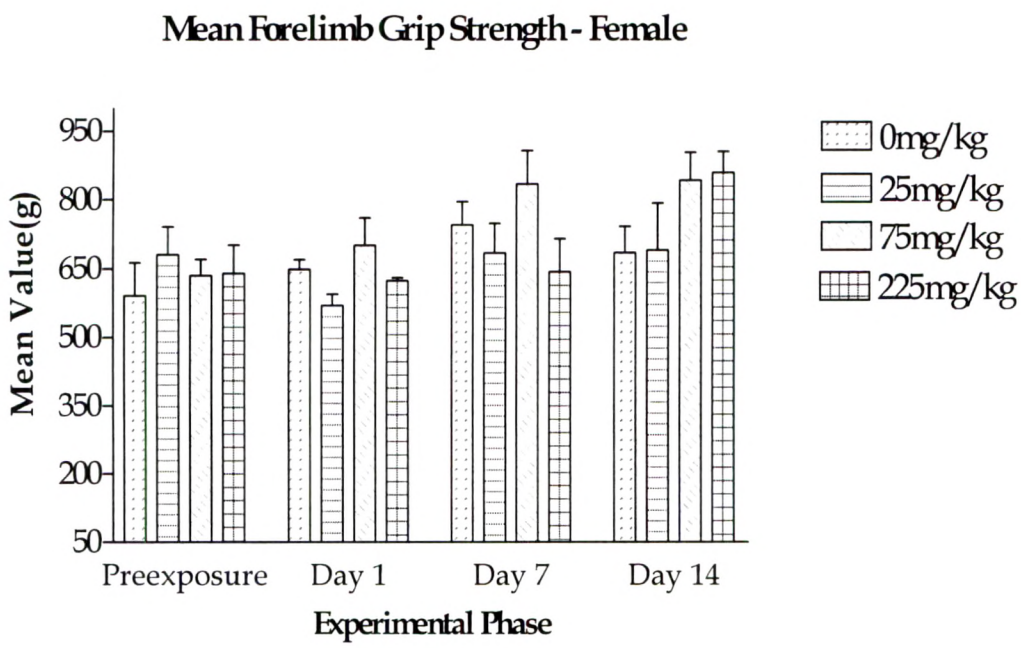
Table 2.7

Effect of Insecticide Combination on Forelimb Grip Strength (g)- Female

Period	Dose (mg/kg body weight)			
	0 (N=5)	25 (N=5)	75 (N=5)	225 (N=5)
Pre-exposure	590.8 ± 71.95	680.4 ± 60.18	634.4 ± 36.13	639.4 ± 61.63
Day 1	648.2 ± 21.03	569.0 ± 25.41*↓	700.2 ± 59.98	623.0 ± 7.00
Day 7	744.4 ± 50.76	683.4 ± 64.91	834.2 ± 72.40	642.6 ± 71.71
Day 14	684.2 ± 57.68	690.0 ± 102.44	842.4 ± 60.69	858.4 ± 47.20

Note: Values are expressed as Mean ± SE
*↓ = Significantly lower than control (P ≤ 0.05)

Figure 1.6



Acute Neurotoxicity Screening Battery Tests in Rats

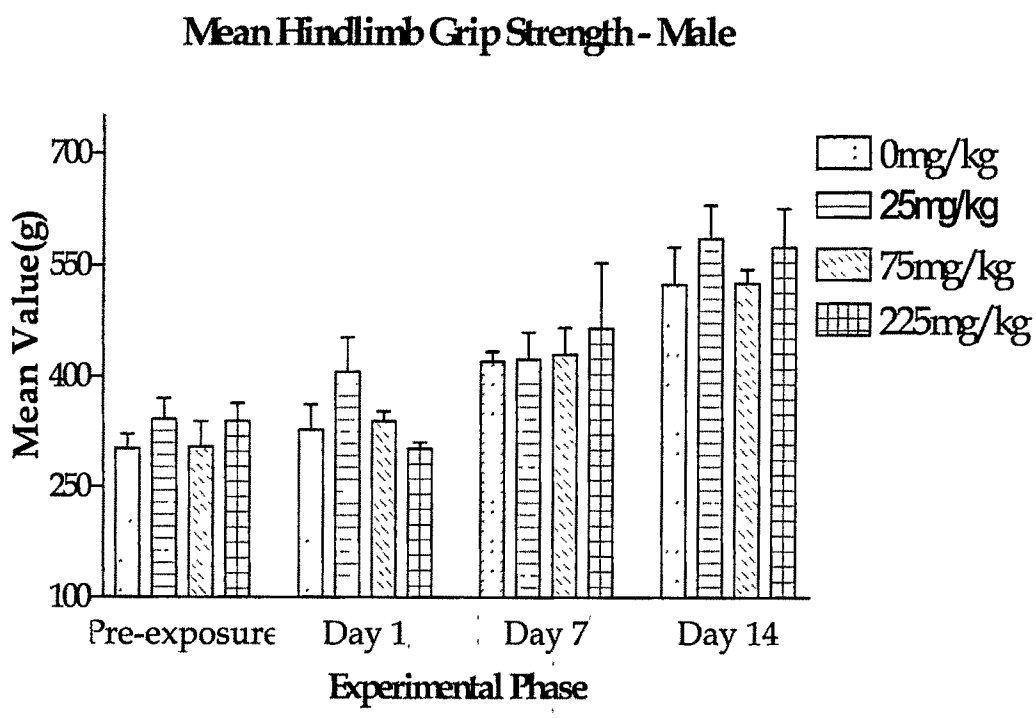
Table 2.8

Effect of Insecticide Combination on Hind limb Grip Strength (g) - Male

Period	Dose (mg/kg body weight)			
	0 (N=5)	25 (N=5)	75 (N=5)	225 (N=5)
Pre-exposure	301.8 ± 18.97	341.6 ± 27.75	304.0 ± 34.58	338.6 ± 24.25
Day 1	327.4 ± 33.84	405.8 ± 45.9	338.8 ± 13.01	301.2 ± 8.663*↓
Day 7	419.4 ± 13.3	422.8 ± 36.29	429.2 ± 36.32	464.2 ± 88.87
Day 14	524.2 ± 50.46	585.8 ± 44.75	526.2 ± 18.07	574.2 ± 52.09

Note: Values are expressed as Mean ± SE
*↓ = Significantly lower than control (P ≤ 0.05)

Figure1.7



Acute Neurotoxicity Screening Battery Tests in Rats

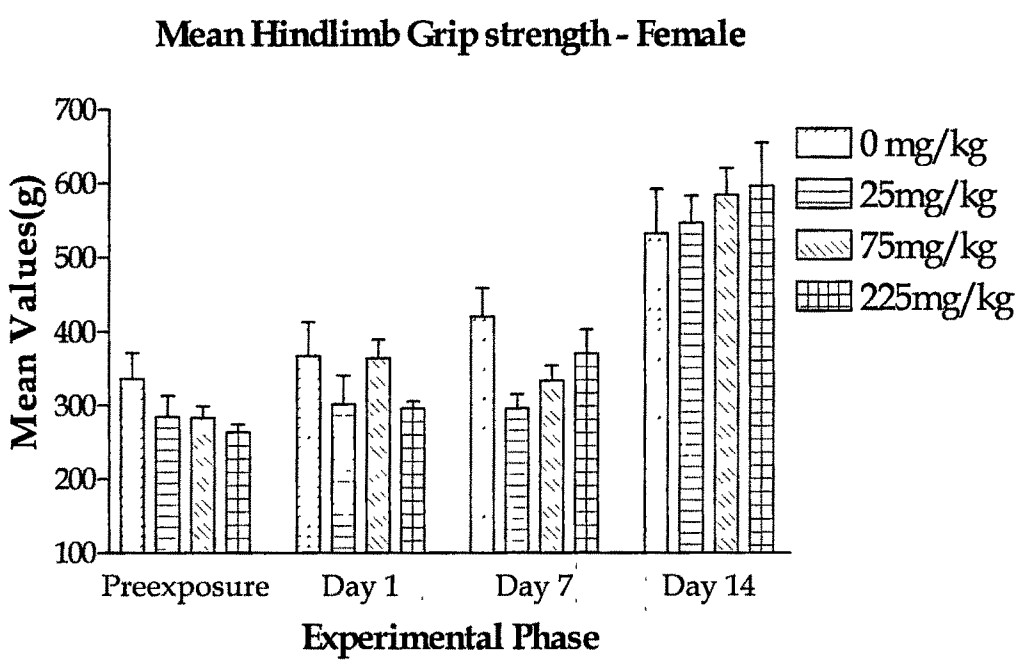
Table 2.9

Effect of Insecticide Combination on Hind limb Grip Strength (g) - Female

Period	Dose (mg/kg body weight)			
	0 (N=5)	25 (N=5)	75 (N=5)	225 (N=5)
Pre-exposure	336.6 ± 36.28	284.8 ± 28.72	283.8 ± 16.11	263.8 ± 11.03
Day 1	368.4 ± 45.44	302.0 ± 39.98	365.2 ± 25.51	296.6 ± 9.54*↓
Day 7	421.4 ± 38.46	296.6 ± 19.07*↓	334.4 ± 21.33*↓	371.6 ± 32.45
Day 14	532.8 ± 60.05	547.2 ± 36.51	584.6 ± 36.64	596.8 ± 58.57

Note: Values are expressed as Mean ± SE
*↓ = Significantly lower than control (P< 0.05)

Figure 1.8



Acute Neurotoxicity Screening Battery Tests in Rats

Table 2.10

Effect of Insecticide Combination on Hind limb Foot Splay (mm)- Male

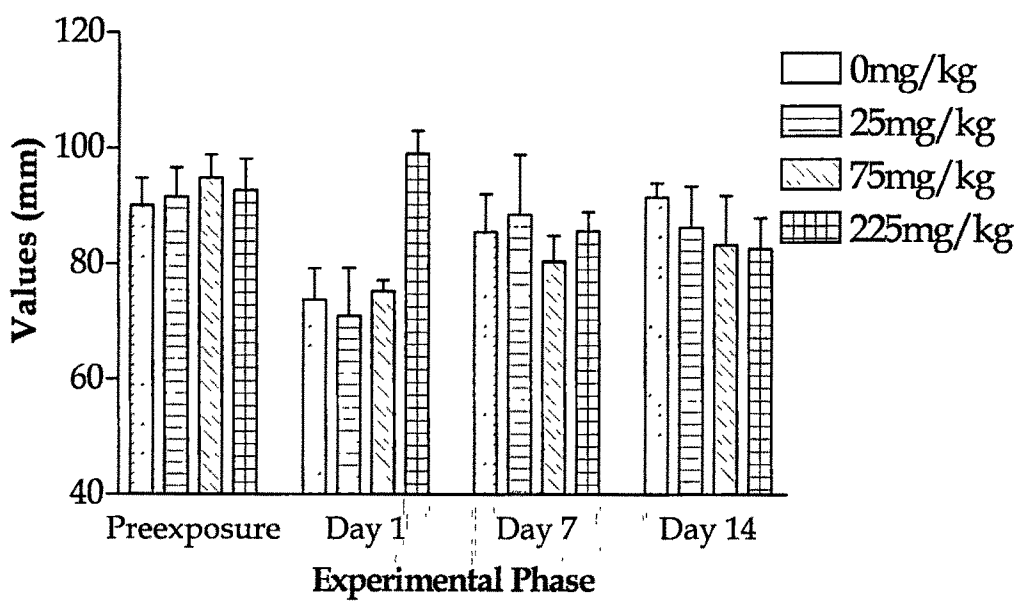
Period	Dose (mg/kg body weight)			
	0 (N=5)	25 (N=5)	75 (N=5)	225 (N=5)
Pre-exposure	90.1 ± 4.73	91.6 ± 5.04	94.9 ± 3.98	92.7 ± 5.48
Day 1	73.8 ± 5.49	71.0 ± 8.35	75.3 ± 1.90	99.0 ± 4.06*↑
Day 7	85.5 ± 6.51	88.5 ± 10.39	80.4 ± 4.49	85.7 ± 3.26
Day 14	91.5 ± 2.55	86.3 ± 7.10	83.3 ± 8.52	82.7 ± 5.29

Note: Values are expressed as Mean ± SE

*↑ = Significantly higher than control ($P \leq 0.05$)

Figure 1.9

Mean Hindlimb Footsplay - Male



Acute Neurotoxicity Screening Battery Tests in Rats

Table 2.11

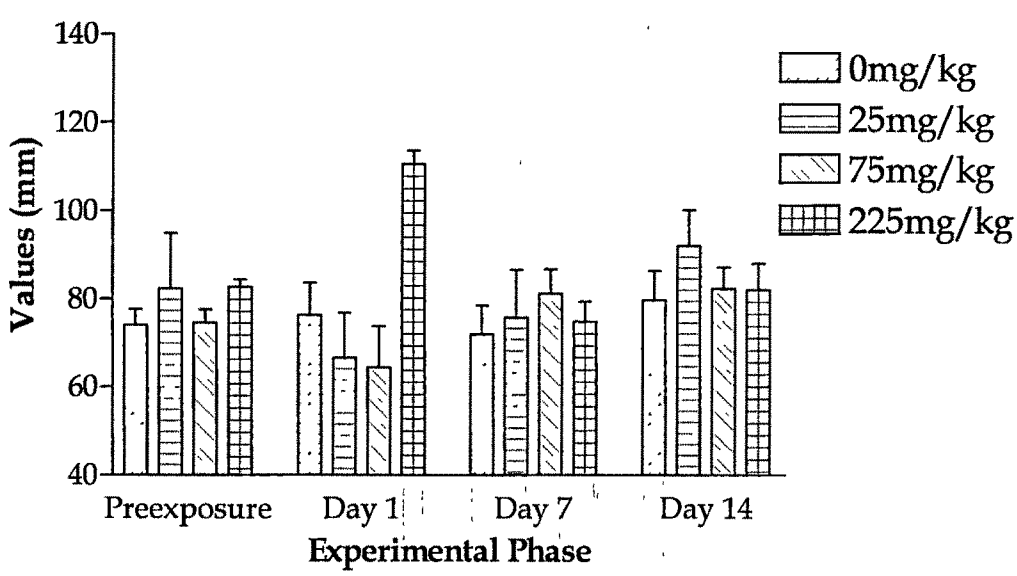
Effect of Insecticide Combination on Hind limb Foot Splay (mm)- Female

Period	Dose (mg/kg body weight)			
	0 (N=5)	25 (N=5)	75 (N=5)	225 (N=5)
Pre-exposure	74.0 ± 3.74	82.4 ± 12.44	74.5 ± 3.01	82.7 ± 1.70
Day 1	76.3 ± 7.34	66.5 ± 10.41	64.4 ± 9.43	110.5 ± 3.11*↑
Day 7	71.9 ± 6.56	75.7 ± 10.80	81.2 ± 5.52	74.9 ± 4.59
Day 14	79.7 ± 6.69	92.0 ± 8.21	82.3 ± 4.79	82.0 ± 6.00

Note: Values are expressed as Mean ± SE
*↑ = Significantly higher than control (P ≤ 0.05)

Figure 1.10

Mean Hindlimb Footsplay - Female



Acute Neurotoxicity Screening Battery Tests in Rats

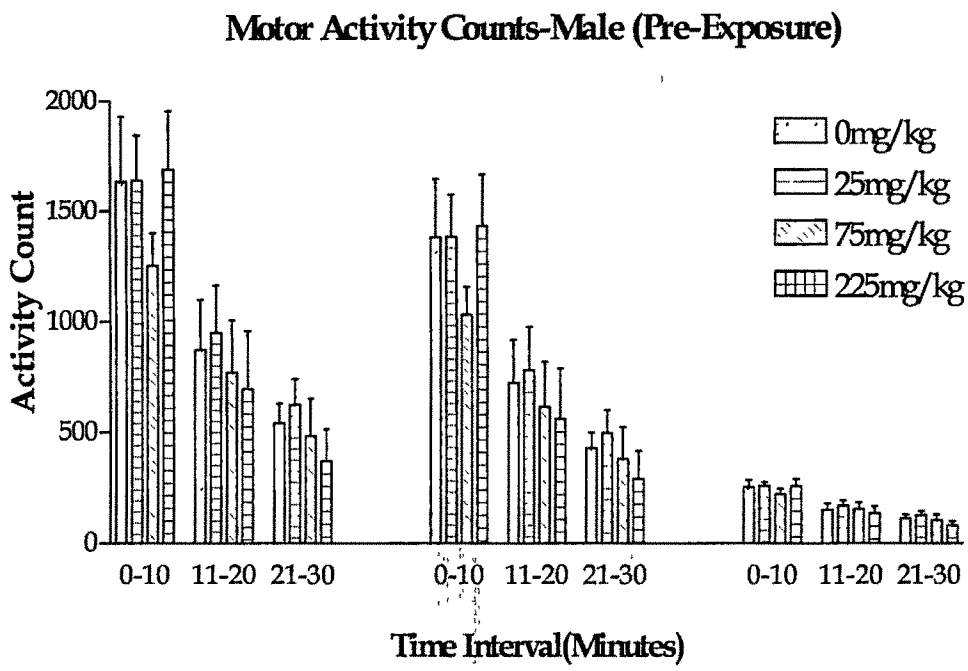
Table 2.12

Effect of Insecticide Combination on Motor Activity (Pre-Exposure)-Male

Time Interval (Min)		Dose (mg/kg body weight)			
		0 (N=5)	25 (N=5)	75 (N=5)	225 (N=5)
Total Activity					
Period	0 - 10	1634.6 ± 295.07	1641.4 ± 204.13	1252.0± 149.35	1689.0 ± 264.46
	11 - 20	873.6 ± 225.22	951 6 ± 212.94	770.4 ± 235.41	695.0 ± 263.19
	21 - 30	542.6 ± 88.42	625.0 ± 115.63	484.6 ± 169.66	371.4 ± 142.19
Ambulatory Activity					
Period	0 - 10	1380.2 ± 265.30	1383.0 ± 189.31	1030.0 ± 127.92	1431.2 ± 233.80
	11 - 20	724.6 ± 193.90	780.2 ± 195.48	616.2 ± 205.32	560.2 ± 229.26
	21 - 30	429.0 ± 72.49	496.8 ± 103.34	380.0 ± 143.60	290.2 ± 124.84
Stereotypic Activity					
Period	0 - 10	254.4 ± 31.84	258.4 ± 18.02	222.0 ± 24.87	257.8 ± 32.24
	11 - 20	149.0 ± 32.30	171.4 ± 20.84	154.2 ± 31.65	134.8 ± 34.15
	21 - 30	113.6 ± 17.86	128.2 ± 16.22	104.6 ± 26.75	81.2 ± 18.32

Note: Values are expressed as Mean ± SE

Figure 1.11


Motor Activity Counts-Male (Pre-Exposure)
Activity Count
2000
1500
1000
500
0
0mg/kg
25mg/kg
75mg/kg
225mg/kg
0-10
11-20
21-30
0-10
11-20
21-30
0-10
11-20
21-30
Time Interval(Minutes)

Acute Neurotoxicity Screening Battery Tests in Rats

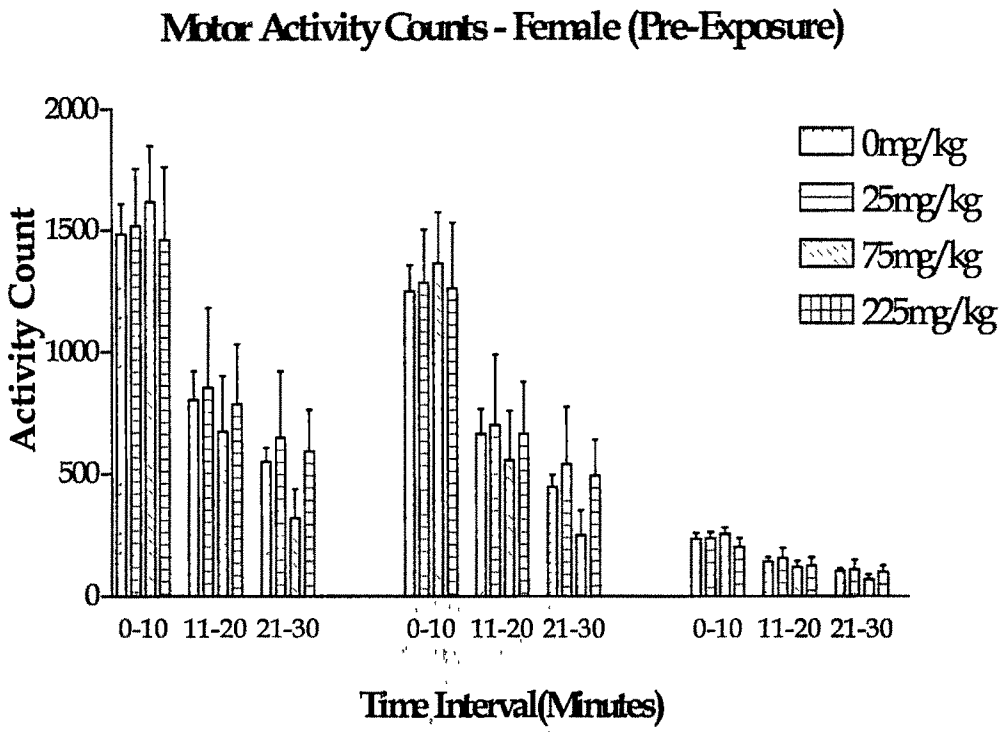
Table 2.13

Effect of Insecticide Combination on Motor Activity (Pre-Exposure)-Female

Time Interval (Min)		Dose (mg/kg body weight)			
		0 (N=5)	25 (N=5)	75 (N=5)	225 (N=5)
Total Activity					
Period	0 - 10	1482.0 ± 125.67	1517.8 ± 237.10	1616.2 ± 233.61	1460.2 ± 302.99
	11 - 20	803.0 ± 118.02	853.2 ± 327.14	673.0 ± 227.18	783.8 ± 247.69
	21 - 30	549.4 ± 56.06	647.4 ± 271.81	317.4 ± 120.86	592.8 ± 167.80
Ambulatory Activity					
Period	0 - 10	1249.8 ± 103.92	1282.6 ± 218.77	1361.8 ± 210.61	1261.0 ± 267.64
	11 - 20	663.0 ± 101.51	699.8 ± 287.23	555.6 ± 202.51	662.0 ± 213.11
	21 - 30	445.4 ± 50.39	538.8 ± 232.97	249.6 ± 100.41	493.2 ± 146.03
Stereotypic Activity					
Period	0 - 10	232.2 ± 25.69	235.2 ± 26.11	254.4 ± 24.88	199.2 ± 36.87
	11 - 20	140.0 ± 17.40	153.4 ± 42.27	117.4 ± 25.13	121.8 ± 35.66
	21 - 30	104.8 ± 7.51	108.6 ± 39.31	67.8 ± 20.69	99.6 ± 24.05

Note: Values are expressed as Mean ± SE

Figure 1.12


Motor Activity Counts - Female (Pre-Exposure)
Activity Count
2000
1500
1000
500
0
0mg/kg
25mg/kg
75mg/kg
225mg/kg
0-10 11-20 21-30
0-10 11-20 21-30
0-10 11-20 21-30
Time Interval(Minutes)

Acute Neurotoxicity Screening Battery Tests in Rats

Table 2.14

Effect of Insecticide Combination on Motor Activity (Day 1)–Male

Time Interval (Min)		Dose (mg/kg body weight)			
		0 (N=5)	25 (N=5)	75 (N=5)	225 (N=5)
Total Activity					
Period	0 - 10	703.8 ± 174.23	958.0 ± 225.97	663.4 ± 143.98	177.8 ± 85.77
	11 - 20	94.6 ± 49.08	234.6 ± 169.23	31.8 ± 12.09	61.8 ± 34.08
	21 - 30	33.8 ± 12.59	23.2 ± 8.28	9.2 ± 5.83	74.0 ± 44.68
Ambulatory Activity					
Period	0 - 10	578.2 ± 151.63	796.0 ± 203.48	549.2 ± 123.37	121.6 ± 70.68
	11 - 20	63.6 ± 36.50	180.4 ± 139.04	15.6 ± 7.48	22.6 ± 16.37
	21 - 30	10.6 ± 5.11	8.8 ± 4.64	2.6 ± 2.60	31.6 ± 23.32
Stereotypic Activity					
Period	0 - 10	125.6 ± 24.08	162.0 ± 23.84	114.2 ± 21.19	56.2 ± 24.17
	11 - 20	31.0 ± 12.59	54.2 ± 32.11	16.2 ± 5.01	39.2 ± 18.61
	21 - 30	23.2 ± 8.73	14.4 ± 4.27	6.6 ± 3.33	42.4 ± 22.49

Note: Values are expressed as Mean ± SE

Figure 1.13

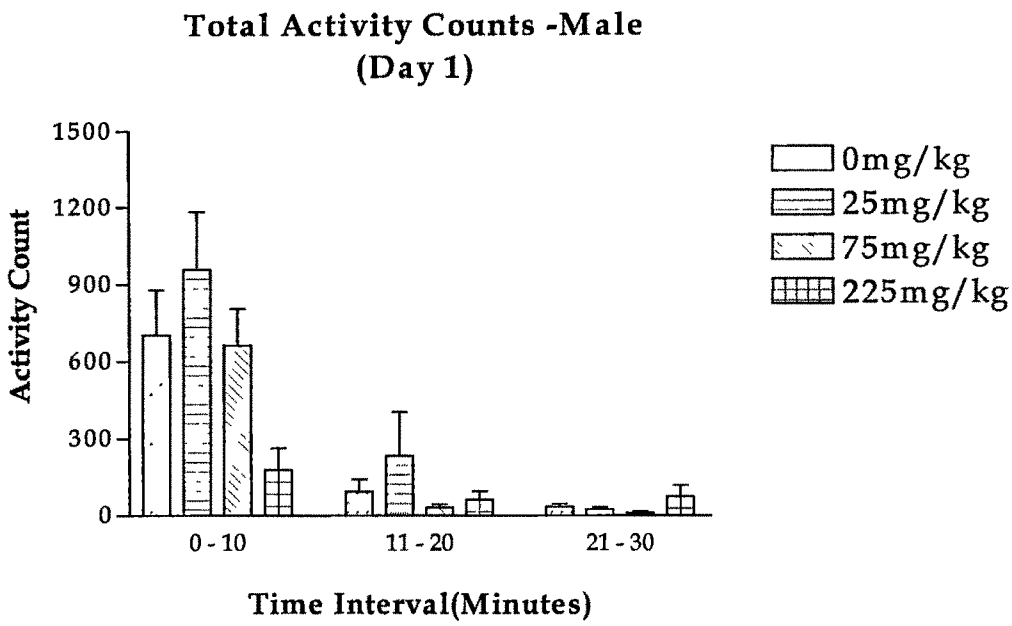


Figure 1.14

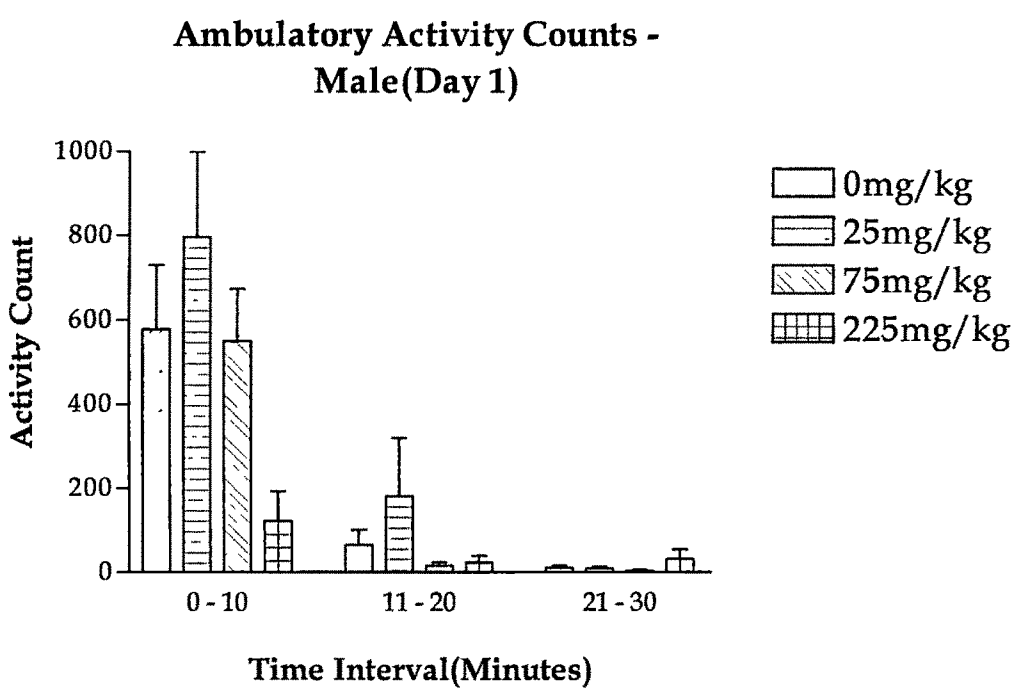
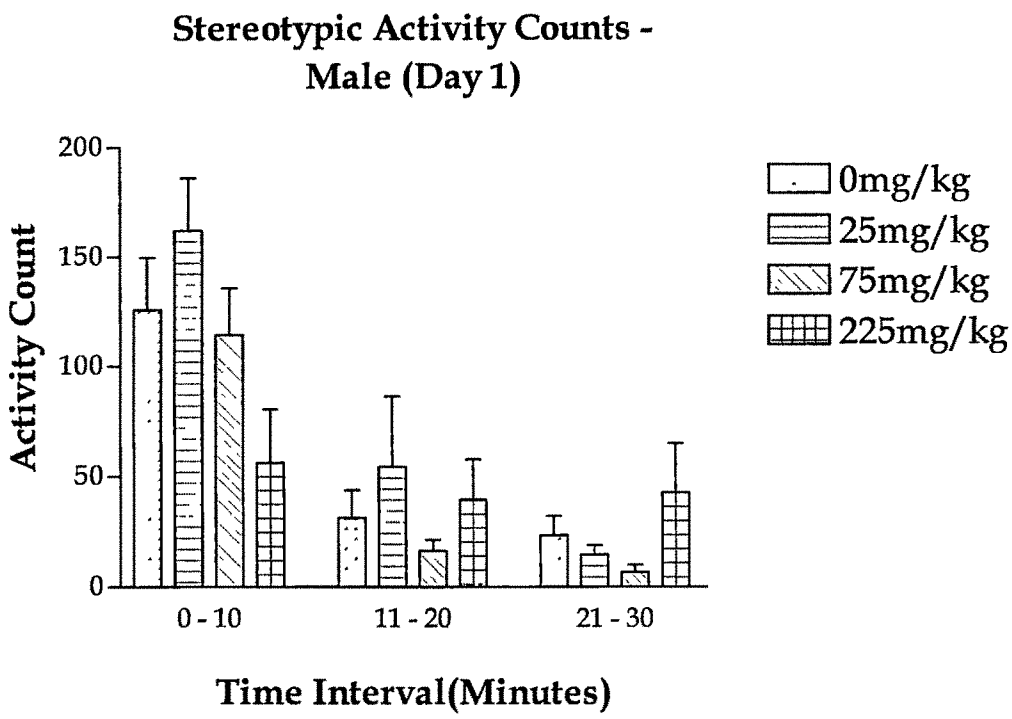


Figure 1.15


Stereotypic Activity Counts - Male (Day 1)
Activity Count
200
150
100
50
0
0 - 10
11 - 20
21 - 30
Time Interval(Minutes)
0mg/kg
25mg/kg
75mg/kg
225mg/kg

Acute Neurotoxicity Screening Battery Tests in Rats

Table-2.15

Effect of Insecticide Combination on Motor Activity (Day 1) - Female

Time Interval (Min)		Dose (mg/kg body weight)			
		0 (N=5)	25 (N=5)	75 (N=5)	225 (N=5)
Total Activity					
Period	0 - 10	709.8 ± 147.04	936.8 ± 283.98	335.8 ± 209.39	173.6 ± 74.30
	11 - 20	138.8 ± 53.43	57.0 ± 35.04	165.4 ± 82.83	113.6 ± 68.51
	21 - 30	19.0 ± 6.15	9.2 ± 2.63	29.4 ± 13.65	149.4 ± 73.60
Ambulatory Activity					
Period	0 - 10	587.0 ± 127.5	774.8 ± 253.08	279.0 ± 178.29	102.4 ± 43.10
	11 - 20	98.4 ± 41.19	19.8 ± 11.88	123.4 ± 68.21	67.8 ± 42.39
	21 - 30	5.6 ± 2.84	1.6 ± 0.75	13.4 ± 6.86	85.6 ± 40.62
Stereotypic Activity					
Period	0 - 10	122.8 ± 20.48	162.0 ± 31.70	56.8 ± 31.24	71.2 ± 36.46
	11 - 20	40.4 ± 13.11	37.2 ± 23.71	42.0 ± 15.06	45.8 ± 26.20
	21 - 30	13.4 ± 4.06	7.6 ± 2.34	16.0 ± 6.98	63.8 V33.67

Note: Values are expressed as Mean ± SE

Figure 1.16

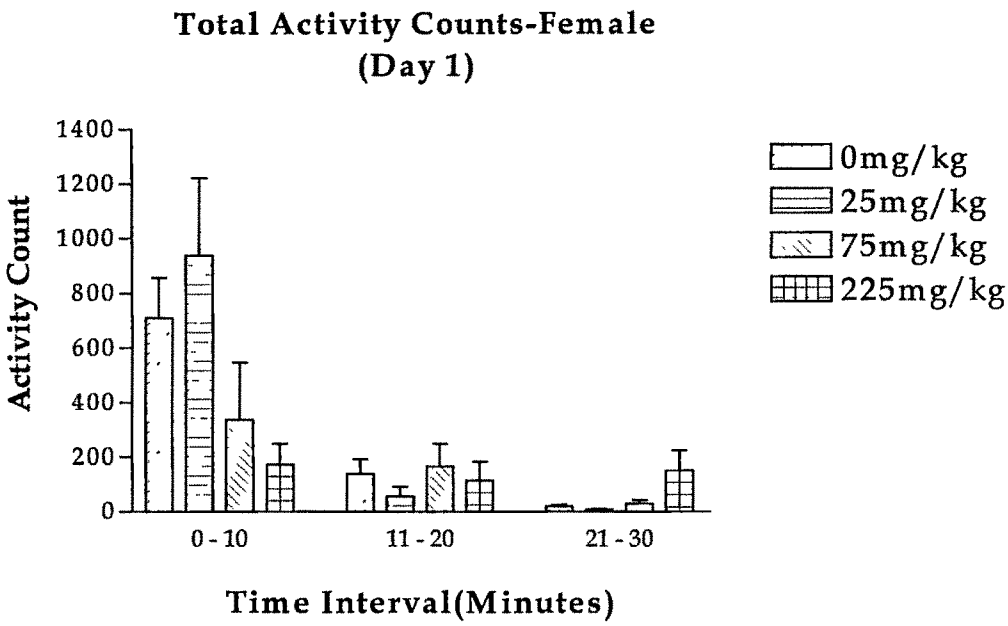


Figure 1.17

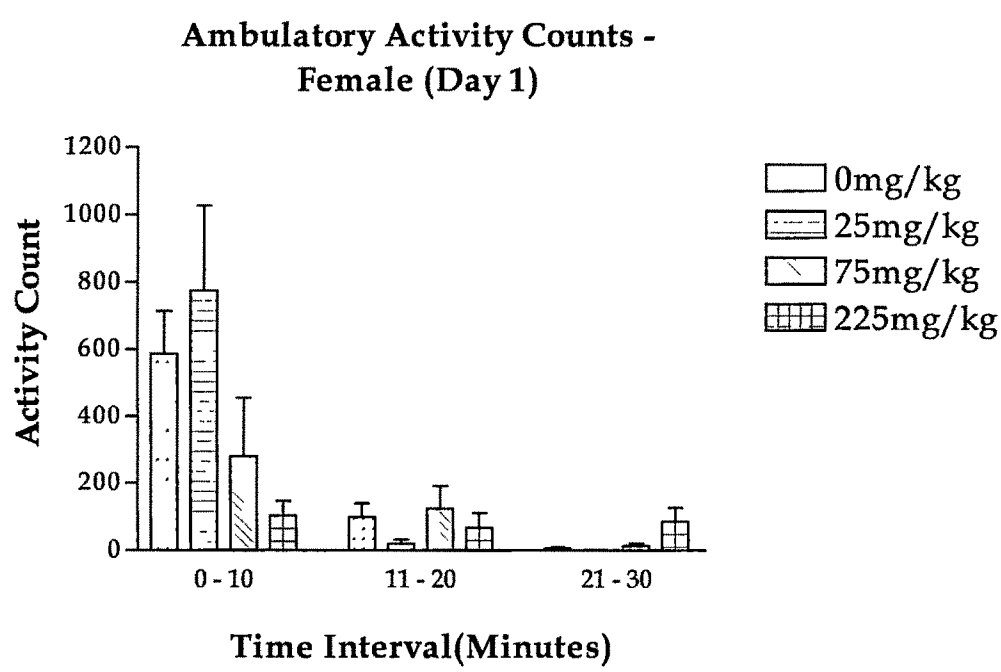
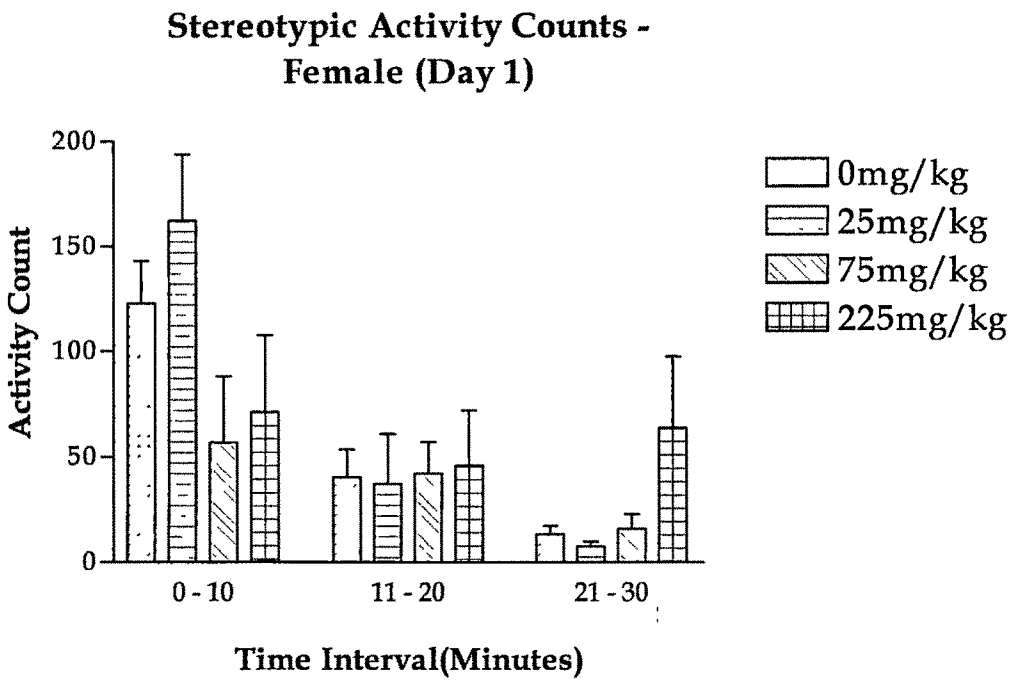


Figure 1.18


Stereotypic Activity Counts - Female (Day 1)
Activity Count
200
150
100
50
0
0 - 10
11 - 20
21 - 30
Time Interval(Minutes)
0mg/kg
25mg/kg
75mg/kg
225mg/kg

Acute Neurotoxicity Screening Battery Tests in Rats

Table-2.16

Effect of Insecticide Combination on Motor Activity (Day 7) - Male

Time Interval (Min)		Dose (mg/kg body weight)			
		0 (N=5)	25 (N=5)	75 (N=5)	225 (N=5)
Total Activity					
Period	0 - 10	1019.2 ± 263.04	827.0 ± 180.82	916.6 ± 100.42	1304.4 ± 175.41
	11 - 20	429.4 ± 163.23	439.4 ± 85.37	326.8 ± 63.75	548.8 ± 153.60
	21 - 30	400.8 ± 188.67	340.6 ± 49.09	294.4 ± 116.24	549.0 ± 163.65
Ambulatory Activity					
Period	0 - 10	843.6 ± 227.22	652.8 ± 156.78	755.6 ± 82.93	1056.2 ± 155.55
	11 - 20	341.2 ± 141.26	323.4 ± 70.24	248.2 ± 55.94	420.8 ± 134.17
	21 - 30	322.6 ± 158.70	250.2 ± 38.75	219.2 ± 93.29	430.6 ± 137.40
Stereotypic Activity					
Period	0 - 10	175.6 ± 37.16	174.2 ± 27.80	161.0 ± 19.89	248.2 ± 24.84
	11 - 20	88.2 ± 24.12	116.0 ± 18.60	78.6 ± 9.61	128.0 ± 21.37
	21 - 30	78.2 ± 30.36	90.4 ± 11.31	75.2 ± 23.25	118.4 ± 26.96

Note: Values are expressed as Mean ± SE

Acute Neurotoxicity Screening Battery Tests in Rats

Table-2.17

Effect of Insecticide Combination on Motor Activity (Day 7) - Female

Time Interval (Min)		Dose (mg/kg body weight)			
		0 (N=5)	25 (N=5)	75 (N=5)	225 (N=5)
Total Activity					
Period	0 - 10	1146.2 ± 232.39	1132.6 ± 270.93	1254.0 ± 186.44	1629.0 ± 127.12
	11 - 20	636.8 ± 149.13	645.6 ± 264.61	665.8 ± 47.63	793.2 ± 185.86
	21 - 30	369.4 ± 91.55	547.6 ± 131.76	610.8 ± 143.32	403.4 ± 126.20
Ambulatory Activity					
Period	0 - 10	956.8 ± 213.05	936.0 ± 238.55	1029.8 ± 162.29	1378.2 ± 113.60
	11 - 20	517.8 ± 131.41	515.0 ± 221.63	539.2 ± 44.30	642.8 ± 164.35
	21 - 30	290.8 ± 81.38	429.2 ± 112.19	489.0 ± 124.99	323.0 ± 109.89
Stereotypic Activity					
Period	0 - 10	189.4 ± 21.42	196.6 ± 35.38	224.6 ± 27.87	250.8 ± 17.91
	11 - 20	119.0 ± 18.72	130.6 ± 43.13	126.6 ± 8.61	150.4 ± 27.14
	21 - 30	78.6 ± 12.56	118.4 ± 25.49	121.8 ± 20.62	80.4 ± 17.11

Note: Values are expressed as Mean ± SE

Acute Neurotoxicity Screening Battery Tests in Rats

Table-2.18

Effect of Insecticide Combination on Motor Activity (Day 14)-Male

Time Interval (Min)		Dose (mg/kg body weight)			
		0 (N=5)	25 (N=5)	75 (N=5)	225 (N=5)
Total Activity					
Period	0 - 10	441.8 ± 274.87	373.0 ± 229.86	139.6 ± 93.40	406.0 ± 264.13
	11 - 20	130.0 ± 89.31	461.4 ± 338.50	278.6 ± 232.29	482.6 ± 307.05
	21 - 30	122.0 ± 122.00	248.8 ± 248.80	219.6 ± 219.60	447.0 ± 447.00
Ambulatory Activity					
Period	0 - 10	222.2 ± 164.18	87.6 ± 59.31	108.2 ± 66.67	110.2 ± 72.94
	11 - 20	68.6 ± 47.71	247.8 ± 193.66	87.0 ± 62.69	351.2 ± 215.56
	21 - 30	76.8 ± 76.80	110.8 ± 110.80	170.2 ± 170.20	261.0 ± 261.00
Stereotypic Activity					
Period	0 - 10	113.4 ± 72.93	56.2 ± 45.17	64.0 ± 39.65	19.8 ± 14.39
	11 - 20	28.4 ± 18.98	188.4 ± 117.19	51.2 ± 34.70	267.2 ± 166.84
	21 - 30	28.6 ± 28.60	43.0 ± 43.00	11.8 ± 11.80	232.8 ± 232.80

Note: Values are expressed as Mean ± SE

Acute Neurotoxicity Screening Battery Tests in Rats

Table-2.19

Effect of Insecticide Combination on Motor Activity (Day 14) - Female

Time Interval (Min)		Dose (mg/kg body weight)			
		0 (N=5)	25 (N=5)	75 (N=5)	225 (N=5)
Total Activity					
Period	0 - 10	477.0 ± 394.93	700.4 ± 429.71	436.8 ± 312.34	1115.4 ± 772.41
	11 - 20	664.6 ± 407.00	489.0 ± 348.62	522.2 ± 330.78	572.2 ± 351.17
	21 - 30	194.8 ± 194.80	140.8 ± 140.80	250.8 ± 250.80	197.6 ± 197.60
Ambulatory Activity					
Period	0 - 10	270.2 ± 194.87	509.2 ± 324.23	224.6 ± 188.04	724.4 ± 530.67
	11 - 20	192.8 ± 130.80	275.8 ± 244.60	269.8 ± 214.68	312.6 ± 209.28
	21 - 30	34.2 ± 34.20	206.8 ± 206.80	163.8 ± 163.80	85.0 ± 85.00
Stereotypic Activity					
Period	0 - 10	61.4 ± 42.78	350.8 ± 215.11	224.8 ± 138.92	478.4 ± 351.31
	11 - 20	117.4 ± 97.76	163.8 ± 153.76	190.6 ± 150.71	92.6 ± 86.25
	21 - 30	21.4 ± 21.40	133.2 ± 133.20	22.8 ± 22.80	15.8 ± 15.80

Note: Values are expressed as Mean ± SE

Acute Neurotoxicity Screening Battery Tests in Rats

Table-2.20

Effect of Insecticide Combination on Neurobehavioural Observations - Pre Exposure

Home Cage Observations:

Parameters	Observation	Male (N=5)				Female (N=5)			
		Dose (mg/kg body weight)							
		0	25	75	225	0	25	75	225
Posture	Sleeping	2	2	1	1	1	3	1	1
	Sitting normally	3	0	0	2	3	0	0	3
	Sitting alert	0	2	4	1	1	1	1	1
	Rearing	0	1	0	1	0	1	3	0
Clonic convulsions	Present	0	0	0	0	0	0	0	0
	Absent	5	5	5	5	5	5	5	5
Tonic convulsions	Present	0	0	0	0	0	0	0	0
	Absent	5	5	5	5	5	5	5	5

Note: Values are expressed as number of animals

Acute Neurotoxicity Screening Battery Tests in Rats

Table-2.20 (Contd.)

Effect of Insecticide Combination on Neurobehavioural Observations- Pre Exposure

Handling Observations:

Parameters	Observation	Male (N=5)				Female (N=5)			
		Dose (mg/kg body weight)							
		0	25	75	225	0	25	75	225
Ease of removal from cage	Very easy	0	1	0	0	1	0	0	0
	Easy	5	4	4	5	4	5	4	4
	Moderately difficult	0	1	1	0	0	0	0	0
	Difficult	0	0	0	0	0	0	1	1
Handling reactivity	Easy	5	5	5	5	5	5	5	4
	Moderately easy	0	0	0	0	0	0	0	1
Palpebral closure	Eyelids wide open	5	5	5	5	5	5	5	5
Lacrimation	None	5	5	5	5	5	5	5	5
Eye examination	Normal	5	5	5	5	5	5	5	5
Piloerection	Absent	5	5	5	5	5	5	5	5
Skin examination	Normal	5	5	5	5	5	5	5	5
Salivation	None	5	5	5	5	5	5	5	5

Note: Values are expressed as number of animals

Acute Neurotoxicity Screening Battery Tests in Rats

Table-2.20 (Contd.)

Effect of Insecticide Combination on Neurobehavioural Observations- Pre Exposure

Open field observations:

Parameters	Observation	Male (N=5)				Female (N=5)			
		Dose (mg/kg body weight)							
		0	25	75	225	0	25	75	225
Gait	Normal	5	5	5	5	5	5	5	5
Mobility	Normal	5	5	5	5	5	5	5	5
Arousal	High	5	5	5	5	5	5	5	5
Respiration	Normal	5	5	5	5	5	5	5	5
Clonic movement	Absent	5	5	5	5	5	5	5	5
Tonic movement	Absent	5	5	5	5	5	5	5	5
stereotypy	Absent	5	5	5	5	5	5	5	5
Bizarre behaviour	Absent	5	5	5	5	5	5	5	5

Note: Values are expressed as number of animals

Acute Neurotoxicity Screening Battery Tests in Rats

Table-2.21

Effect of Insecticide Combination on Sensory Reactivity Measurements-Pre Exposure

Parameters	Observation	Male (N= 5)				Female (N= 5)			
		Dose (mg/kg body weight)							
		0	25	75	225	0	25	75	225
Approach response	No reaction	0	1	0	0	0	0	0	0
	Energetic	5	4	5	5	5	5	5	5
Touch response	Energetic	5	5	5	5	5	5	5	5
Click response	Slight reaction	1	1	0	0	1	0	1	0
	Energetic	4	4	5	5	4	5	4	5
Pupil response	Normal	5	5	5	5	5	5	5	5
Air righting reflex	Normal	5	5	5	5	5	5	5	5
Tail pinch response	No reaction	1	0	0	0	0	0	0	0
	Slight reaction	1	0	0	0	0	0	0	0
	Flinch	3	5	5	5	5	5	5	5

Note: Values are expressed as number of animals

Acute Neurotoxicity Screening Battery Tests in Rats

Table-2.22

Effect of Insecticide Combination on Neurobehavioural Observations- Day 1

Home Cage Observations:

Parameters	Observation	Male (N=5)				Female (N=5)			
		Dose (mg/kg body weight)							
		0	25	75	225	0	25	75	225
Posture	Sitting normally	1	0	0	0	0	0	0	0
	Sitting head hung down	0	0	0	1	0	0	0	0
	Sitting alert	4	5	5	0	5	5	5	0
	Vertical jumping	0	0	0	2	0	0	0	3
	Writhing	0	0	0	2	0	0	0	2
Clonic convulsions	Present	0	0	0	5	0	0	2	5
	Absent	5	5	5	0	5	5	3	0
Tonic convulsions	Present	0	0	0	5	0	0	2	5
	Absent	5	5	5	0	5	5	3	0

Note: Values are expressed as number of animals

Acute Neurotoxicity Screening Battery Tests in Rats

Table-2.22 (Contd.)

Effect of Insecticide Combination on Neurobehavioural Observations-Day 1

Handling Observations:

Parameter	Observation	Male (N=5)				Female (N=5)			
		Dose (mg/kg body weight)							
		0	25	75	225	0	25	75	225
Removal from home cage	Easy	5	5	3	1	5	4	2	0
	Moderately difficult	0	0	2	0	0	1	2	0
	Difficult	0	0	0	0	0	0	1	3
	Very difficult	0	0	0	4	0	0	0	2
Handling reactivity	Easy	5	5	3	0	5	4	3	0
	Moderate easy	0	0	2	1	0	1	0	0
	Difficult	0	0	0	4	0	0	2	5
Palpebral closure	Eye lids Wide open	5	5	5	4	5	5	5	5
	Ptosis	0	0	0	1	0	0	0	0
Lacrimation	None	5	5	5	5	5	5	5	1
	Slight	0	0	0	0	0	0	0	4
Eye examination	Normal	5	5	5	5	5	5	5	5
Piloerection	Absent	5	5	4	1	5	5	4	0
	Present	0	0	1	4	0	0	1	5
Skin examination	Normal	5	5	5	5	5	5	5	5
Salivation	None	5	5	5	5	5	5	2	5
	Slight	0	0	0	0	0	0	2	0
	Severe	0	0	0	0	0	0	1	0

Note: Values are expressed as number of animals

Acute Neurotoxicity Screening Battery Tests in Rats

Table-2.22 (Contd.)

Effect of Insecticide Combination on Neurobehavioural Observations-Day 1

Open field observations:

Parameters	Observation	Male (N=5)				Female (N=5)			
		Dose (mg/kg body weight)							
		0	25	75	225	0	25	75	225
Gait	Normal	5	4	3	0	5	4	0	0
	Slightly abnormal	0	1	2	0	0	1	4	0
	Severely abnormal	0	0	0	5	0	0	1	5
Mobility	Normal	5	5	3	0	5	5	2	0
	Slight impaired	0	0	2	1	0	0	3	0
	Totally impaired	0	0	0	4	0	0	0	5
Arousal	High	4	5	4	2	5	5	1	0
	Low	1	0	1	0	0	0	4	2
	Very low	0	0	0	3	0	0	0	3
Respiration	Normal	5	5	5	5	5	5	5	5
Clonic movement	Absent	5	4	5	0	5	5	2	0
	Clonic tremors whole body	0	0	0	5	0	0	3	5
	Mild clonic tremors-limbs	0	1	0	0	0	0	0	0
Tonic movement	Absent	5	5	5	2	5	5	2	0
	Tonic contraction of hind limbs	0	0	0	1	0	0	3	1
	Emprosthotonous	0	0	0	2	0	0	0	4
Stereotypy	Absent	5	5	5	5	5	5	5	5
Bizarre behaviour	Absent	5	5	5	5	5	5	5	5

Note: Values are expressed as number of animals

Acute Neurotoxicity Screening Battery Tests in Rats

Table-2.23

Effect of Insecticide Combination on Sensory Reactivity Measurements- Day 1

Parameters	Observation	Male (N=5)				Female (N=5)			
		Dose (mg/kg body weight)							
		0	25	75	225	0	25	75	225
Approach response	No reaction	0	1	0	1	0	0	0	1
	Slight reaction	0	1	2	0	0	0	1	1
	Energetic	5	3	3	4	5	5	4	3
Touch response	No reaction	0	0	0	1	0	0	0	0
	Slight reaction	0	1	0	0	0	0	0	0
	Energetic	5	4	5	0	5	0	2	0
	Exaggerated	0	0	0	4	0	0	3	5
Click response	Slight reaction	0	0	0	0	0	5	0	0
	Energetic	5	5	5	1	5	5	4	0
	Exaggerated	0	0	0	4	0	0	1	5
Pupil response	Normal	5	5	3	0	5	5	2	0
	Slight response	0	0	2	1	0	0	3	3
	No constriction	0	0	0	4	0	0	0	2
Air righting reflex	Normal	5	5	5	0	5	5	0	0
	Landed on side	0	0	0	5	0	0	0	2
	Landed on back	0	0	0	0	0	0	0	3
Tail pinch response	No reaction	0	0	0	3	0	0	0	2
	Slight reaction	0	0	1	1	0	0	1	3
	Flinch	5	5	4	1	5	4	4	0
	Exaggerated	0	0	0	0	0	1	0	0

Note: Values are expressed as number of animals

Acute Neurotoxicity Screening Battery Tests in Rats

Table-2.24

Effect of Insecticide Combination on Neurobehavioural Observations-Day 7

Home Cage Observations:

Parameters	Observation	Male (N=5)				Female (N=5)			
		Dose (mg/kg body weight)							
		0	25	75	225	0	25	75	225
Posture	Sitting normally	3	1	3	2	0	0	0	0
	Sitting alert	2	4	2	3	4	3	5	3
	Rearing	0	0	0	0	1	2	0	2
Clonic convulsions	Absent	5	5	5	5	5	5	5	5
	Absent	5	5	5	5	5	5	5	5

Handling Observations:

Parameters	Observation	Male (N=5)				Female (N=5)			
		Dose (mg/kg body weight)							
		0	25	75	225	0	25	75	225
Removal from home cage	Easy	5	5	5	5	5	4	5	5
	Very difficult	0	0	0	0	0	1	0	0
Handling reactivity	Easy	5	5	5	0	5	4	5	0
	Difficult	0	0	0	0	0	1	0	0
Palpebral closure	Eye lids wide open	5	5	5	5	5	5	5	5
Lacrimation	None	5	5	5	5	5	5	5	5
Eye examination	Normal	5	5	5	5	5	5	5	5
Piloerection	Absent	5	5	5	5	5	5	5	5
Skin examination	Normal	5	5	5	5	5	5	5	5
Salivation	None	5	5	5	5	5	5	5	5

Note: Values are expressed as number of animals

Acute Neurotoxicity Screening Battery Tests in Rats

Table-2.24 (Contd.)

Effect of Insecticide Combination on Neurobehavioural Observations- Day 7

Open field observations:

Parameters	Observation	Male (N=5)				Female (N=5)			
		Dose (mg/kg body weight)							
		0	25	75	225	0	25	75	225
Gait	Normal	5	5	5	5	5	5	5	5
Mobility	Normal	5	5	5	5	5	5	5	5
Arousal	High	5	5	3	5	5	5	3	5
	Low	0	0	2	0	0	0	2	0
Respiration	Normal	5	5	5	5	5	5	5	5
Clonic movement	Absent	5	5	5	5	5	5	5	5
Tonic movement	Absent	5	5	5	5	5	5	5	5
Stereotypy	Absent	5	5	5	5	5	5	5	5
Bizarre behaviour	Absent	5	5	5	5	5	5	5	5

Note: Values are expressed as number of animals

Acute Neurotoxicity Screening Battery Tests in Rats

Table-2.25

Effect of Insecticide Combination on Sensory Reactivity Measurements- Day 7

Parameters	Observation	Male (N=5)				Female (N=5)			
		Dose (mg/kg body weight)							
		0	25	75	225	0	25	75	225
Approach response	No reaction	0	1	0	0	0	0	0	0
	Energetic	5	4	5	5	5	5	5	5
Touch response	Energetic	5	5	5	5	5	5	5	5
Click response	Slight reaction	1	1	0	0	1	0	1	0
	Energetic	4	4	5	5	4	5	4	5
Pupil response	Normal	5	5	5	5	5	5	5	5
Air righting reflex	Normal	5	5	5	5	5	5	5	5
Tail pinch response	No reaction	1	0	0	0	0	0	0	0
	Slight reaction	1	0	0	0	0	0	0	0
	Flinch	3	5	5	5	5	5	5	5

Note: Values are expressed as number of animals

Acute Neurotoxicity Screening Battery Tests in Rats

Table-2.26

Effect of Insecticide Combination on Neurobehavioural Observations- Day 14

Home Cage Observations:

Parameters	Observation	Male (N=5)				Female (N=5)			
		Dose (mg/kg body weight)							
		0	25	75	225	0	25	75	225
Posture	Sitting normally	1	3	2	3	3	1	1	2
	Sitting alert	4	1	1	2	2	3	2	1
	Rearing	0	1	2	0	0	1	2	2
Clonic convulsions	Absent	5	5	5	5	5	5	5	5
	Absent	5	5	5	5	5	5	5	5

Handling Observations:

Parameters	Observation	Male (N=5)				Female (N=5)			
		Dose (mg/kg body weight)							
		0	25	75	225	0	25	75	225
Removal from home cage	Easy	5	5	5	5	5	4	5	5
	Very difficult	0	0	0	0	0	1	0	0
Handling reactivity	Easy	5	5	5	4	5	3	5	5
	Difficult	0	0	0	1	0	2	0	0
Palpebral closure	Eye lids wide open	5	5	5	5	5	5	5	5
Lacrimation	None	5	5	5	5	5	5	5	5
Eye examination	Normal	5	5	5	5	5	5	5	5
Piloerection	Absent	5	5	5	5	5	5	5	5
Skin examination	Normal	5	5	5	5	5	5	5	5
Salivation	None	5	5	5	5	5	5	5	5

Note: Values are expressed as number of animals

Acute Neurotoxicity Screening Battery Tests in Rats

Table-2.26 (Contd.)

Effect of Insecticide Combination on Neurobehavioural Observations- Day 14

Open field observations:

Parameters	Observation	Male (N=5)				Female (N=5)			
		Dose (mg/kg body weight)							
		0	25	75	225	0	25	75	225
Gait	Normal	5	5	5	5	5	5	5	5
Mobility	Normal	5	5	5	5	5	5	5	5
Arousal	High	5	3	3	5	5	4	3	5
	Low	0	2	2	0	0	1	2	0
Respiration	Normal	5	5	5	5	5	5	5	5
Clonic movement	Absent	5	5	5	5	5	5	5	5
Tonic movement	Absent	5	5	5	5	5	5	5	5
Stereotypy	Absent	5	5	5	5	5	5	5	5
Bizarre behaviour	Absent	5	5	5	5	5	5	5	5

Note: Values are expressed as number of animals

Acute Neurotoxicity Screening Battery Tests in Rats

Table-2.27

Effect of Insecticide Combination on Sensory Reactivity Measurements - Day 14

Parameters	Observation	Male (N=5)				Female (N=5)			
		Dose (mg/kg body weight)							
		0	25	75	225	0	25	75	225
Approach response	No reaction	0	1	0	0	0	0	0	0
	Energetic	5	4	5	5	5	5	5	5
Touch response	Energetic	5	5	5	5	5	5	5	5
Click response	Slight reaction	1	1	0	0	0	0	1	0
	Energetic	4	4	5	5	5	5	4	5
Pupil response	Normal	5	5	5	5	5	5	5	5
Air righting reflex	Normal	5	5	5	5	5	5	5	5
Tail pinch response	No reaction	1	0	0	0	0	0	0	0
	Slight reaction	1	0	0	0	0	0	0	0
	Flinch	3	5	5	5	5	5	5	5

Note: Values are expressed as number of animals

3.3 Repeated Dose 90-Day Neurotoxicity Study of Combination Insecticide in Rats

A total of 60 male and 100 females were administered with the test article at various dose concentrations for about 90 days. All the animals were observed for clinical signs once daily. At the end of 90 consecutive days of treatment, various clinical toxicological parameters were studied. A group of animals at high dose level and another group of animals served as control were kept for a period of 28 following 90 days of treatment to study the recovery from effects observed.

Effects of the test article on various toxicological parameters investigated were as follows:

3.3.1 Mortality

Reference Table: 3.1

No mortalities were observed in any of the treatment and control group during 90 days of treatment.

3.3.2 Clinical Symptoms:

Reference Table: 3.2

All the rats were observed once daily for the onset of treatment related symptoms. Symptoms such as nasal discharge, snuffles and soft stools were observed sporadically in few experimental animals irrespective of dose group and sex.

3.3.3 Ophthalmology:

A complete ophthalmologic examination was performed on each rat using an ophthalmoscope once before commencement of treatment and at the end of 90 days treatment. No treatment related ophthalmological abnormalities were observed in any of the experimental animals.

3.3.4 Body Weights

Male: Reference Tables: 3.3 and 3.5

Weekly mean body weights in 6-mg/kg body weight dose group were significantly reduced during weeks 7 - 13 of treatment period and in 15

mg/kg b. wt dose group during week 7 of treatment as compared to control group. However, weekly mean body weights in 38-mg/kg body weight dose groups were comparable to control throughout the treatment period.

Female: Reference Tables: 3.4 and 3.6

Weekly mean body weights of female rats from treatment groups were comparable to control group.

3.3.5 Food Consumption

Male: Reference Tables: 3.7

Food consumption was significantly reduced in 15 and 38 mg/kg body weight dose groups during weeks 8 and 9 of treatment period as compared to control group.

Females: Reference Tables: 3.8

Food consumption of females in treatment groups was comparable to control group throughout the experimental period.

3.3.6 Hematology Values

Male: Reference Tables: 3.9 and 3.11

Mean blood clotting time was significantly increased in 6-mg/kg body weight dose group as compared to control group. In high dose (38 mg/kg body weight) recovery group, mean white blood corpuscular (WBC) count was significantly reduced as compared to respective control recovery group.

All other blood parameters and their indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were comparable to control group.

Female: Reference Tables: 3.10 and 3.12

Animals treated at 6-mg/kg body weight dose group showed significantly increased values of RBC, hemoglobin (Hb), mean corpuscular hemoglobin concentration (MCHC) and platelet count and significantly reduced mean corpuscular volume (MCV) values were as compared to control group. Animals treated at 15 mg/kg body weight revealed significantly reduced RBC, hemoglobin, HCT, MCV, MCHC and platelet counts as compared to control group. Whereas, animals treated at 38 mg/kg body weight dose group revealed significantly lower hemoglobin, HCT and MCV and significantly higher MCHC and platelet counts when compared to control group. Hematology investigations performed at the end of 28 days of recovery period on high dose recovery group (38 mg/kg body weight) were comparable to control recovery group.

3.3.7 Differential Leucocytes Count

Male: Reference Tables: 3.13 and 3.15

Differential leucocytes count from treatment groups at termination as well as at the end of recovery period was comparable to respective control group.

Female: Reference Tables: 3.14 and 3.16

Differential leucocytes count from treatment groups at termination as well as at the end of recovery period was comparable to respective control group.

3.3.8 Clinical Chemistry Values

Male: Reference Tables: 3.17 and 3.19

Clinical chemistry estimations performed at termination revealed significantly increased mean values of total protein and albumin in 6, 15 and 38 mg/kg body weight dose groups as compared to control group. In addition, mean values of ALT, ALP, total bilirubin and creatinine were increased in 38 mg/kg body weight dose group. Mean values of cholinesterase values were

significantly reduced in 38 mg/kg body weight dose group when compared to control group.

In high dose recovery group (38 mg/kg body weight), clinical chemistry estimations performed revealed increased glucose and total protein when compared to control recovery group.

Female: Reference Tables: 3.18 and 3.20

During terminal estimations, significantly higher values of glucose were observed in 15 and 38 mg/kg body weight dose group as compared to control group. Further, mean values of total protein were significantly increased and cholinesterase values were reduced in 6, 15 and 38 mg/kg body weight dose groups, while sodium values were reduced only in 38 mg/kg body weight dose group when compared to control group. Also, mean values of ALT, GGT and albumin were considerably increased; however, they were statistically insignificant.

In high dose recovery group females, glucose was significantly increased as compared to control recovery group.

3.3.9 Functional Observational Battery Tests (FOB)

Functional observational battery test/neurobehavioral observations performed during 13th week of treatment were as described below:

3.3.9.1 Home Cage Observations:

All the animals from control and treatment groups revealed normal posture in the home cage and did not reveal any clonic or tonic movements.

3.3.9.2 Handling Observations:

Ease of removal from home cage and handling reactivity was very easy/easy for all the animals. Palpebral closure was wide open for all the animals, eyes and skin was normal. None of the animals exhibited lacrimation, piloerection or salivation.

3.3.9.3 Open Field Observations:

Observations performed in open field revealed normal gait, mobility and high arousal in all the animals. None of the animals revealed clonic/tonic or stereotypic behaviour.

3.3.9.4 Sensory Reactivity Measurements:

Majority of animals from control and treatment groups responded energetically to approach, touch and click stimulus. Pupil response was normal in all animals. Majority of animals responded as flinch to tail pinch and revealed normal air righting reflex.

3.3.9.5 Motor Activity

Reference Tables: 3.21 and 3.22

Total activity, ambulatory activity and stereotypic activity in mid and high dose group rats were significantly increased as compared to control group.

3.3.9.6 Forelimb Grip Strength

Reference Tables: 3.23 and 3.24

No statistically significant variation was observed in the forelimb grip strength of either sex animals from treatment groups as compared to respective controls.

3.3.9.7 Hind limb Grip strength

Reference Tables: 3.23 and 3.24

Hind limb grip strength in either sex animals was comparable to control.

3.3.9.8 Hind limb Foot Splay

Reference Tables: 3.25 and 3.26

No statistically significant variation was noticed in the hind limb foot splay of treatment group animals as compared to control group.

3.3.10 Absolute Organ Weights

Male (Recovery Groups): Reference Tables: 3.27

Absolute weights of organs in high dose recovery group (38 mg/kg body weight) were comparable to control recovery group.

Female (Recovery Groups): Reference Tables: 3.28

Absolute weights of organs in high dose recovery group (38 mg/kg body weight) were comparable to control recovery group.

3.3.11 Relative Organ Weights

Male (Recovery Groups): Reference Tables: 3.29

Relative weights of organs in high dose recovery group (38 mg/kg body weight) were comparable to control recovery group.

Female (Recovery Groups): Reference Tables: 3.30

Relative weights of organs in high dose recovery group (38 mg/kg body weight) were comparable to control recovery group.

Repeated Dose 90-Day Neurotoxicity Study in Rats

Table-3.1

Summary of Mortality

Dose (mg/kg b. wt/day)	Male	Female
0 (Control)	0/10	0/20
6 (Low dose)	0/10	0/20
15 (Mid dose)	0/10	0/20
38 (High dose)	0/10	0/20

Table-3.2

Summary of Clinical Symptoms

Symptom	Male				Female			
	Dose (mg/kg body weight/day)				Dose (mg/kg body weight/day)			
	0	6	15	38	0	6	15	38
Snuffles	1/10	1/10	0/10	2/10	0/20	0/20	2/20	3/20
Nasal discharge	2/10	0/10	1/10	1/10	0/20	2/20	2/20	1/20
Soft stool/ Diarrhea	0/10	0/10	2/10	1/10	1/20	0/20	3/20	2/20

Repeated Dose 90-Day Neurotoxicity Study in Rats

Table-3.3

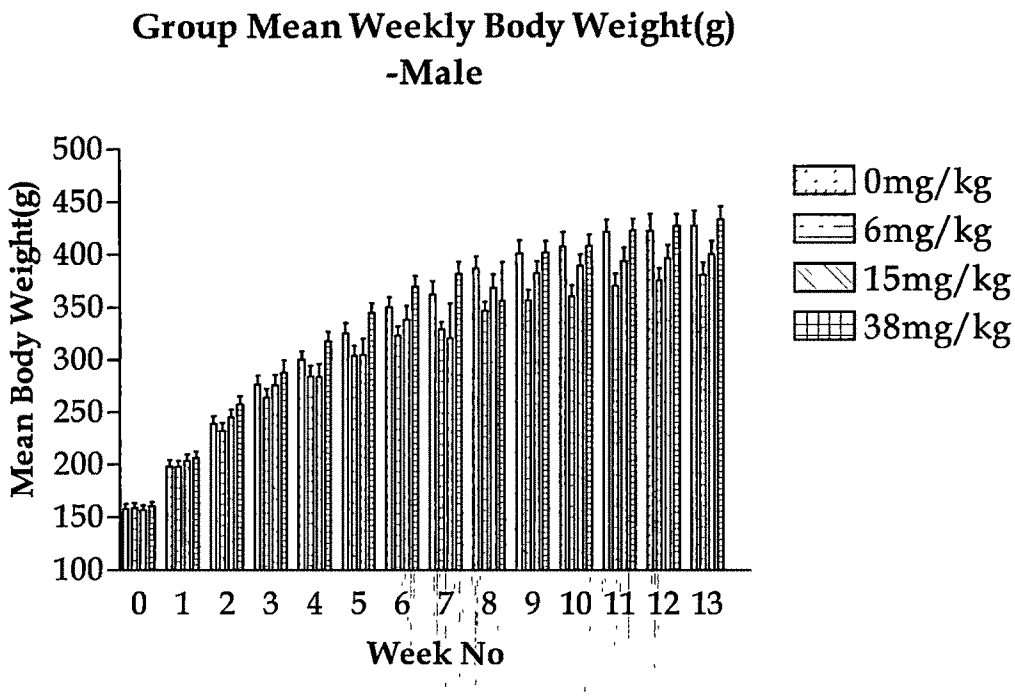
Effect of Insecticide Combination on Weekly Body Weight (g) - Male

Week N°	Dose (mg/kg body weight/day)			
	0 (N= 10)	6 (N= 10)	15 (N= 10)	38 (N= 10)
0	158 ± 4.68	158.8 ± 5.03	157.2 ± 4.18	160.4 ± 4.20
1	198 ± 6.61	197.7 ± 6.19	203.8± 5.82	206.4 ± 6.22
2	239 ± 7.29	232.2 ± 7.75	244.8 ± 7.56	257.4 ± 7.65
3	276 ± 8.85	263.7 ± 8.96	275.6 ± 10.37	287.7 ± 11.64
4	300 ± 8.33	284.2 ± 9.91	283.6 ± 12.51	317.6 ± 9.14
5	325 ± 10.33	303.4 ± 9.65	304.6 ± 15.57	344.8 ± 9.40
6	350 ± 9.97	323.2 ± 8.51	338 ± 13.36	369.4 ± 10.39
7	362 ± 12.86	329.1 ± 7.07*↓	320.6 ± 33.13*↓	381.6 ± 11.54
8	387 ± 11.01	346.5 ± 8.75*↓	368.6 ± 12.59	356.2 ± 36.63
9	401 ± 12.71	356.4 ± 10.33*↓	382 ± 11.52	402.0 ± 10.68
10	407 ± 14.03	360.3 ± 10.50*↓	389.4 ± 10.68	408.2 ± 10.58
11	421 ± 11.85	370.2 ± 11.76*↓	393.4 ± 13.25	422.7 ± 11.25
12	422 ± 16.02	375.4 ± 11.71*↓	396.2 ± 12.81	426.7 ± 11.31
13	427 ± 14.53	380.8 ± 11.62*↓	400 ± 12.88	432.8 ± 12.61

Note: Values are expressed as Mean ± SE

*↓ = Significantly lower than control ($P \leq 0.05$)

Figure-2.1


Group Mean Weekly Body Weight(g)
-Male
Mean Body Weight(g)
500
450
400
350
300
250
200
150
100
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Week No
0mg/kg
6mg/kg
15mg/kg
38mg/kg

Repeated Dose 90-Day Neurotoxicity Study in Rats

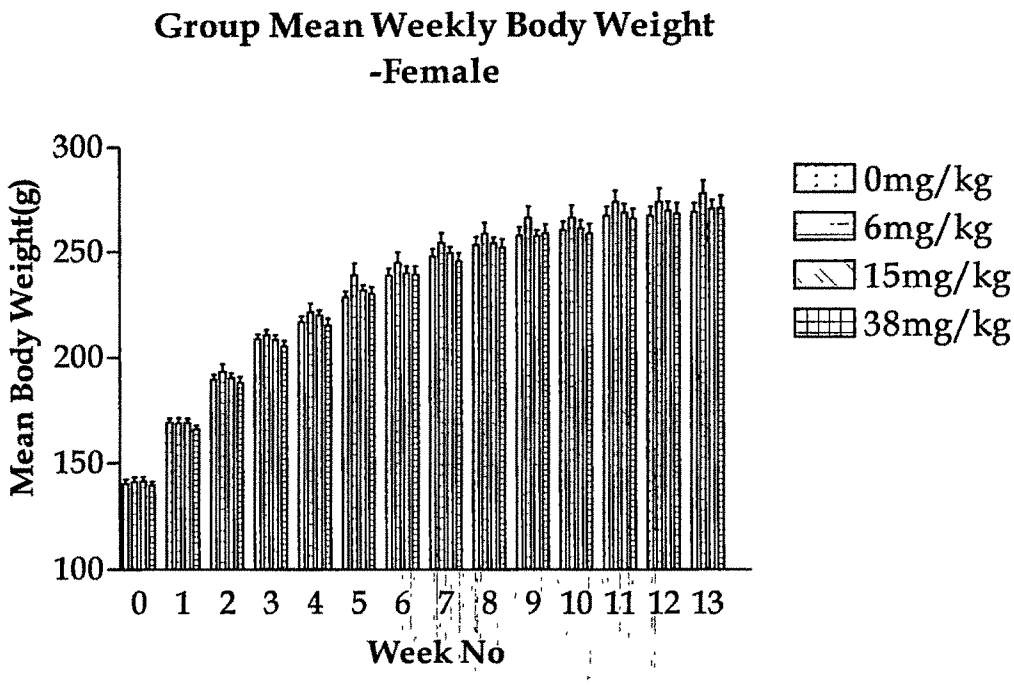
Table-3.4

Effect of Insecticide Combination on Weekly Body Weight (g) - Female

Week N°	Dose (mg/kg body weight/day)			
	0 (N=20)	6 (N=20)	15 (N=20)	38 (N=20)
0	140.4 ± 2.05	141.2 ± 2.21	141.5 ± 2.14	139.6 ± 1.75
1	169.2 ± 2.12	168.8 ± 2.55	169 ± 2.08	166.0 ± 1.70
2	189.6 ± 2.39	193.4 ± 3.53	190.3 ± 2.24	188.1 ± 2.88
3	208.5 ± 2.60	210.5 ± 2.60	208.2 ± 2.33	205.3 ± 2.40
4	216.7 ± 2.95	221.4 ± 4.17	219.9 ± 2.51	215.1 ± 3.33
5	228.5 ± 2.81	239.1 ± 5.56	231.7 ± 2.63	230.0 ± 3.42
6	238.7 ± 3.35	244.9 ± 5.05	239.9 ± 3.10	239.3 ± 3.75
7	247.7 ± 3.74	254.2 ± 4.89	249.4 ± 2.97	245.5 ± 3.99
8	253.2 ± 3.80	258.6 ± 5.35	254 ± 2.90	252.1 ± 3.92
9	257.7 ± 4.06	266.3 ± 5.43	257.6 ± 2.75	258.8 ± 4.39
10	260.4 ± 4.07	266.3 ± 5.92	261.3 ± 3.77	258.8 ± 4.64
11	267.3 ± 4.32	273.7 ± 5.56	268.8 ± 4.14	265.8 ± 4.85
12	267.1 ± 4.32	273.8 ± 6.56	269.5 ± 4.43	268.3 ± 5.07
13	268.9 ± 4.43	277.7 ± 6.38	270.4 ± 4.34	270.8 ± 6.09

Note: Values are expressed as Mean ± SE

Figure-2.2


Group Mean Weekly Body Weight
-Female
Mean Body Weight(g)
300
250
200
150
100
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Week No
0mg/kg
6mg/kg
15mg/kg
38mg/kg

Repeated Dose 90-Day Oral Neurotoxicity in Rats

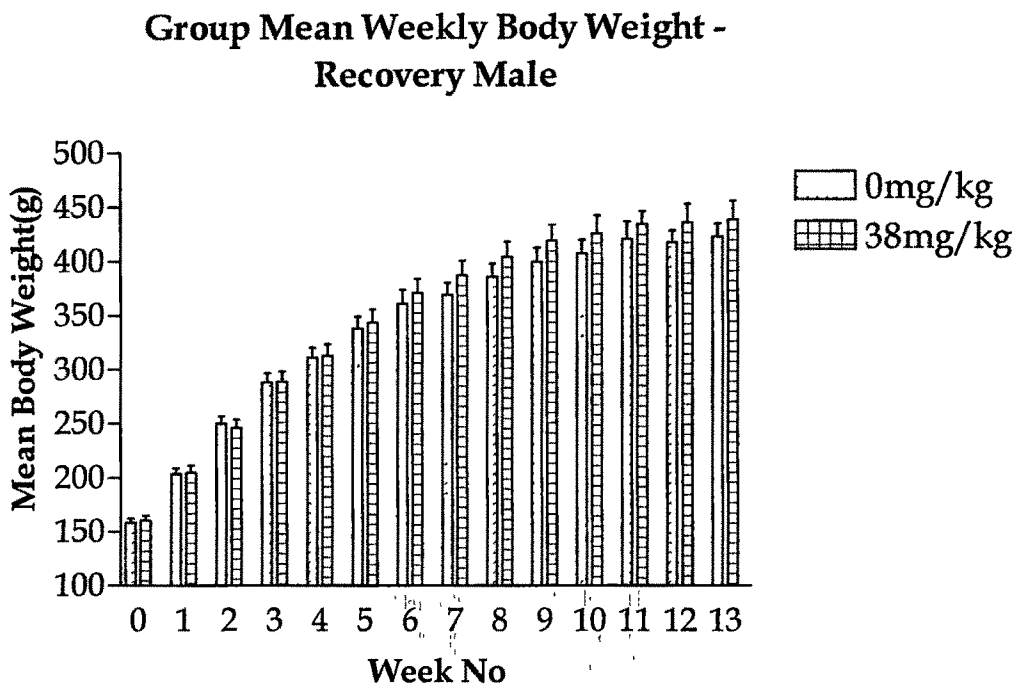
Table-3.5

Effect of Insecticide Combination on Weekly Body Weight (g) - Recovery Group (Male)

Week N°	Dose (mg/kg body weight/day)	
	0 (N=10)	38 (N=10)
0	158.6 ± 3.74	160.6 ± 4.41
1	203.6 ± 5.37	204.8 ± 6.63
2	250.0 ± 7.02	246.0 ± 7.96
3	288.2 ± 8.28	288.6 ± 9.46
4	311.1 ± 9.27	313.2 ± 10.47
5	338.0 ± 11.09	343.7 ± 12.43
6	361.2 ± 12.61	371.2 ± 12.93
7	369.6 ± 10.96	387.2 ± 13.75
8	385.8 ± 11.99	404.0 ± 14.07
9	399.6 ± 12.84	418.8 ± 14.87
10	407.2 ± 12.55	425.8 ± 16.37
11	420.6 ± 16.37	434.2 ± 12.09
12	417.3 ± 10.84	435.7 ± 17.05
13	422.6 ± 12.11	438.3 ± 17.22

Note: Values are expressed as Mean ± SE

Figure-2.3


Group Mean Weekly Body Weight - Recovery Male
Mean Body Weight(g)
500
450
400
350
300
250
200
150
100
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Week No
0mg/kg
38mg/kg

Repeated Dose 90-Day Neurotoxicity Study in Rats

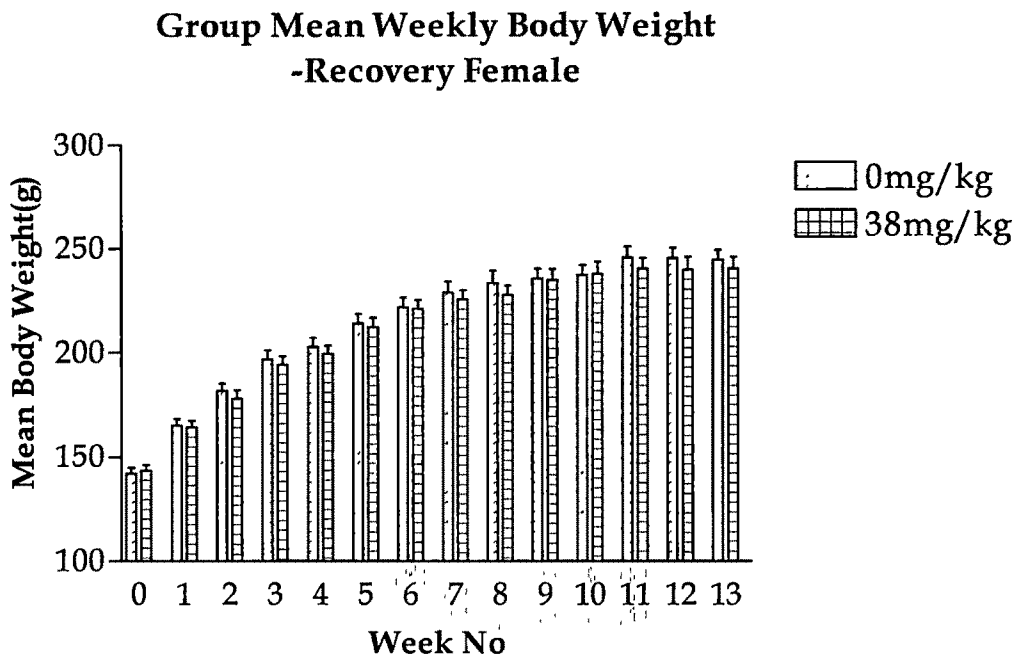
Table-3.6

Effect of Insecticide Combination on Weekly Body Weight (g) - Recovery Group (Female)

Week N°	Dose (mg/kg body weight/day)	
	0 (N=10)	38 (N=10)
0	142.0 ± 2.97	143.6 ± 2.60
1	165.0 ± 3.20	164.2 ± 3.42
2	181.8 ± 3.49	178.0 ± 4.01
3	196.8 ± 4.41	194.2 ± 4.16
4	202.8 ± 4.39	199.6 ± 3.87
5	214.0 ± 4.57	212.2 ± 4.57
6	221.5 ± 4.97	221.0 ± 4.26
7	228.8 ± 5.20	225.4 ± 4.54
8	233.2 ± 6.15	227.8 ± 4.34
9	235.6 ± 4.61	234.8 ± 5.33
10	237.3 ± 4.71	237.8 ± 5.87
11	245.6 ± 5.32	240.2 ± 5.19
12	245.3 ± 5.14	239.7 ± 6.20
13	244.6 ± 4.68	240.2 ± 5.76

Note: Values are expressed as Mean ± SE

Figure-2.4


Group Mean Weekly Body Weight
-Recovery Female
Mean Body Weight(g)
300
250
200
150
100
0 1 2 3 4 5 6 7 8 9 10 11 12 13
Week No
0mg/kg
38mg/kg

Repeated Dose 90-Day Neurotoxicity Study in Rats

Table-3.7

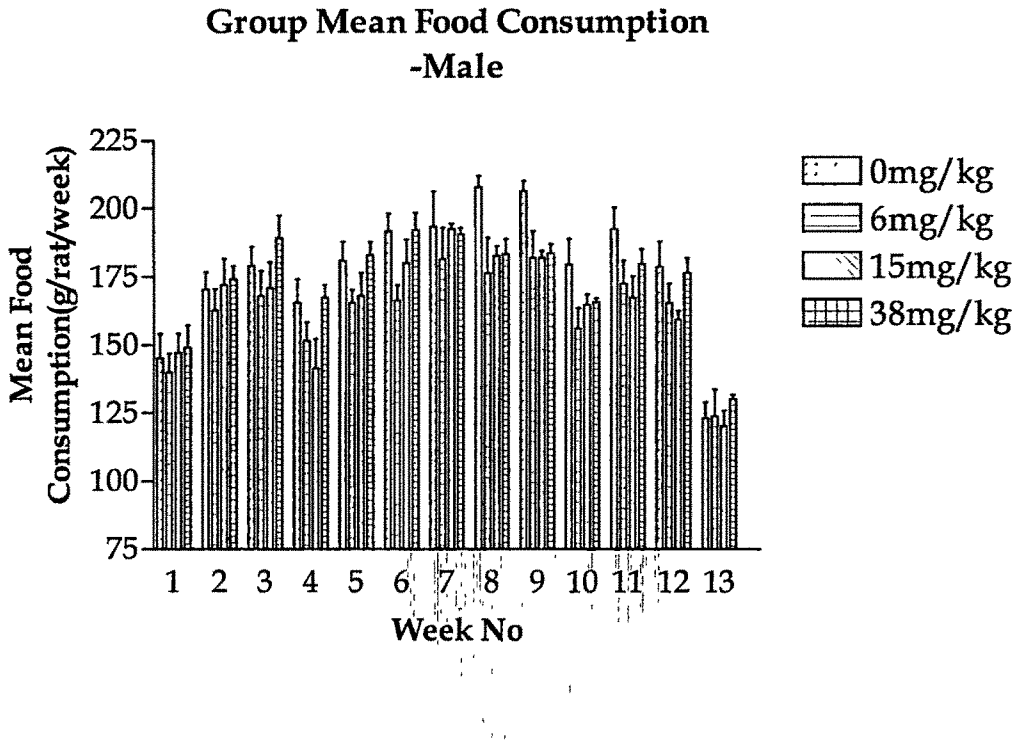
Effect of Insecticide Combination on Food Consumption (g/rat/week) - Male

Week N°	Dose (mg/kg body weight/day)			
	0 (N=5)	6 (N=5)	15 (N=5)	38 (N=5)
1	145.2 ± 8.93	140.0 ± 6.91	147.2 ± 7.06	149.0 ± 8.33
2	170.4 ± 6.35	162.8 ± 7.87	172.0 ± 9.69	174.2 ± 4.91
3	179.0 ± 7.08	168.0 ± 9.16	171.1 ± 9.33	189.3 ± 8.28
4	165.6 ± 8.58	151.6 ± 6.80	141.4 ± 11.04	167.6 ± 4.53
5	180.9 ± 7.04	165.6 ± 4.71	168.0 ± 8.56	183.1 ± 4.66
6	191.8 ± 6.33	166.4 ± 5.60	180.2 ± 8.57	192.2 ± 6.40
7	193.3 ± 13.01	181.6 ± 11.51	192.6 ± 1.81	190.7 ± 2.34
8	207.8 ± 4.15	176.4 ± 12.98	182.8 ± 3.53*↓	183.4 ± 5.55*↓
9	206.3 ± 3.77	182.0 ± 9.87	182.0 ± 2.74*↓	183.8 ± 3.23*↓
10	179.6 ± 9.29	156.0 ± 7.72	164.8 ± 3.84	165.8 ± 1.56
11	192.5 ± 7.79	172.6 ± 8.48	167.5 ± 7.84	179.8 ± 5.42
12	178.8 ± 9.22	165.5 ± 7.00	159.4 ± 3.31	176.5 ± 5.42
13	123.2 ± 5.89	123.8 ± 9.94	120.2 ± 5.70	130.2 ± 1.46

Note: Values are expressed as Mean ± SE

*↓ = Significantly lower than control ($P \leq 0.05$)

Figure-2.5


Group Mean Food Consumption
-Male
Mean Food Consumption(g/rat/week)
225
200
175
150
125
100
75
1 2 3 4 5 6 7 8 9 10 11 12 13
Week No
0mg/kg
6mg/kg
15mg/kg
38mg/kg

Repeated Dose 90-Day Neurotoxicity Study in Rats

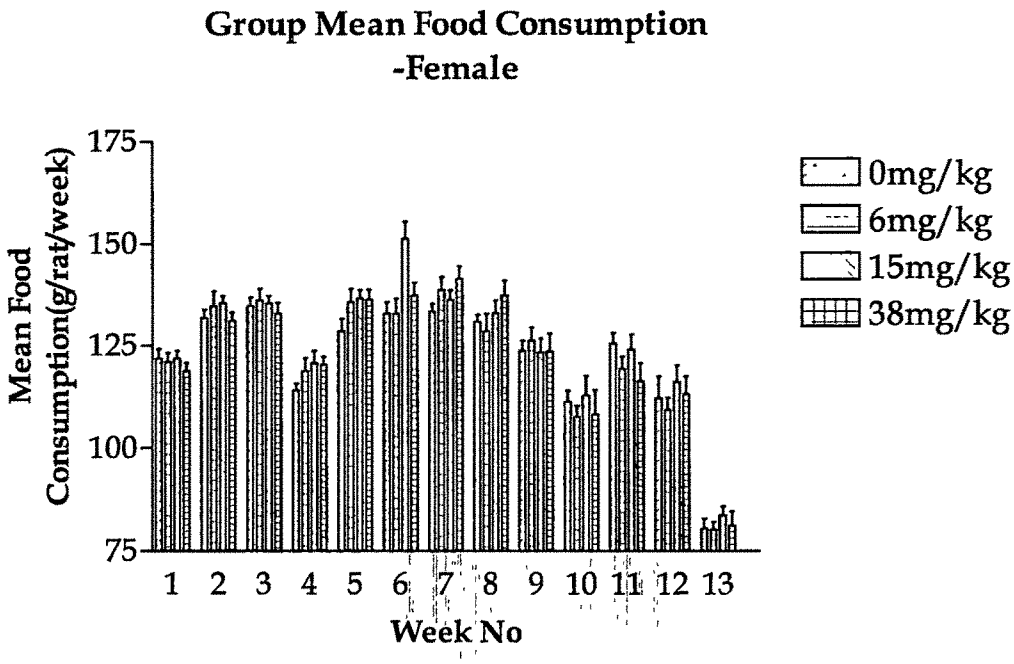
Table-3.8

Effect of Insecticide Combination on Food Consumption (g/rat/week)- Female

Week N°	Dose (mg/kg body weight/day)			
	0 (N=10)	6 (N=10)	15 (N=10)	38 (N=10)
1	121.8 ± 2.38	121.0 ± 2.33	121.9 ± 1.80	118.8 ± 2.00
2	131.8 ± 2.01	134.6 ± 3.66	135.4 ± 1.69	131.0 ± 2.02
3	134.7 ± 2.09	136.0 ± 2.97	135.3 ± 1.94	132.8 ± 2.71
4	114.1 ± 1.75	118.8 ± 3.15	120.7 ± 2.98	120.4 ± 1.90
5	128.5 ± 3.01	135.6 ± 3.39	136.5 ± 2.11	136.2 ± 2.50
6	132.8 ± 2.80	132.8 ± 3.73	151.2 ± 4.29	137.3 ± 3.23
7	133.3 ± 1.91	138.6 ± 3.30	136.1 ± 2.42	141.4 ± 3.11
8	130.8 ± 1.70	128.5 ± 4.29	132.9 ± 3.12	137.3 ± 3.74
9	123.7 ± 2.49	126.2 ± 3.21	123.4 ± 3.31	123.5 ± 4.45
10	111.4 ± 2.68	107.6 ± 2.77	112.8 ± 4.81	108.2 ± 6.10
11	125.5 ± 2.59	119.3 ± 3.01	124.0 ± 3.68	116.3 ± 4.39
12	112.1 ± 5.36	109.3 ± 3.02	116.1 ± 4.12	113.1 ± 4.37
13	80.4 ± 2.35	80.2 ± 1.88	83.6 ± 2.21	81.1 ± 3.54

Note: Values are expressed as Mean ± SE

Figure-2.6


Group Mean Food Consumption
-Female
Mean Food Consumption(g/rat/week)
175
150
125
100
75
1 2 3 4 5 6 7 8 9 10 11 12 13
Week No
0mg/kg
6mg/kg
15mg/kg
38mg/kg

Repeated Dose 90-Day Neurotoxicity Study in Rats

Table-3.9

Effect of Insecticide Combination on Hematology Parameters –Male

Parameter	Dose (mg/kg body weight/day)			
	0 (N=10)	6 (N=10)	15 (N=10)	38 (N=10)
WBC (10^3/µL)	13.11 ± 1.53	15.22 ± 1.18	14.19 ± 0.97	11.36 ± 0.90
RBC (10^6/µL)	8.61 ± 0.21	8.66 ± 0.11	8.50 ± 0.10	8.63 ± 0.15
HGB (g/dL)	15.75 ± 0.38	16.00 ± 0.24	15.96 ± 0.23	15.85 ± 0.21
HCT (%)	43.04 ± 0.91	42.63 ± 0.66	41.85 ± 0.56	42.83 ± 0.47
MCV (fL)	47.06 ± 3.05	49.23 ± 0.60	49.25 ± 0.43	49.66 ± 0.42
MCH (pg)	18.31 ± 0.13	18.48 ± 0.18	18.78 ± 0.19	18.37 ± 0.16
MCHC (g/dL)	34.65 ± 1.84	37.56 ± 0.29	38.14 ± 0.20	37.01 ± 0.19
PLT (10^3/µL)	970.9 ± 86.77	1044.5 ± 58.67	1039.4 ± 76.96	973.2 ± 94.68
Clotting time (sec)	86.20 ± 7.00	108.50 ± 4.38*↑	97.30 ± 6.14	93.20 ± 6.63

Note: Values are expressed as Mean ± SE

*↑ = Significantly higher than control ($P \leq 0.05$)

Repeated Dose 90-Day Neurotoxicity Study in Rats

Table-3.10

Effect of Insecticide Combination on Hematology Parameters–Female

Parameter	Dose (mg/kg body weight/day)			
	0 (N=10)	6 (N=10)	15 (N=10)	38 (N=10)
WBC (10^3/µL)	12.09 ± 0.75	10.72 ± 0.97	8.54 ± 1.57	15.33 ± 1.68
RBC (10^6/µL)	7.52 ± 0.07	7.89 ± 0.12*↑	6.95 ± 0.41*↓	7.38 ± 0.09
HGB (g/dL)	14.79 ± 0.16	15.41 ± 0.18*↑	12.83 ± 1.03*↓	14.20 ± 0.17*↓
HCT (%)	40.08 ± 0.37	39.81 ± 0.27	35.58 ± 1.96*↓	37.17 ± 0.50*↓
MCV (fL)	53.34 ± 0.63	50 59 ± 0.61*↓	51.31 ± 0.54*↓	50.37 ± 0.25*↓
MCH (pg)	19.81 ± 0.27	19.58 ± 0.24	18.26 ± 0.77	19.25 ± 0.11
MCHC (g/dL)	37.13 ± 0.21	38.71 ± 0.24*↑	35.66 ± 1.56*↓	38.21 ± 0.14*↑
PLT (10^3/µL)	818.4 ± 30 88	956.1 ± 28.56*↑	686.1 ± 90.88*↓	932.2 ± 29.38*↑
Clotting time (sec)	99.80 ± 5.91	99.70 ± 7.23	95.80 ± 5.96	111.60 ± 6.26

Note: Values are expressed as Mean ± SE
*↓ = Significantly lower than control ($P \leq 0.05$),
*↑ = Significantly higher than control ($P \leq 0.05$)

Repeated Dose 90-Day Neurotoxicity Study in Rats

Table-3.11

Effect of Insecticide Combination on Hematology Parameters-Recovery Group (Male)

Parameter	Dose (mg/kg body weight/day)	
	0 (N=9)	38 (N=8)
WBC ($10^3/\mu L$)	12 83 ± 0.62	9.21 ± 0.57*↓
RBC ($10^6/\mu L$)	8.23 ± 0.35	8.60 ± 0.18
HGB (g/dL)	15.34 ± 0.38	15.81 ± 0.21
HCT (%)	43.53 ± 0.71	43.29 ± 0.61
MCV (fL)	50.93 ± 0.36	50.40 ± 0.44
MCH (pg)	17.94 ± 0.44	18.41 ± 0.22
MCHC (g/dL)	35.30 ± 0.87	36.54 ± 0.15
PLT ($10^3/\mu L$)	990.11 ± 20.15	1001.00 ± 40.02
Clotting time (sec)	102.56 ± 8.36	104.75 ± 7.26

Note: Values are expressed as Mean ± SE
*↓ = Significantly lower than control ($P \leq 0.05$)

Repeated Dose 90-Day Neurotoxicity Study in Rats

Table-3.12

Effect of Insecticide Combination on Hematology Parameters – Recovery Group (Female)

Parameter	Dose (mg/kg body weight/day)	
	0 (N=10)	38 (N=10)
WBC ($10^3/\mu L$)	10.96 ± 0.95	10.47 ± 0.76
RBC ($10^6/\mu L$)	7.293 ± 0.09	7.24 ± 0.29
HGB (g/dL)	14.81 ± 0.19	13.73 ± 0.92
HCT (%)	40.01 ± 0.55	38.37 ± 1.56
MCV (fL)	54.87 ± 0.59	53.98 ± 0.27
MCH (pg)	20.31 ± 0.22	18.71 ± 0.79
MCHC (g/dL)	37.01 ± 0.16	35.29 ± 1.43
PLT ($10^3/\mu L$)	801.1 ± 22.50	840.00 ± 43.52
Clotting time (sec)	100.1 ± 7.01	111.30 ± 7.58

Note: Values are expressed as Mean ± SE

Repeated Dose 90-Day Neurotoxicity Study in Rats

Table-3.13

Effect of Insecticide Combination on DLC Values -Male

Parameter	Dose (mg/kg body weight/day)			
	0 (N=10)	6 (N=10)	15 (N=10)	38 (N=10)
Lymphocytes (%)	80.7 ± 0.9	80.5 ± 1.3	80.8 ± 1.2	79.7 ± 1.9
Neutrophils (%)	17.0 ± 1.0	17.2 ± 1.1	17.0 ± 1.2	17.4 ± 1.6
Monocytes (%)	1.4 ± 0.3	1.5 ± 0.3	1.1 ± 0.3	1.6 ± 0.5
Basophils (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Eosinophils (%)	0.9 ± 0.3	0.8 ± 0.2	1.1 ± 0.3	1.3 ± 0.3

Note: Values are expressed as Mean ± SE

Table - 3.14

Effect of Insecticide Combination on DLC Values -Female

Parameter	Dose (mg/kg body weight/day))			
	0 (N=10)	6 (N=10)	15 (N=10)	38 (N=10)
Lymphocytes (%)	77.3 ± 1.4	77.0 ± 2.6	77.9 ± 1.5	77.3 ± 1.3
Neutrophils (%)	20.3 ± 1.1	20.4 ± 2.4	19.4 ± 1.5	20.6 ± 1.2
Monocytes (%)	1.4 ± 0.3	1.3 ± 0.4	1.7 ± 0.3	1.4 ± 0.3
Basophils (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Eosinophils (%)	1.0 ± 0.3	1.3 ± 0.3	1.1 ± 0.3	0.8 ± 0.2

Note: Values are expressed as Mean ± SE

Table-3.15

Effect of Insecticide Combination on DLC Values – Recovery Group (Male)

Parameter	Dose (mg/kg body weight/day)	
	0 (N=9)	38 (N=8)
Lymphocytes (%)	78.67 ± 1.20	75.5 ± 2.40
Neutrophils (%)	18.89 ± 1.18	21.25 ± 2.35
Monocytes (%)	1.56 ± 0.34	1.75 ± 0.31
Basophils (%)	0.00 ± 0.00	0 ± 0.00
Eosinophils (%)	0.89 ± 0.31	1.5 ± 0.46

Note: Values are expressed as Mean ± SE

Table-3.16

Effect of Insecticide Combination on DLC Values – Recovery Group (Female)

Parameter	Dose (mg/kg body weight/day)	
	0 (N=10)	38 (N=10)
Lymphocytes (%)	76.70 ± 1.20	78.3 ± 1.40
Neutrophils (%)	20.50 ± 0.92	18.7 ± 1.45
Monocytes (%)	1.70 ± 0.30	1.6 ± 0.27
Basophils (%)	0.00 ± 0.00	0 ± 0.00
Eosinophils (%)	1.10 ± 0.31	1.4 ± 0.31

Note: Values are expressed as Mean ± SE

Repeated Dose 90-Day Neurotoxicity Study in Rats

Table-3.17

Effect of Insecticide Combination on Clinical Chemistry Parameters – Male

Parameter	Dose (mg/kg body weight/day)			
	0 (N=10)	6 (N=10)	15 (N=10)	38 (N=10)
GLU (mg/dL)	84.30 ± 3.53	84.47 ± 5.32	96.95 ± 3.80	78.56 ± 3.66
ALT (IU/L)	50.84 ± 2.23	51.75 ± 1.35	52.38 ± 1.08	56.95 ± 0.91*↑
AST (IU/L)	197.71 ± 10.82	199.97 ± 9.45	200.78 ± 9.15	223.25 ± 7.96
GGT (IU/L)	5.79 ± 0.74	5.66 ± 0.69	5.45 ± 0.42	4.40 ± 0.59
T.BIL (mg/dL)	0.16 ± 0.01	0.17 ± 0.00	0.17 ± 0.01	0.20 ± 0.01*↑
Creatinine (mg/dL)	0.59 ± 0.01	0.60 ± 0.01	0.60 ± 0.02	0.65 ± 0.02*↑
BUN (mg/dL)	24.33 ± 0.45	24.11 ± 0.51	23.45 ± 0.76	24.55 ± 0.66
T.Protein (g/dL)	7.78 ± 0.09	8.28 ± 0.09*↑	8.29 ± 0.06*↑	8.48 ± 0.12*↑
ALB (g/dL)	3.55 ± 0.04	3.72 ± 0.05*↑	3.74 ± 0.03*↑	3.79 ± 0.04*↑
Cholesterol (mg/dL)	58.51 ± 2.87	59.28 ± 2.53	63.06 ± 2.54	66.62 ± 2.83
Urea (mg/dL)	52.10 ± 1.02	51.80 ± 1.06	50.20 ± 1.62	52.60 ± 1.42
ChE (IU/L)	169.90 ± 18.89	148.10 ± 29.36	142.50 ± 13.24	86.80 ± 9.12*↓
ALP (IU/L)	119.75 ± 11.09	125.92 ± 17.18	131.76 ± 8.84	134.06 ± 9.03*↑
Cl (mEq/L)	118.43 ± 4.05	126.63 ± 8.22	118.67 ± 4.61	129.93 ± 7.17
Na (mEq/L)	138.39 ± 1.23	137.38 ± 1.25	137.09 ± 1.48	124.81 ± 12.30
K (mEq/L)	4.59 ± 0.14	4.60 ± 0.09	4.71 ± 0.27	4.51 ± 0.10

Note: Values are expressed as Mean ± SE
*↓ = Significantly lower than control (P< 0.05),
*↑ = Significantly higher than control (P< 0.05)

Figure-2.7

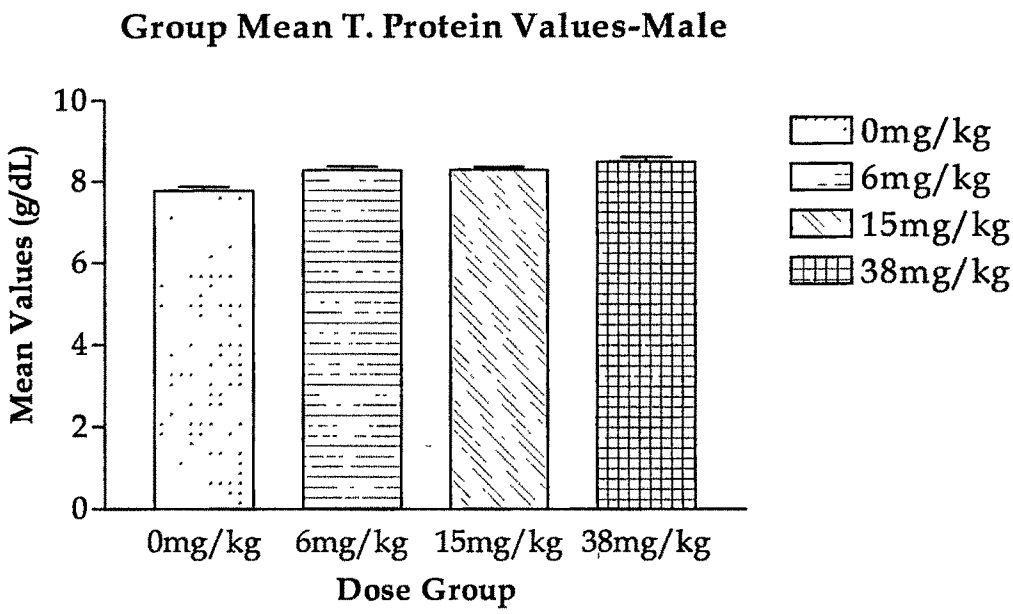


Figure-2.8

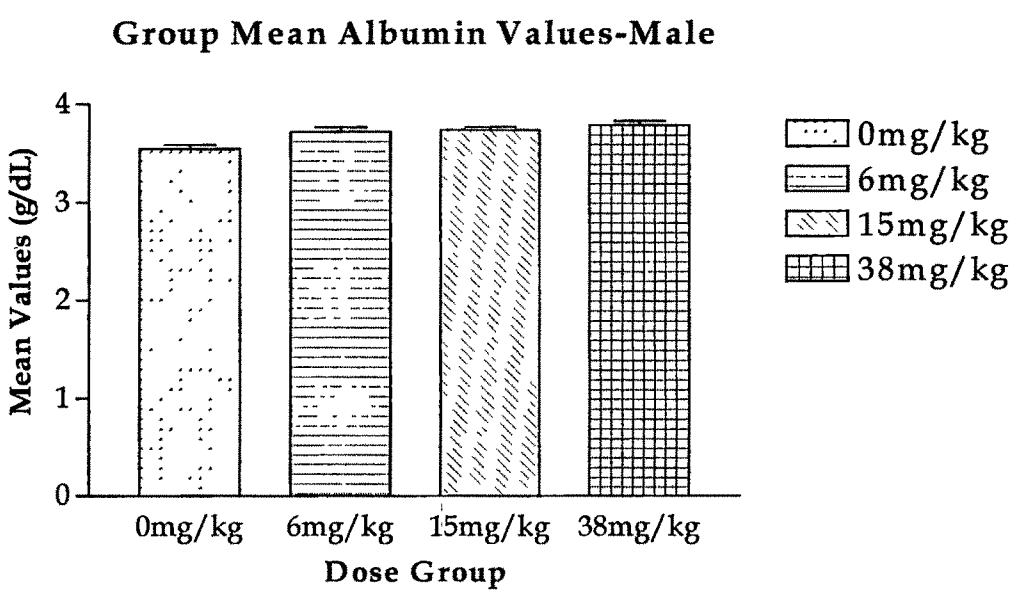


Figure-2.9

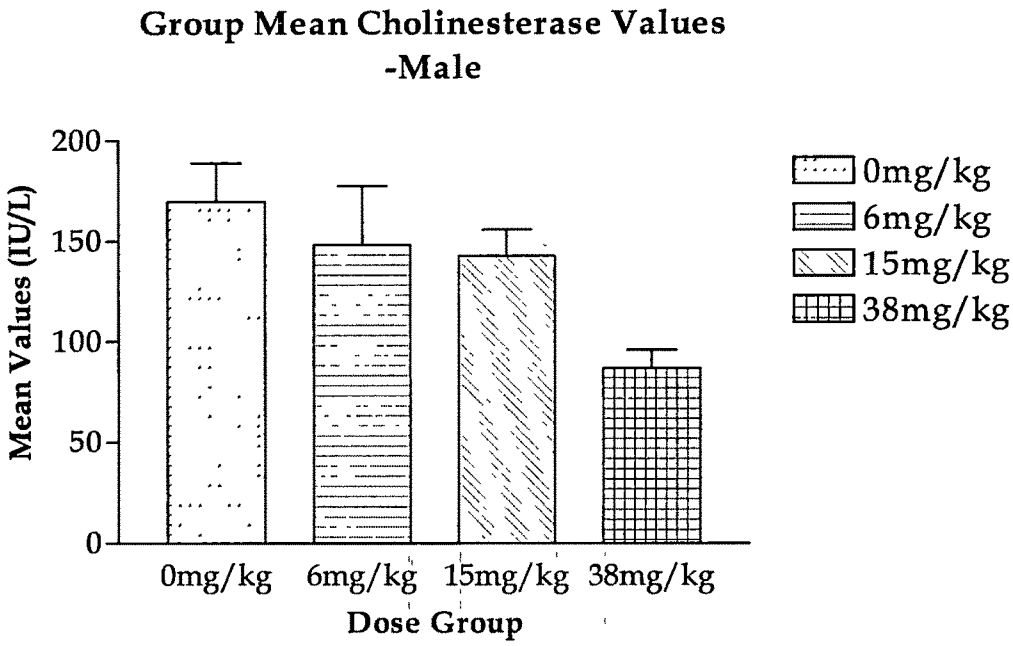


Figure 2.10

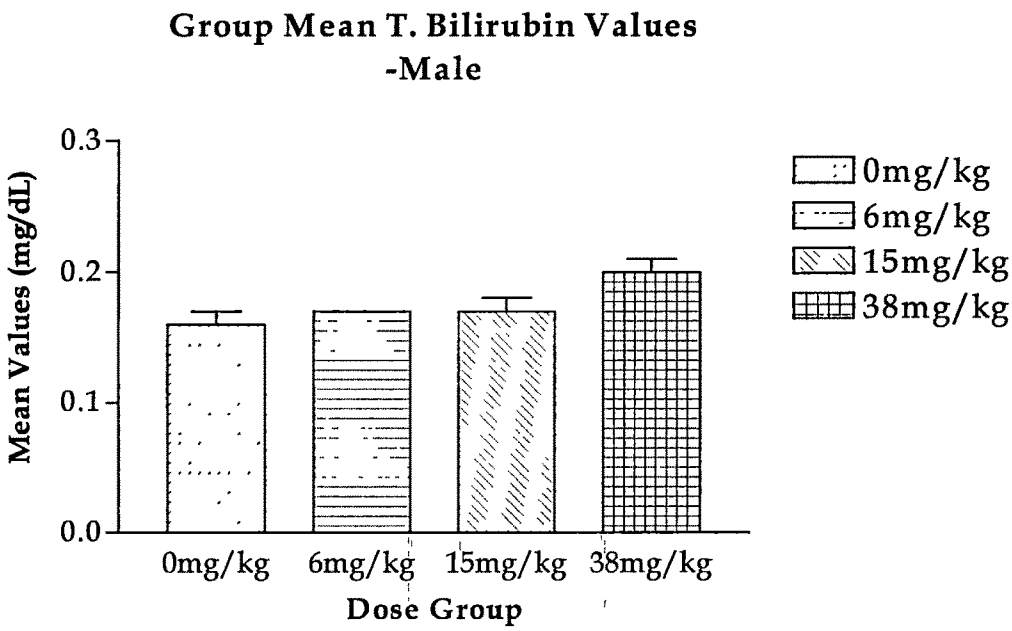


Figure 2.11

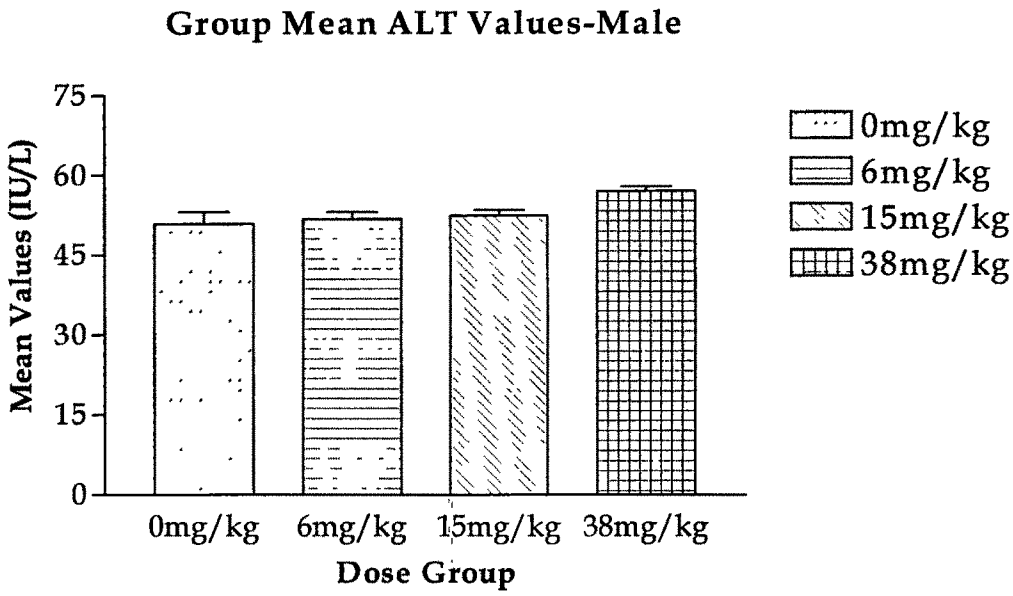
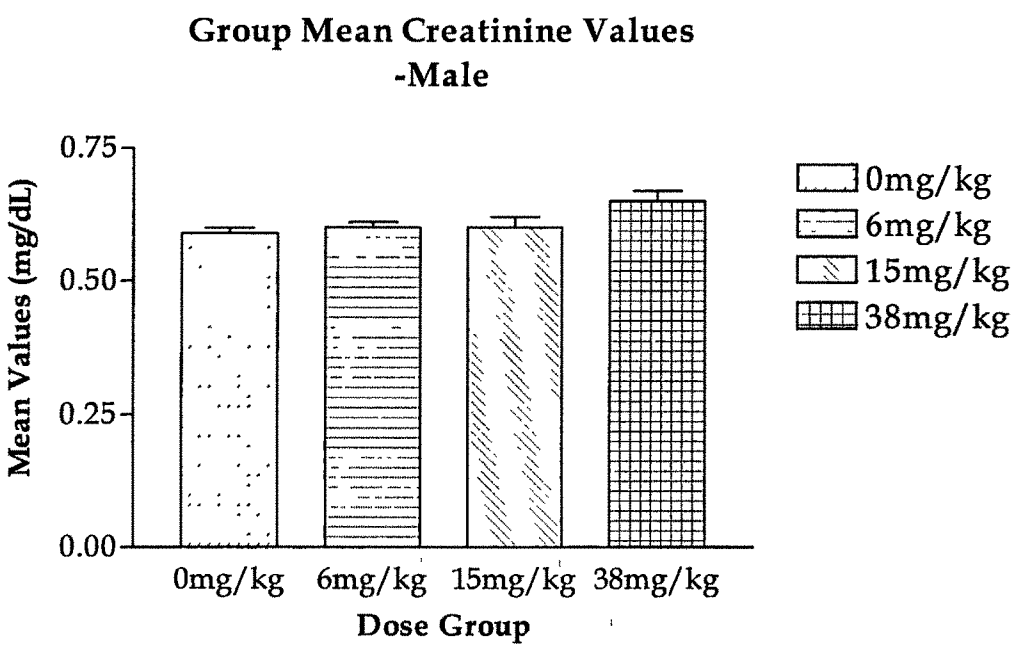


Figure-2.12



Repeated Dose 90-Day Neurotoxicity Study in Rats

Table-3.18

Effect of Insecticide Combination on Clinical Chemistry Parameters – Female

Parameter	Dose (mg/kg body weight/day)			
	0 (N=10)	6 (N=10)	15 (N=10)	38 (N=10)
GLU (mg/dL)	86.66 ± 3.35	98.01 ± 5.69	105.43 ± 4.77*↑	106.11 ± 2.41*↑
ALT (IU/L)	70.77 ± 5.06	71.57 ±3.59	71.16 ± 3.15	75.57 ± 3.63
AST (IU/L)	254.34 ± 12.95	243.95 ± 15.81	222.52 ± 10.94	255.09 ± 10.50
GGT (IU/L)	6.66 ± 0.52	7.76 ± 0.64	7.97 ± 0.86	8.65 ± 0.63
T.BIL (mg/dL)	0.23 ± 0.02	0.20 ± 0.01	0.20 ± 0.01	0.23 ± 0.01
Creatinine (mg/dL)	0.71 ± 0.03	0.73 ± 0.03	0.72 ± 0.02	0.77 ± 0.03
BUN (mg/dL)	28.39 ± 1.30	28.00 ± 1.08	27.46 ± 0.43	30.44 ± 1.82
T.Protein (g/dL)	8.20 ± 0.09	8.92 ± 0.15*↑	9.13 ± 0.13*↑	8.95 ± 0.13*↑
ALB (g/dL)	3.97 ± 0.09	4.23 ± 0.06	4.19 ± 0.09	4.23 ± 0.10
Cholesterol (mg/dL)	71.70 ± 4.58	78.63 ± 4.01	72.53 ± 3.39	68.45 ± 3.65
Urea (mg/dL)	60.80 ± 2.78	59.8 ± 2.27	58.7 ± 0.93	65.2 ± 3.86
ChE(IU/L)	1146.4 ± 125.71	755.7 ± 99.67*↓	681.4 ± 59.83*↓	316.4 ± 38.98*↓
ALP (IU/L)	122.88 ± 9.78	130.56 ± 7.31	136.37 ± 6.70	142.71 ± 4.18
Cl (mEq/L)	127.11 ± 5.80	136.49 ± 8.01	121.59 ± 1.88	138.86 ± 13.46
Na (mEq/L)	140.0 ± 1.82	138.4 ± 1.84	135.6 ± 1.15	134.4 ± 1.46*↓
K (mEq/L)	4.58 ± 0.08	4.58 ± 0.10	4.45 ± 0.14	4.50 ± 0.10

Note: Values are expressed as Mean ± SE
*↓ = Significantly lower than control (P ≤ 0.05),
*↑ = Significantly higher than control (P ≤ 0.05)

Figure-2.13

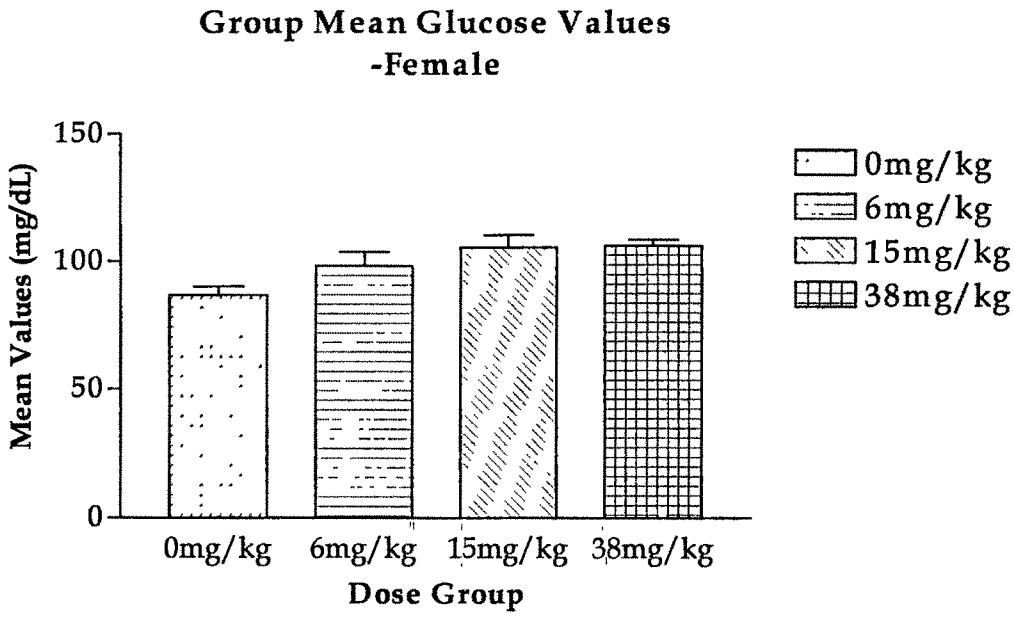


Figure-2.14

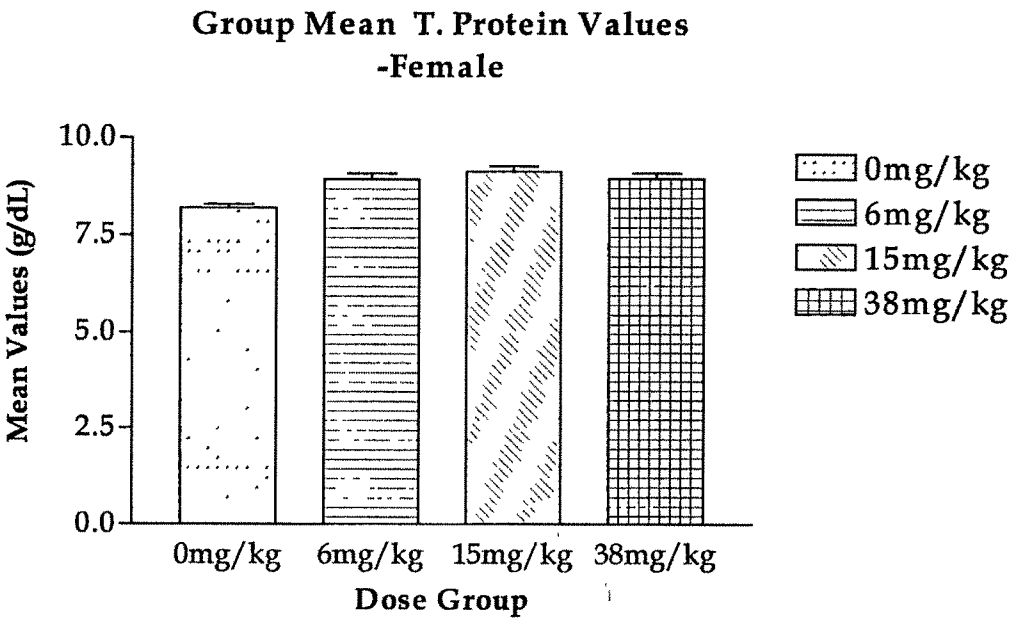


Figure-2.15

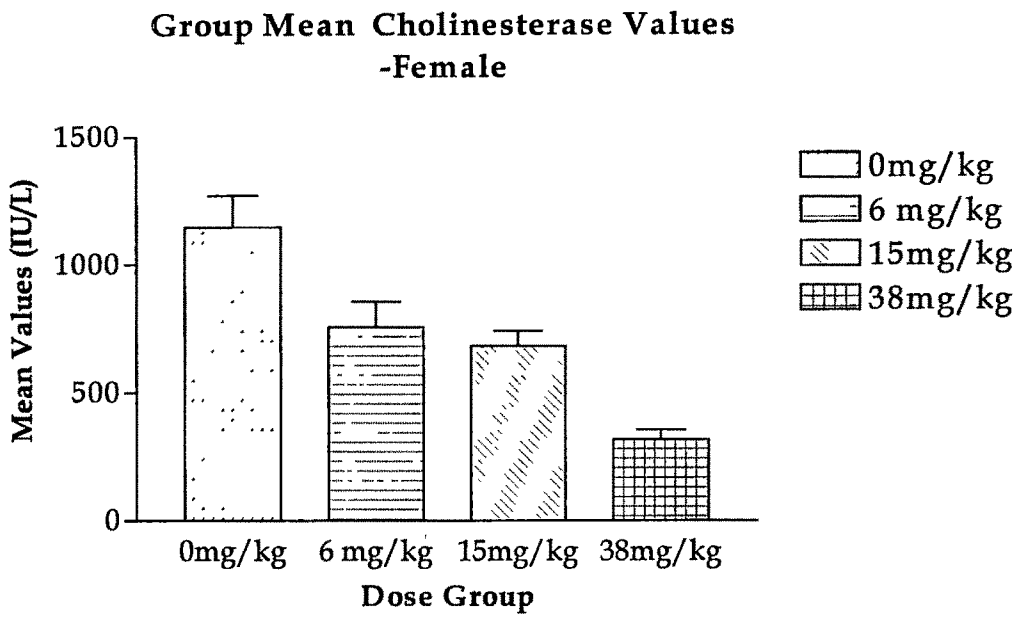
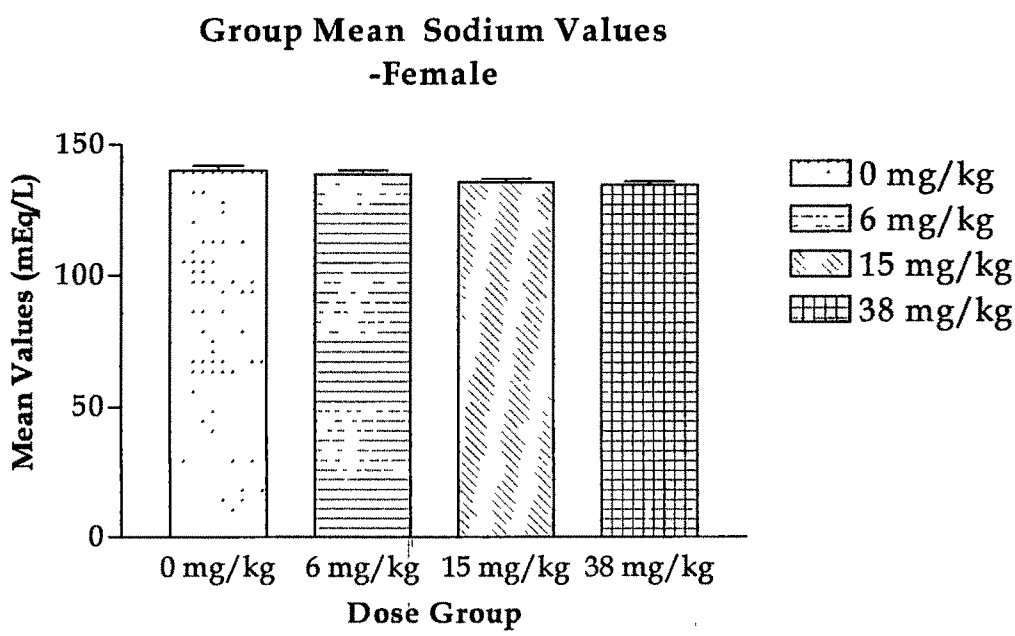


Figure 2.16



Repeated Dose 90-Day Neurotoxicity Study in Rats

Table-3.19

Effect of Insecticide Combination on Clinical Chemistry Parameters – Recovery Group (Male)

Parameters	Dose (mg/kg body weight/day)	
	0 (N=9)	38 (N=8)
GLU (mg/dL)	91.57 ± 3.71	123.09 ± 8.77*↑
ALT (IU/L)	90.35 ± 4.96	80.03 ± 4.11
AST (IU/L)	218.50 ± 9.51	220.79 ± 10.16
GGT (IU/L)	3.81 ± 0.98	3.06 ± 0.64
T.BIL (mg/dL)	0.05 ± 0.01	0.08 ± 0.01
Creatinine (mg/dL)	0.74 ± 0.01	0.75 ± 0.02
BUN (mg/dL)	24.26 ± 0.55	23.36 ± 0.49
T.Protein(g/dL)	8.42 ± 0.06	8.73 ± 0.16*↑
ALB (d/dL)	3.67 ± 0.04	3.94 ± 0.07
Cholesterol (mg/dL)	70.20 ±2.32	68.56 ± 2.96
Urea (mg/dL)	50.78 ± 1.05	50.00 ± 1.07
ChE(IU/L)	212.78 ± 26.46	277.75 ± 24.25
ALP (IU/L)	123.84 ± 11.72	137.53 ± 7.86
Cl (mEq/L)	109.61 ± 2.16	112.01 ± 4.81
Na (mEq/L)	135.90 ± 2.25	131.48 ± 1.56
K (mEq/L)	4.36 ± 0.25	3.94 ± 0.19

Note: Values are expressed as Mean ± SE
*↓ = Significantly lower than control ($P \leq 0.05$),
*↑ = Significantly higher than control ($P \leq 0.05$)

Repeated Dose 90-Day Neurotoxicity Study in Rats

Table-3.20

Effect of Insecticide Combination on Clinical Chemistry Parameters – Recovery Group (Female)

Parameter	Dose (mg/kg body weight/day)	
	0 (N=10)	38 (N=10)
GLU (mg/dL)	107.71 ± 4.28	126.09 ± 3.32*↑
ALT (IU/L)	75.96 ± 3.57	74.79 ± 5.12
AST (IU/L)	232.56 ± 6.61	248.93 ± 7.19
GGT (IU/L)	4.25 ± 1.29	3.94 ± 0.97
T.BIL (mg/dL)	0.16 ± 0.02	0.18 ± 0.02
Creatinine (mg/dL)	0.81 ± 0.02	0.84 ± 0.01
BUN (mg/dL)	23.21 ± 0.71	25.46 ± 1.46
T.Protein(g/dL)	8.99 ± 0.14	8.76 ± 0.11
ALB (d/dL)	4.32 ± 0.12	4.57 ± 0.07
Cholesterol (mg/dL)	69.36 ± 4.04	69.21 ± 2.32
Urea (mg/dL)	49.90 ± 1.49	54.40 ± 3.11
ChE(IU/L)	1393.50 ± 189.49	1131.80 ± 174.44
ALP (IU/L)	121.14 ± 5.17	130.74 ± 3.84
Cl (mEq/L)	105.25 ± 3.42	111.47 ± 2.27
Na (mEq/L)	135.52 ± 2.21	130.25 ± 3.98
K (mEq/L)	4.11 ± 0.16	3.96 ± 0.23

Note: Values are expressed as Mean ± SE
*↓ = Significantly lower than control ($P \leq 0.05$),
*↑ = Significantly higher than control ($P \leq 0.05$)

Repeated Dose 90-Day Neurotoxicity Study in Rats

Table-3.21

Effect of Insecticide Combination on Motor Activity - Male

Time Interval (Min)		Dose (mg/kg body weight/day)			
		0 (N=5)	6 (N=5)	15 (N=5)	38 (N=5)
Total Activity					
Period	0 - 10	468.8 ± 142.71	681.6 ± 102.46	1263.8 ± 126.50*↑	1319.0 ± 50.99*↑
	11 - 20	434.2 ± 103.90	304.4 ± 83.90	497.4 ± 62.73	747.2 ± 55.89*↑
	21 - 30	226.4 ± 95.91	242.4 ± 106.84	423.6 ± 17.97	530.4 ± 126.52
Ambulatory Activity					
Period	0 - 10	383.0 ± 122.73	574.6 ± 91.93	1062.2 ± 116.69*↑	1118.2 ± 46.53*↑
	11 - 20	355.4 ± 90.23	245.0 ± 68.36	393.8 ± 54.45	629.0 ± 46.11*↑
	21 - 30	172.4 ± 83.37	190.4 ± 87.17	347.4 ± 17.70	430.6 ± 108.46
Stereotypic Activity					
Period	0 - 10	85.8 ± 21.42	107.0 ± 13.71	201.6 ± 11.82*↑	200.8 ± 15.91*↑
	11 - 20	78.8 ± 15.14	59.4 ± 16.05	103.6 ± 10.65	118.2 ± 10.20
	21 - 30	54.0 ± 13.82	52.0 ± 20.39	76.2 ± 4.12	99.8 ± 20.98

Note: Values are expressed as Mean ± SE
*↓ = Significantly lower than control ($P \leq 0.05$),
*↑ = Significantly higher than control ($P \leq 0.05$)

Figure-2.17

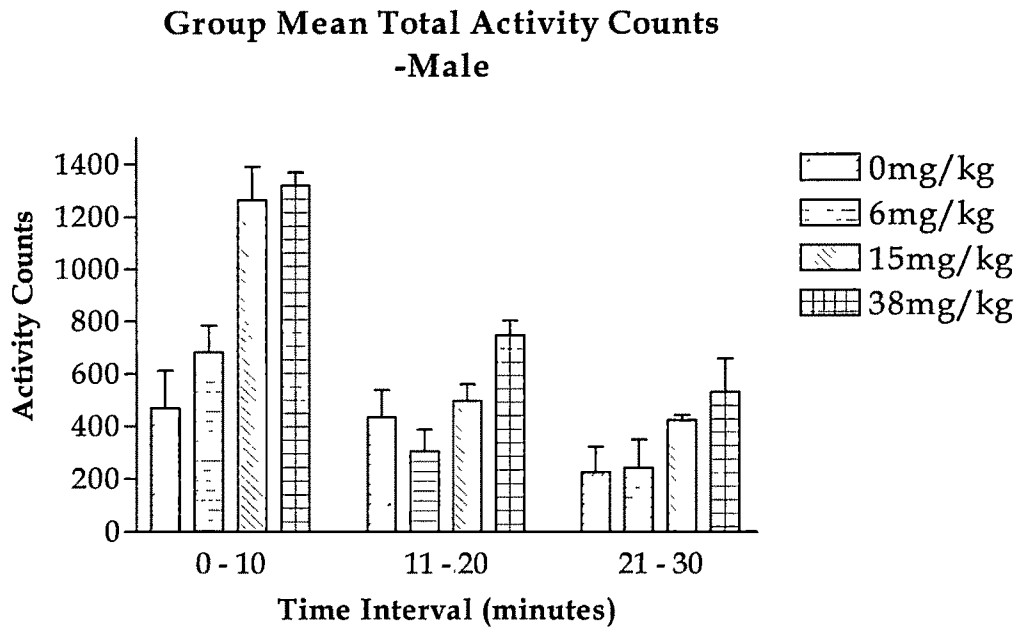


Figure 2.18

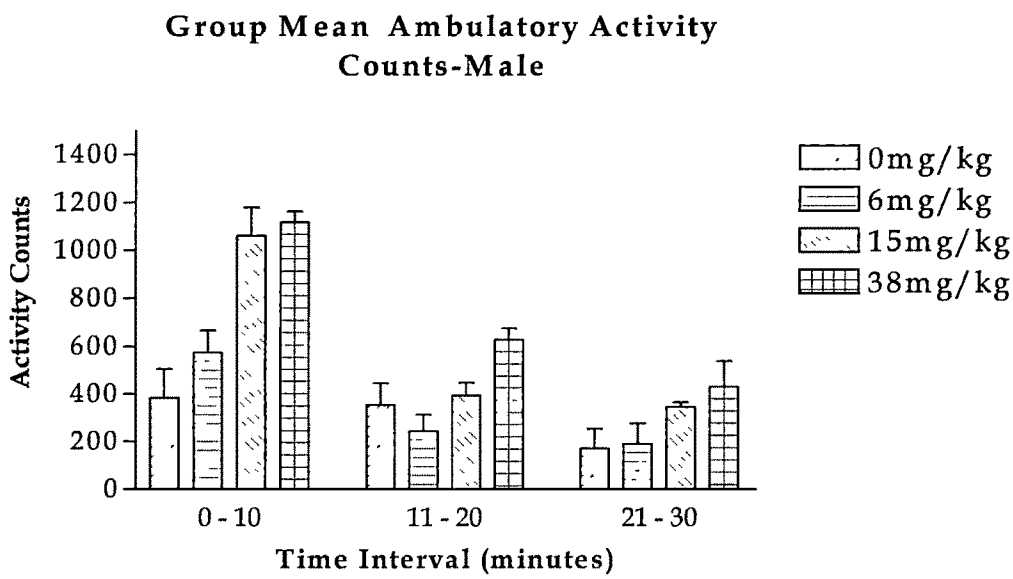
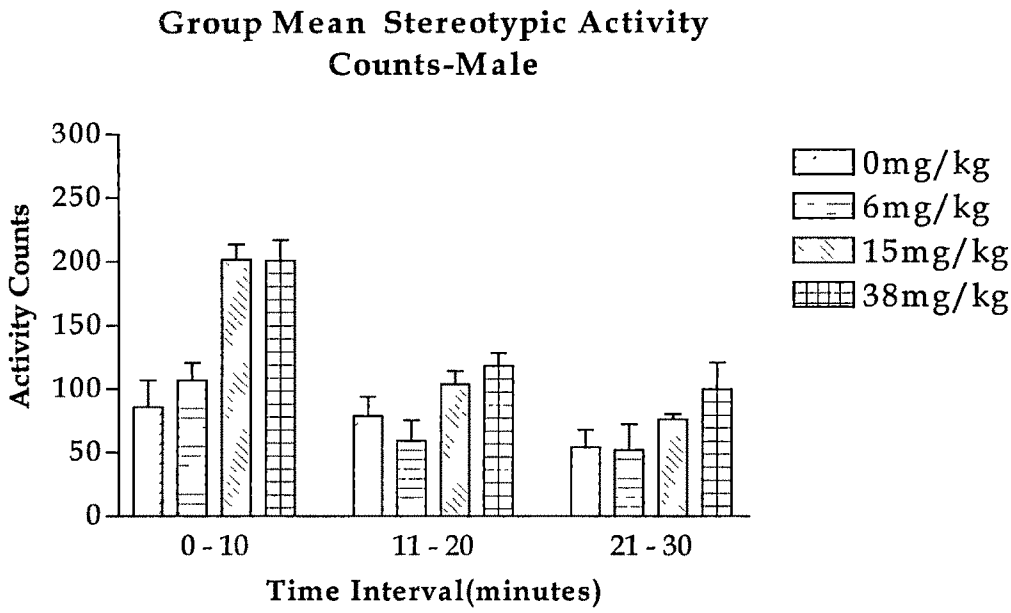


Figure-2.19


Group Mean Stereotypic Activity Counts-Male
Activity Counts
300
250
200
150
100
50
0
0 - 10
11 - 20
21 - 30
Time Interval(minutes)
0mg/kg
6mg/kg
15mg/kg
38mg/kg

Repeated Dose 90-Day Neurotoxicity Study in Rats

Table-3.22

Effect of Insecticide Combination on Motor Activity - Female

Time Interval (Min)		Dose (mg/kg body weight/day)			
		0 (N=5)	6 (N=5)	15 (N=5)	38 (N=5)
Total Activity					
Period	0 - 10	1382.6 ± 252.99	1270.2 ± 256.02	1381.0 ± 247.46	1554.4 ± 172.79
	11 - 20	702.2 ± 249.36	673.6 ± 137.89	798.4 ± 133.18	896.8 ± 350.40
	21 - 30	659.4 ± 235.87	293.2 ± 91.65	627.0 ± 163.47	563.2 ± 235.39
Ambulatory Activity					
Period	0 - 10	1175.0 ± 215.47	1073.0 ± 224.28	1195.2 ± 228.63	1331.6 ± 158.52
	11 - 20	600.0 ± 212.20	556.6 ± 122.95	665.4 ± 113.41	759.2 ± 306.49
	21 - 30	568.8 ± 201.35	231.2 ± 78.83	527.4 ± 145.01	478.6 ± 211.98
Stereotypic Activity					
Period	0 - 10	207.6 ± 38.01	197.2 ± 32.04	185.8 ± 20.25	222.8 ± 19.55
	11 - 20	102.2 ± 37.39	117.0 ± 18.77	133.0 ± 20.99	137.6 ± 44.24
	21 - 30	90.6 ± 34.82	62.0 ± 14.21	99.6 ± 19.64	84.6 ± 23.71

Note: Values are expressed as Mean ± SE

Figure-2.20

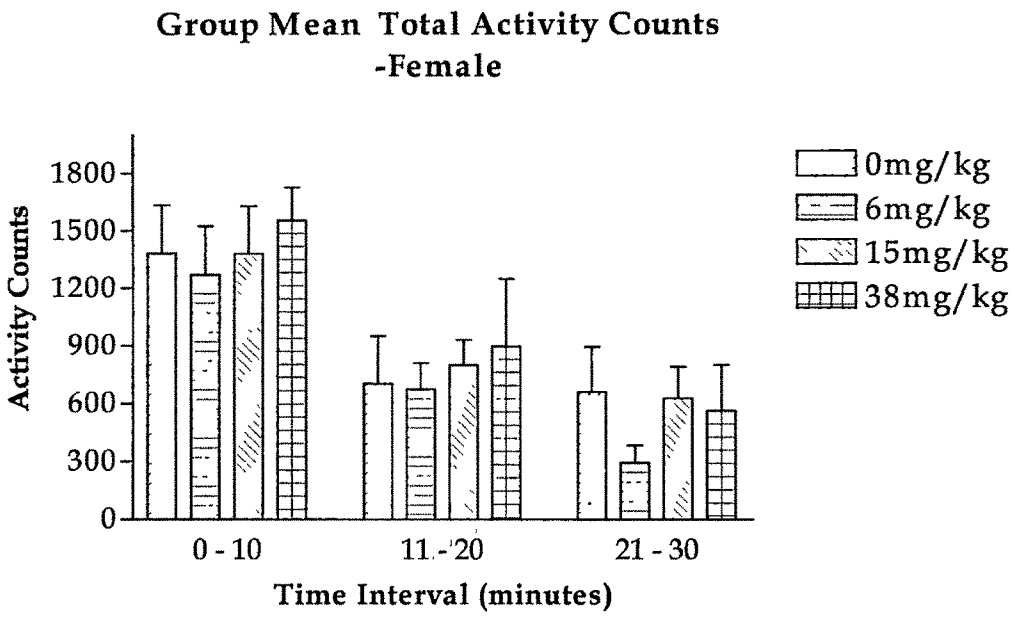


Figure 2.21

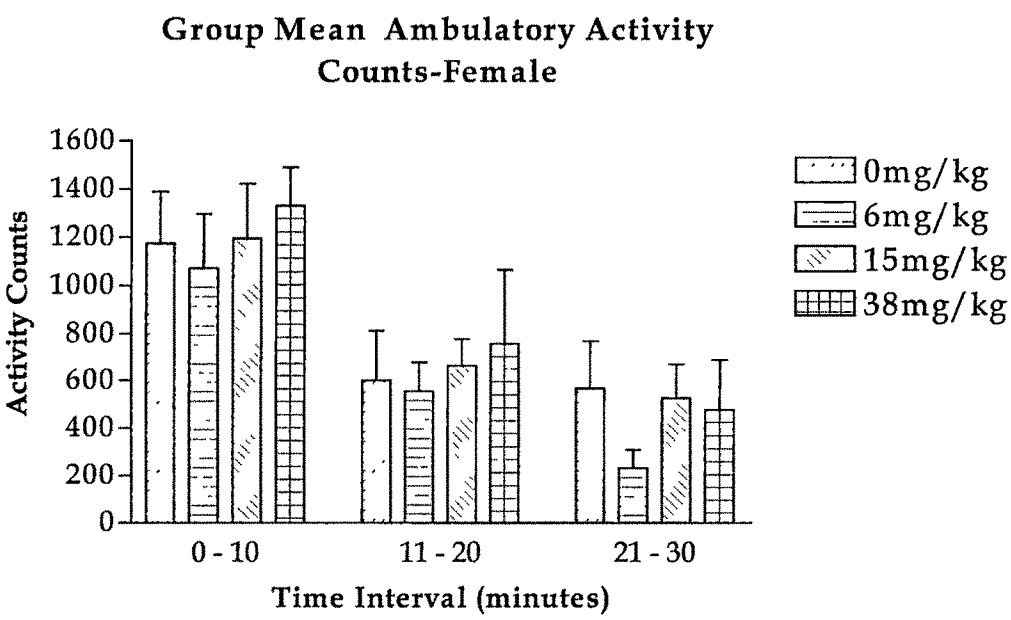
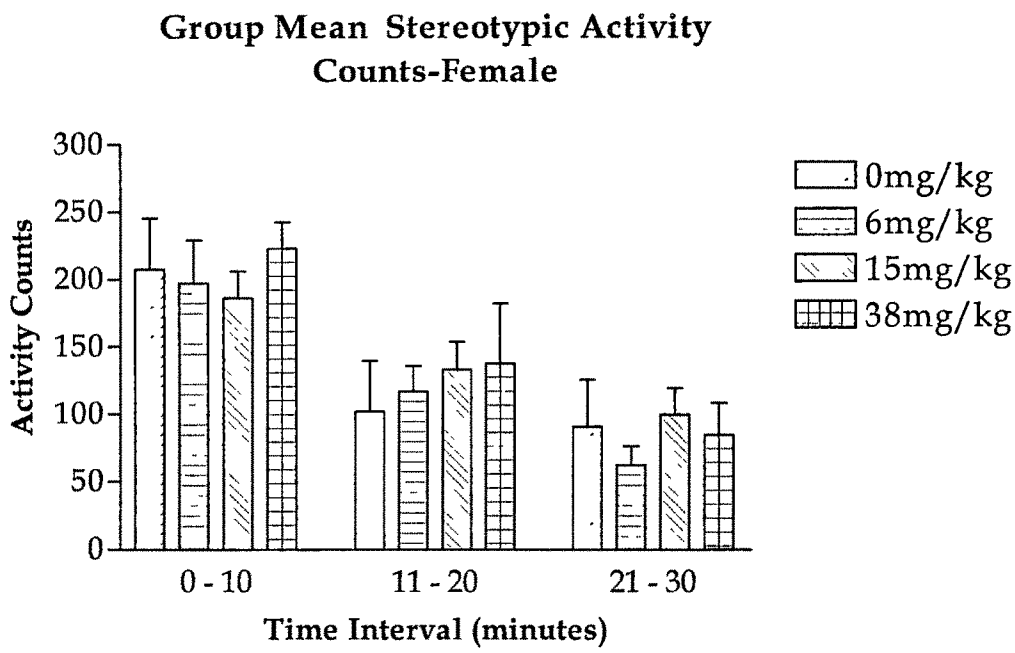


Figure-2.22


Group Mean Stereotypic Activity Counts-Female
Activity Counts
300
250
200
150
100
50
0
0 - 10
11 - 20
21 - 30
Time Interval (minutes)
0mg/kg
6mg/kg
15mg/kg
38mg/kg

Repeated Dose 90-Day Neurotoxicity Study in Rats

Table-3.23

Effect of Insecticide Combination on Grip strength (g) - Male

Parameter	Dose (mg/kg body weight/day)			
	0 (N=5)	6 (N=5)	15 (N=5)	38 (N=5)
Fore limb	1056 ± 15.11	946.6 ± 39.10	966 ± 35.34	994.4 ± 59.91
Hind limb	760.4 ± 16.56	684.8 ± 41.05	795.8 ± 77.35	656.6 ± 35.90

Note: Values are expressed as Mean ± SE

Figure 2.23

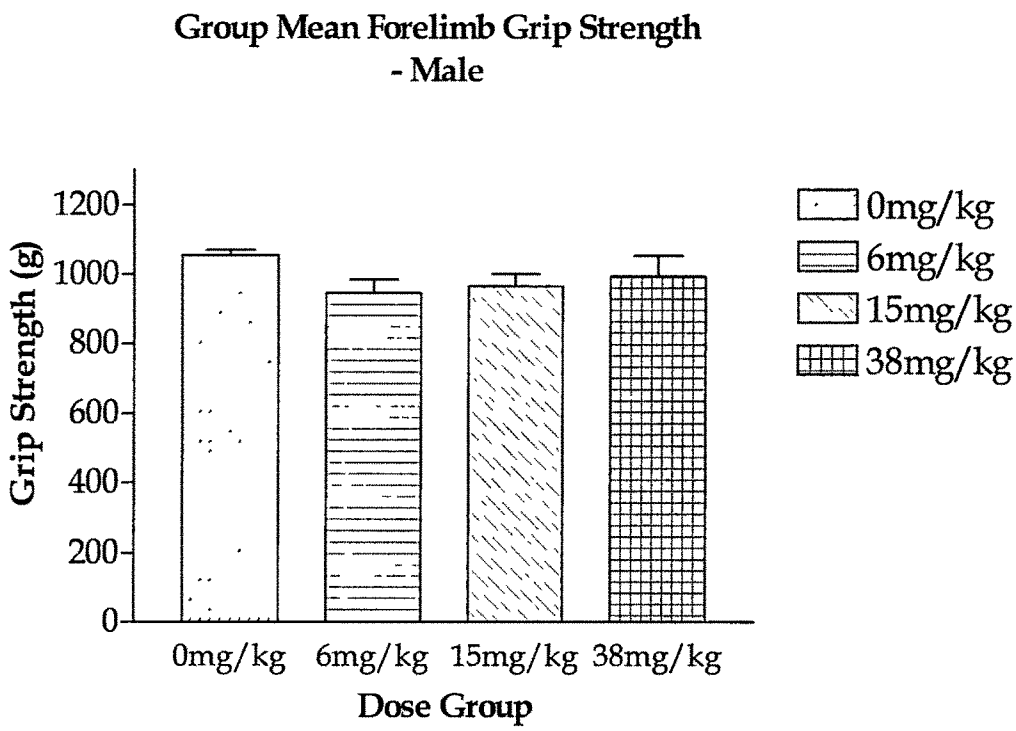
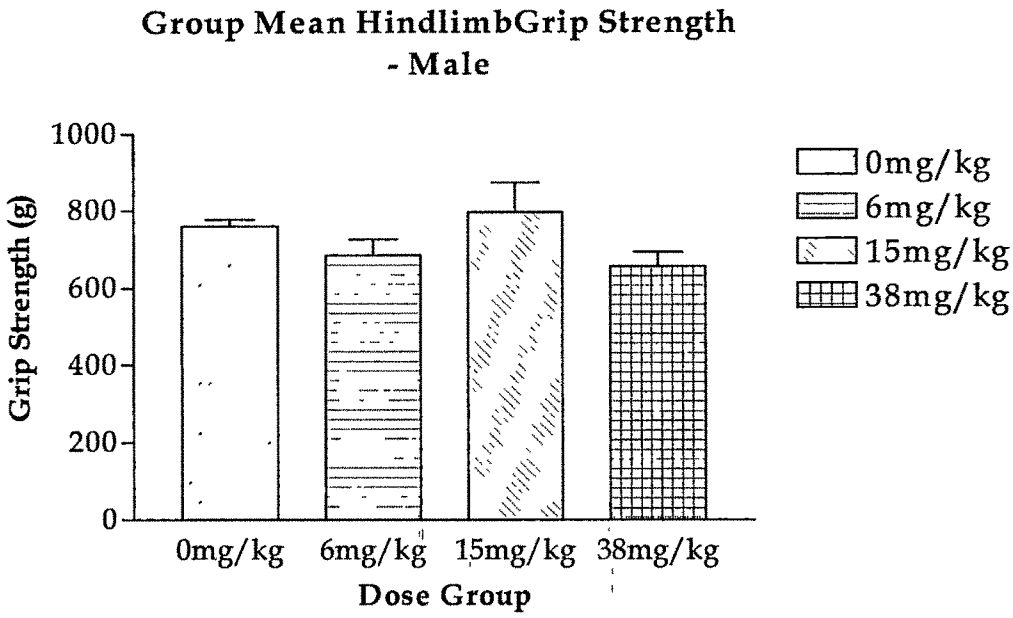


Figure 2.24


Group Mean HindlimbGrip Strength
- Male
Grip Strength (g)
1000
800
600
400
200
0
0mg/kg
6mg/kg
15mg/kg
38mg/kg
Dose Group
0mg/kg
6mg/kg
15mg/kg
38mg/kg

Repeated Dose 90-Day Neurotoxicity Study in Rats

Table-3.24

Effect of Insecticide Combination on Grip strength (g) - Female

Parameter	Dose (mg/kg body weight/day))			
	0 (N=5)	6 (N=5)	15 (N=5)	38 (N=5)
Fore limb	932.6 ± 43.82	915.8 ± 44.79	853.2 ± 74.49	937.6 ± 59.30
Hind limb	523.2 ± 44.77	549 ± 25.62	574 ± 66.52	670 ± 15.93

Note: Values are expressed as Mean ± SE

Figure 2.25

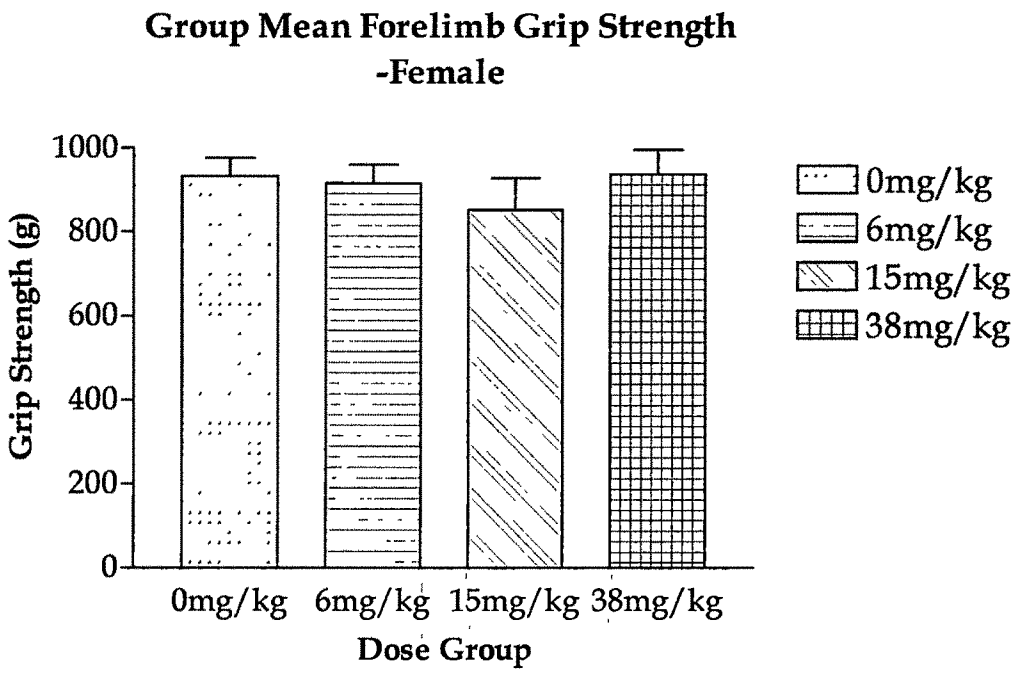
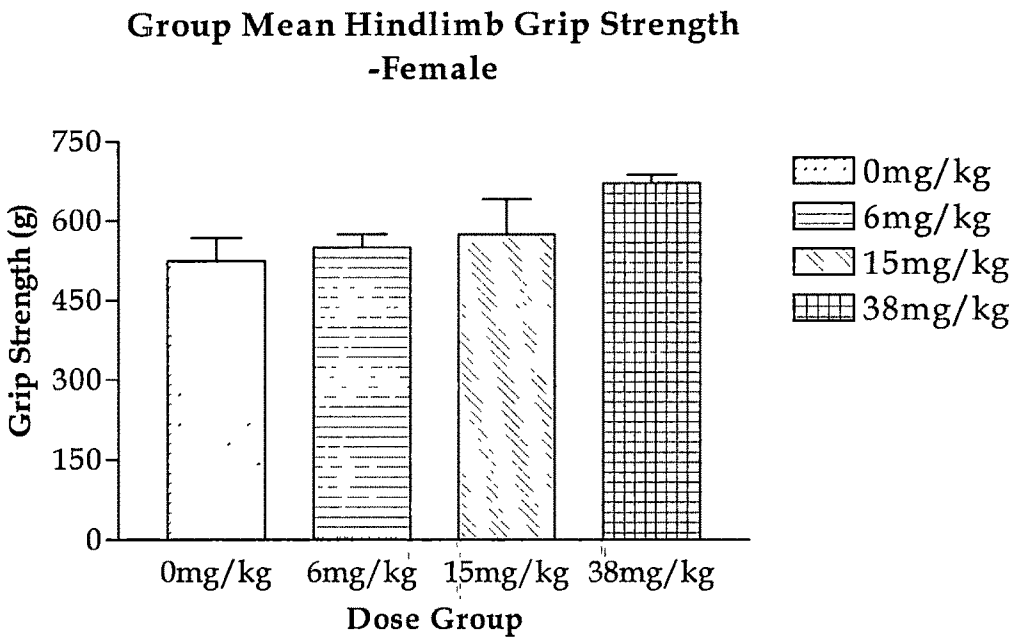


Figure 2.26


Group Mean Hindlimb Grip Strength
-Female
Grip Strength (g)
750
600
450
300
150
0
0mg/kg
6mg/kg
15mg/kg
38mg/kg
Dose Group
0mg/kg
6mg/kg
15mg/kg
38mg/kg

Repeated Dose 90-Day Neurotoxicity Study in Rats

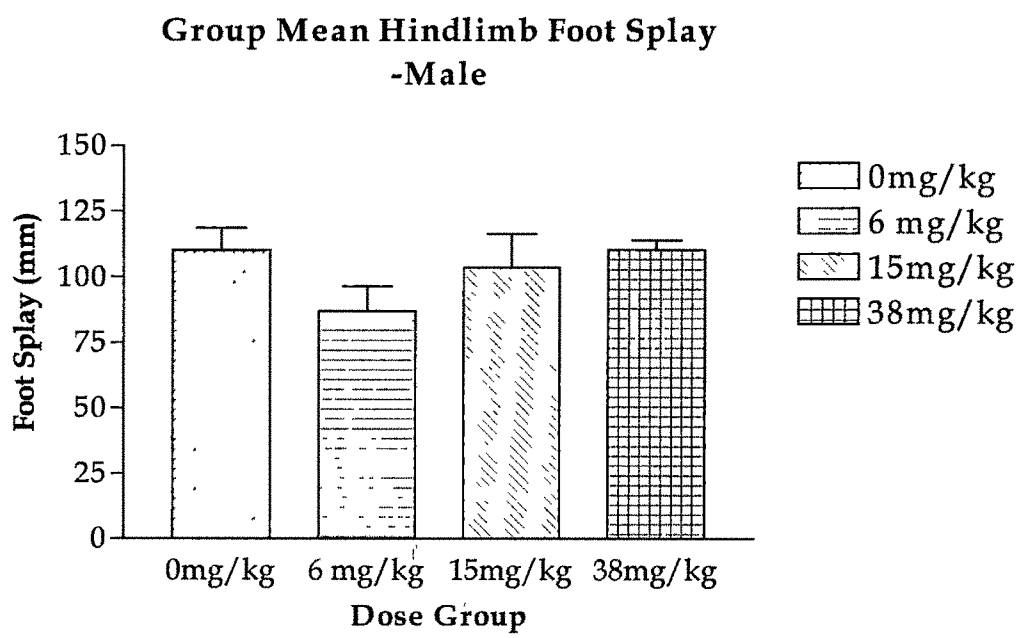
Table-3.25

Effects of Insecticide Combination on Hind limb Foot splay (mm) - Male

Parameter	Dose (mg/kg body weight/day)			
	0 (N=5)	6 (N=5)	15 (N=5)	38 (N=5)
Hind limb Foot splay	110.2 ± 8.43	87 ± 9.49	103.8 ± 12.61	110.4 ± 3.73

Note: Values are expressed as Mean ± SE

Figure 2.27



Repeated Dose 90-Day Neurotoxicity Study in Rats

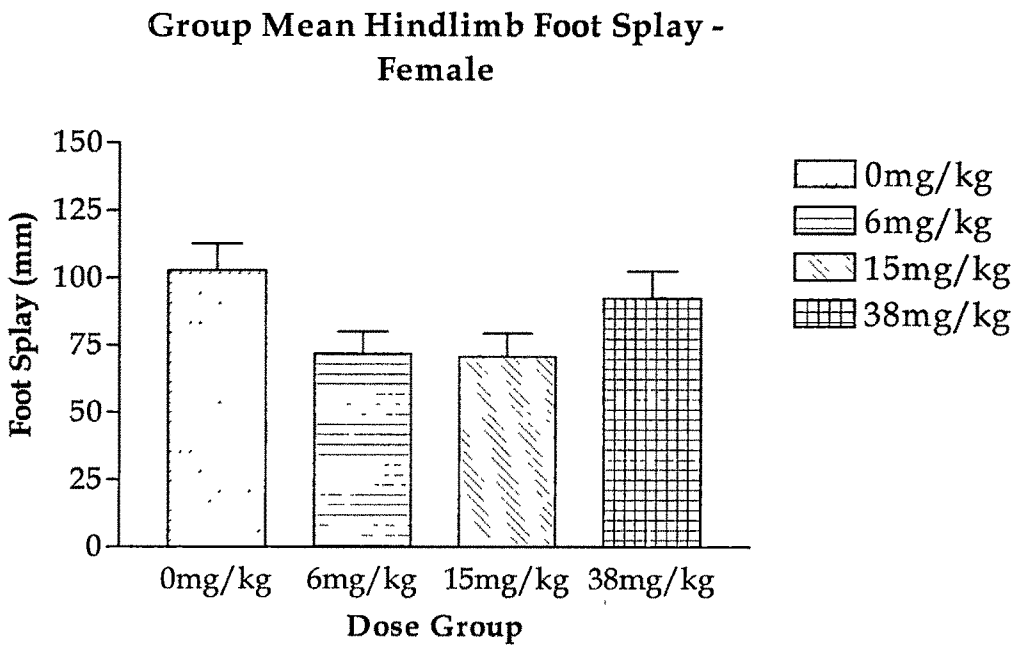
Table-3.26

Effect of Insecticide Combination on Hind limb Foot splays (mm)- Female

Parameter	Dose (mg/kg body weight/day)			
	0	6	15	38
Hind limb Foot splay	102.8 ± 10 07	71.8 ± 8 43	70.8 ± 8.58	92.4 ± 10.11

Note: Values are expressed as Mean ± SE

Figure 2.28



Repeated Dose 90-Day Neurotoxicity Study in Rats

Table-3.27

Effect of Insecticide Combination on Absolute Organ Weight (g) - Recovery Group (Male)

Parameter	Dose (mg/kg body weight/day)	
	0 (N=9)	38 (N=8)
Body Weight	454.67 ± 15.04	480.00 ± 21.19
Adrenals	0.07 ± 0.00	0.07 ± 0.01
Testes	3.48 ± 0.17	3.40 ± 0.15
Epididymis	1.33 ± 0.04	1.30 ± 0.03
Kidneys	3.44 ± 0.12	3.41 ± 0.13
Brain	2.03 ± 0 05	2.11 ± 0.04
Heart	1.45 ± 0.06	1.45 ± 0.03
Spleen	1.39 ± 0.06	1.29 ± 0.07
Liver	16.30 ± 0.45	16.14 ± 0.66
Lungs	2.14 ± 0.08	2.39 ± 0.14
Thymus	0.34 ± 0.03	0.41 ± 0.05

Note: Values are expressed as Mean ± SE

Repeated Dose 90-Day Neurotoxicity Study in Rats

Table-3.28

Effect of Insecticide Combination on Absolute Organ Weight (g) - Recovery Group (Female)

Parameter	Dose (mg/kg body weight/day)	
	0 (N=10)	38 (N=10)
Body Weight	242.20 ± 4.18	251.40 ± 5.31
Adrenals	0.07 ± 0.00	0.06 ± 0.00
Ovary	0.09 ± 0.01	0.10 ± 0.01
Uterus	0.60 ± 0.05	0.61 ± 0.04
Kidneys	1.69 ± 0.04	1.76 ± 0.04
Brain	1.81 ± 0.03	1.77 ± 0.02
Heart	0.91 ± 0.03	0.94 ± 0.02
Spleen	0.97 ± 0.03	0.91 ± 0.04
Liver	7.89 ± 0.30	8.41 ± 0.29
Lungs	1.68 ± 0.08	1.72 ± 0.10
Thymus	0.30 ± 0.02	0.30 ± 0.04

Note: Values are expressed as Mean ± SE

Repeated Dose 90-Day Neurotoxicity Study in Rats

Table-3.29

Effect of Insecticide Combination on Relative Organ Weight (%)- Recovery Group (Male)

Parameter	Dose (mg/kg body weight/day)	
	0 (N=9)	38 (N=8)
Adrenals	0.016 ± 0.001	0.014 ± 0.001*↓
Testes	0.771 ± 0.039	0.725 ± 0.059
Epididymis	0.295 ± 0 010	0.276 ± 0.014
Kidneys	0.758 ± 0 017	0.716 ± 0.026
Brain	0.448 ± 0.009	0.445 ± 0.018
Heart	0.319 ± 0.009	0.304 ± 0.010
Spleen	0.307 ± 0.015	0.274 ± 0.021
Liver	3.598 ± 0.091	3.371 ± 0.073
Lungs	0.473 ± 0.016	0.503 ± 0.029
Thymus	0.075 ± 0.006	0.084 ± 0.009

Note: Values are expressed as Mean ± SE
*↓ = Significantly lower than control ($P \leq 0.05$)

Repeated Dose 90-Day Neurotoxicity Study in Rats

Table-3.30

Effect of Insecticide Combination on Relative Organ Weight (%)- Recovery Group (Female)

Parameter	Dose (mg/kg body weight/day)	
	0 (N=10)	38 (N=10)
Adrenals	0.027 ± 0.001	0.025 ± 0.001
Testes	0.039 ± 0.003	0.042 ± 0.004
Epididymis	0.248 ± 0.018	0.243 ± 0.016
Kidneys	0.700 ± 0.018	0.704 ± 0.025
Brain	0.752 ± 0.019	0.707 ± 0.019
Heart	0.375 ± 0 014	0.377 ± 0.012
Spleen	0.403 ± 0.012	0.362 ± 0.018
Liver	3.258 ± 0.106	3.356 ± 0.122
Lungs	0.691 ± 0.026	0.688 ± 0.042
Thymus	0.124 ± 0.010	0.122 ± 0.018

Note: Values are expressed as Mean ± SE

3.4 Developmental Neurotoxicity of Combination Insecticide in Rats

Following 90 days of test article administration, males and females in the main groups (*i.e.,* G1, G2, G3 and G4) were kept for cohabitation at 1:2 ratios. Treatment was continued during their mating, during resultant pregnancies, lactation and through the weaning of F_1 offspring.

Various reproductive parameters were investigated during mating, gestation and lactation period. Brief descriptions of the same were as follows:

3.4.1 Estrous Cycle:

Females from control and test article treated groups showed normal estrous cyclic phases.

3.4.2 Gestation Body Weights: Reference Table: 4.1

On gestation days 0, 7, 14 and 20 mean body weights of dams from treatment groups were comparable to control group.

3.4.3 Gestation Food Consumption: Reference Table: 4.3

No statistically significant difference was observed in average food consumption of test article treated group dams during gestation days 0-7, 7-14 and 14-20 as compared to control group.

3.4.4 Lactation Body Weights: Reference Table: 4.2

Mean body weights of dams from test article treated groups on lactation days 0, 4, 7, 14 and 21 were comparable to control group.

3.4.5 Lactation Food Consumption: Reference Table: 4.4

Average food consumption of treatment group dams during lactation periods 0-4, 4-7, 7-14 and 14-21 were comparable to control.

3.4.6 Fertility Index: Reference Table: 4.5

Fertility indices in treatment groups were 100 %, 85 % and 90 % in 6, 15 and 38 mg/kg body weight dose group as compared to 100 % in control group. No significant association was noticed between incidences.

3.4.7 Mating Index: Reference Table: 4.5

Mating indices were 100%, 95% and 100% for 6, 15 and 38 mg/kg body weight dose groups respectively as compared to 100 % in control group. No significant association was noticed between incidence and dose.

3.4.8 Gestation Index: Reference Table: 4.5

No dose dependent or significant variation was observed in gestation index of test article treated animals (i.e., 95 %, 89.47% and 90 % for 6, 15 and 38 mg/kg body weight dose groups, respectively) as compared to control (100%).

3.4.9 Lactation Index Reference Table: 4.5

There is a significant reduction in lactation index of high dose (38 mg/kg body weight) group (75.27%) as compared to control group (79.72 %).

3.4.10 Live Birth Index: Reference Table: 4.12

Live birth indices of control group (97.7%) and test article treated groups (*i.e.*, 98.62%, 95.56% and 97.85% for 6, 15 and 38 mg/kg body weight dose groups, respectively) were comparable.

3.4.11 Duration of Gestation: Reference Table: 4.5

Mean duration of gestation in treatment group dams was comparable to control group.

3.4.12 Litter Data:

Survival Index: Reference Tables: 4.5 and 4.6

Reduced survival indices were noticed in 38 mg/kg body weight dose group on days 4 (86.81%), 7 (84.62%) and 14 (82.97%) as compared to control group (96.23%, 92.45 % and 89.15 %).

Mean Litter Size (number):

Male + Female: Reference Tables: 4.13 and 4.14

The mean litter size of was comparatively less in high dose (38 mg/kg body weight) group than control during day 0, 4, 7, 14 and 21.

Male Reference Table: 4.15

Average litter size (number) of male pups in high dose (38 mg/kg body weight) group was comparatively low on day 0, 4, 7, 14 and 21 of lactation.

Female Reference Table: 4.16

Similar to male pups, average litter size of female pups was observed in high dose (38 mg/kg body weight) group during day 0, 4, 7, 14 and 21.

Mean Litter Weight:

Male and Female: Reference Table: 4.18

Average litter weight of male and female pups from test article treated groups during lactation days 0, 4, 7, 14 and 21 were comparable to control group.

Male Reference Table: 4.19

Average litter weight of male from test article treated groups during lactation days 0, 4, 7, 14 and 21 were comparable to control group.

Female Reference Table: 4.20

Like males, average litter weights of female pups in treatment group were comparable to control group.

Mortality Index

Male+ Female: Reference Table: 4.9

Per cent pup mortality was considerably high (13.19 %) in 38 mg/kg body weight dose group during lactation period day 1- day 4 as compared to control group (3.77%).

Male: Reference Table: 4.10

Significantly higher percent (12.64%) mortality was observed during lactation period day 1- day 4 for male pups at 38 mg/kg body weight dose group as compared to control group (4.55%).

Female: Reference Table: 4.11

During lactation period, day 0-4 percent mortality of female was significantly higher (12.64%) at 38 mg/kg body weight dose group as compared to control group (4.55%).

3.4.13 Absolute Organ Weights - Pups:

Male: Reference Tables: 4.27

Absolute weights of spleen (0.20g) and liver (2.11g) were significantly increased in 38 mg/kg dose group as compared to control group (0.15g and 1.67g).

Female: Reference Tables: 4.28

Absolute weights of liver in 15 (1.93g) and 38 (1.94 g) mg/kg dose group were significantly increased as compared to control group (1.57g).

3.4.14 Relative Organ weights-Pups:

Male: Reference Tables: 4.29

Relative weight of kidney (1.69%), spleen (0.62%) and liver (6.59%) were significantly increased in 38 mg/kg dose group as compared to control group (1.42, 0.44 and 4.98%).

Female: Reference Tables: 4.30

Relative weight of kidney (1.78%) and liver (6.40%) were significantly increased in 38mg/kg dose group as compared to control group (1.52 and 5.00%).

3.4.15 Organ to Brain Weight Ratio - Pups:

Male: Reference Tables: 4.31

Organ to brain weight ratio of spleen (13.42%) and liver (144.66%) in 38 mg/kg body weight dose group pups were significantly higher as compared to control (9.86% and 112.67%)

Female: Reference Tables: 4.32

Organ to brain weight ratio of kidney (39.22% and 38.78%) at 15 and 38 mg/kg dose groups were significantly increased as compared to control group (33.03%). Relative brain weight of liver (135.52% and 138.04%) from 15 and 38 mg/kg body weight dose group pups were significantly higher than control (109.05%).

3.4.16 Organ Weights - Parents

Male Reference Tables: 4.21, 4.22 and 4.23

Absolute as well as relative weights of organs from test article treated group parental males were comparable to control group. No significant variation was noticed in any of the dose groups.

Female Reference Tables: 4.24, 4.25 and 4.26

Like males, test article treated females (dams) also did not show any significant difference in absolute and relative weights of organs weighed at terminal necropsy.

3.4.17 Pathology

3.4.17.1 Gross Pathology - Parents Reference Table: 4.33 and 4.34

The gross pathology of the animals treated at 38 mg/kg body weight dose group showed varying degrees of different lesions in liver (hyperemic, congestion, consolidation, mottling, pin point/ petechial hemorrhages), spleen (diffused white foci, congestion, atrophy and enlarged), kidneys (pallor and congestion), lungs (diffused pneumonic foci, edematous and

hemorrhages), Uterus (thickened wall with abscess formation of pyometra and hydrometra) and intestine (hyperemic).

3.4.17.2 Gross Pathology – Pups Reference Tables. 4.34

The gross pathology of the pups from 38 mg/kg body weight dose group showed varying degrees of different lesions in liver (congestion, mottling, consolidation and mottling), spleen (enlarged and hyperemic), kidneys (pallor and congestion), lungs (sub plural hemorrhages, diffused pneumonic foci and hemorrhage).

3.4.17.3 Histopathology – Parents Reference Tables: 4.35 and 4.36

Histopathology of organs from control and test article treated groups revealed lesions in **liver** (congestion, minimal fatty change, centrilobular hypertrophy, multi focal degeneration and necrotic foci), **kidney** (cortical tubular dilatation with flattened epithelium, congestion and multifocal regenerating tubules), **nerve** (demyelination, vacuolation and axonal degeneration), **brain** (inflammatory cell infiltration and gliosis), **ovary** (atretic follicle), and **thymus** (atrophy and epithiolisation).

3.4.17.4 Histopathology –Pups Reference Tables: 4.37

Microscopic examination of different organs from test article treated group pups showed lesions in **brain** (multiple pale acellular areas, gliosis, demyelination), **liver** (cystic tubules, extramedullary hematopoisis, sinusoidal dilation, vacoulation and congestion, sinusoidal dilation), **thymus** (atrophy characterized by epithiolisation), **kidney** (tubular degeneration with flattened epithelium) and **spleen** (extramedullary hematopoesis, megalokaryocytosis).

Developmental Neurotoxicity Study in Rats

Table-4.1

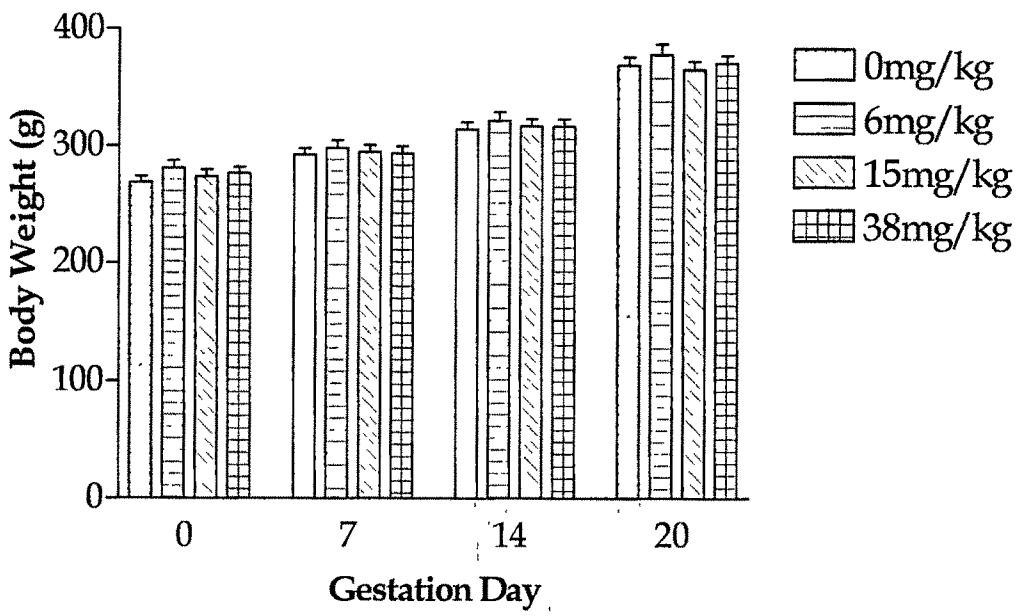
Effect of Insecticide Combination on Gestation Body Weights (g)

Gestation Day	Dose (mg/kg b. wt/day)			
	0 (N=17)	6 (N=19)	15 (N=16)	38 (N=20)
0	268.94 ± 4.83	281.05 ± 6.44	273.50 ± 6.15	276.40 ± 5.32
7	292.65 ± 5.11	298.11 ± 6.77	294.75 ± 6.17	293.90 ± 5.85
14	314.12 ± 5.88	321.63 ± 7.34	316.94 ± 6.29	316.50 ± 6.63
20	368.94 ± 7.35	377.79 ± 9.34	365.06 ± 7.26	370.40 ± 6.91

Note: Values are expressed as Mean ± SE

Figure 3.1

Group Mean Gestation Body Weight



Developmental Neurotoxicity Study in Wistar Rats

Table-4.2

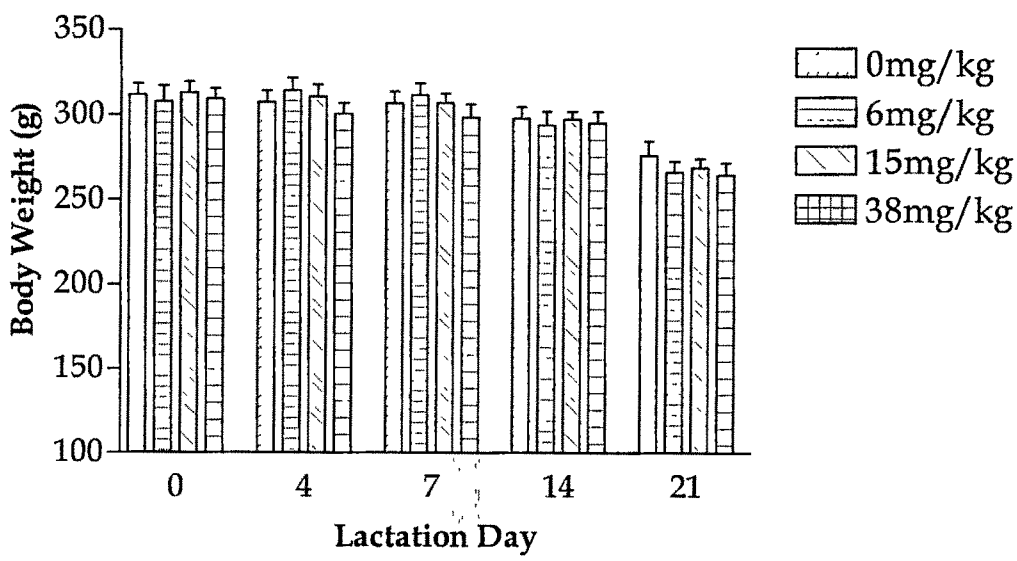
Effect of Insecticide Combination on Lactation Body Weights (g)

Lactation Day	Dose (mg/kg b. wt/day)			
	0 (N=19)	6 (N=17)	15 (N=16)	38 (N=16)
0	311.84 ± 6.66	307.74 ± 9.54	312.94 ± 6.59	309.38 ± 6.24
4	307.68 ± 6.73	314.42 ± 7.37	310.75 ± 7.24	300.69 ± 6.20
7	306.68 ± 7.07	311 79 ± 7.04	306.94 ± 5.63	298.25 ± 8.14
14	297.79 ± 6.84	294.05 ± 8.10	297.38 ± 4.81	295.38 ± 6.89
21	276.16 ± 8.47	266.32 ± 6.46	268.94 ± 5.42	264.50 ± 7.54

Note: Values are expressed as Mean ± SE

Figure 3.2

Group Mean Lactation Body Weight



Developmental Neurotoxicity Study in Rats

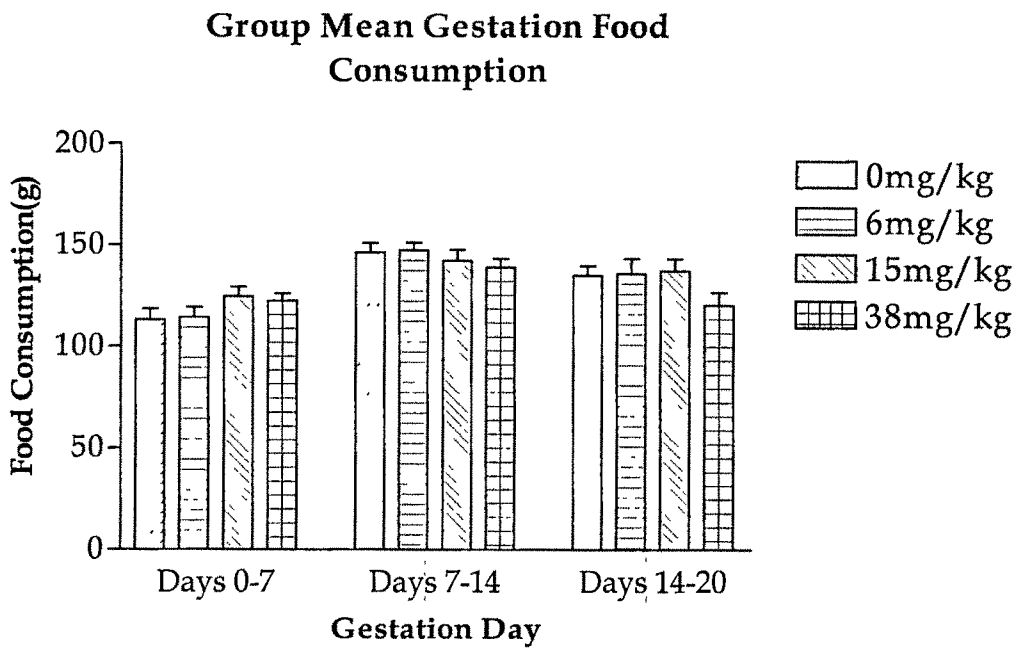
Table-4.3

Effect of Insecticide Combination on Gestation Food Consumption (g)

Gestation Period	Dose (mg/kg b. wt/day)			
	0 (N=17)	6 (N=19)	15 (N=17)	38 (N=20)
Days 0-7	113.18 ± 5.46	114.42 ± 4.86	124.5 ± 4.86	122.4 ± 3.68
Days 7-14	146.35 ± 4.67	147.37 ± 4.07	142.19 ± 5.59	139.1 ± 4.54
Days 14-20	135.06 ± 4.85	135.95 ± 7.52	137.44 ± 5.96	120.40 ± 6.30

Note: Values are expressed as Mean ± SE

Figure 3.3



Developmental Neurotoxicity Study in Rats

Table-4.4

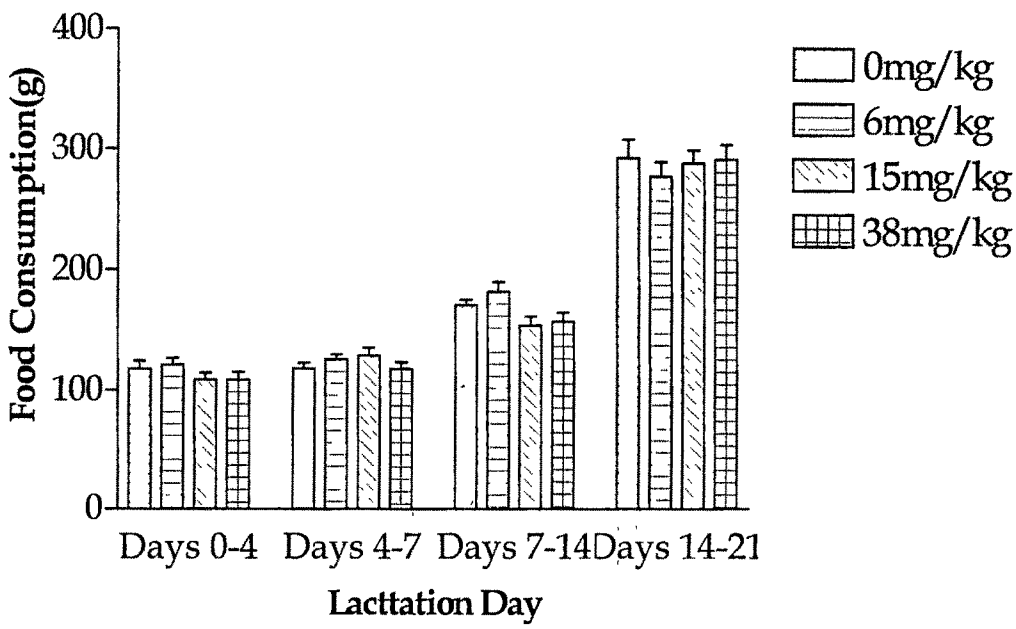
Effect of Insecticide Combination on Lactation Food Consumption (g)

Lactation Period	Dose (mg/kg b. wt/day)			
	0 (N=19)	6 (N=20)	15 (N=17)	38 (N=16)
Days 0-4	117.74 ± 7.03	121.63 ± 5.18	108.75 ± 6.02	108.69 ± 6.96
Days 4-7	118.00 ± 4.83	125.89 ± 3.84	128.50 ± 6.55	117.69 ± 5.68
Days 7-14	170.68 ± 4.47	181.89 ± 7.98	153.69 ± 7.96	157.06 ± 7.84
Days 14-21	292.53 ± 14.96	277.11 ± 12.04	288.13 ± 10.37	290.81 ± 12.37

Note: Values are expressed as Mean ± SE

Figure 3.4

Group Mean Lactation Food Consumption



Developmental Neurotoxicity Study in Rats

Table-4.5

Effect of Insecticide Combination on Fertility Parameters

Parameter	Dose (mg/kg b. wt/day)			
	0	6	15	38
N° of Males housed with females	10	10	10	10
N° of males impregnating females	10	10	10	10
N° of females housed with males	20	20	20	20
N° cohabitated females confirmed pregnant	20	19	17	18
N° of females with sperm +ve	20	20	19	20
N° of Females delivering young pups	20	19	17	18
N° of females giving birth to viable pups	19	19	17	18
N° of Live fetus on Day 0	212	214	172	182
N° of Live fetus on Day 21	169	160	137	137
N° of Still births on Day 0	5	3	8	4
Mating Index %	100	100	95	100
Fertility Index %	100	100	85	90
Gestation Index %	100	95	89.47	90
Lactation Index %	79.72	74.77	79.65	75.27
Live birth index %	97.7	98.62	95.56	97.85
4-Day Survival Index %	96.23	97.66	93.6	86.81
7 Day Survival Index %	92.45	89.72	90.70	84.62
14 Day Survival Index %	89.15	85.05	86.63	82.97
21 Day Survival Index%	79.72	74.77	79.65	75.27
Sex Ratio %	107.84	94.55	91.11	91.58

Developmental Neurotoxicity Study in Rats

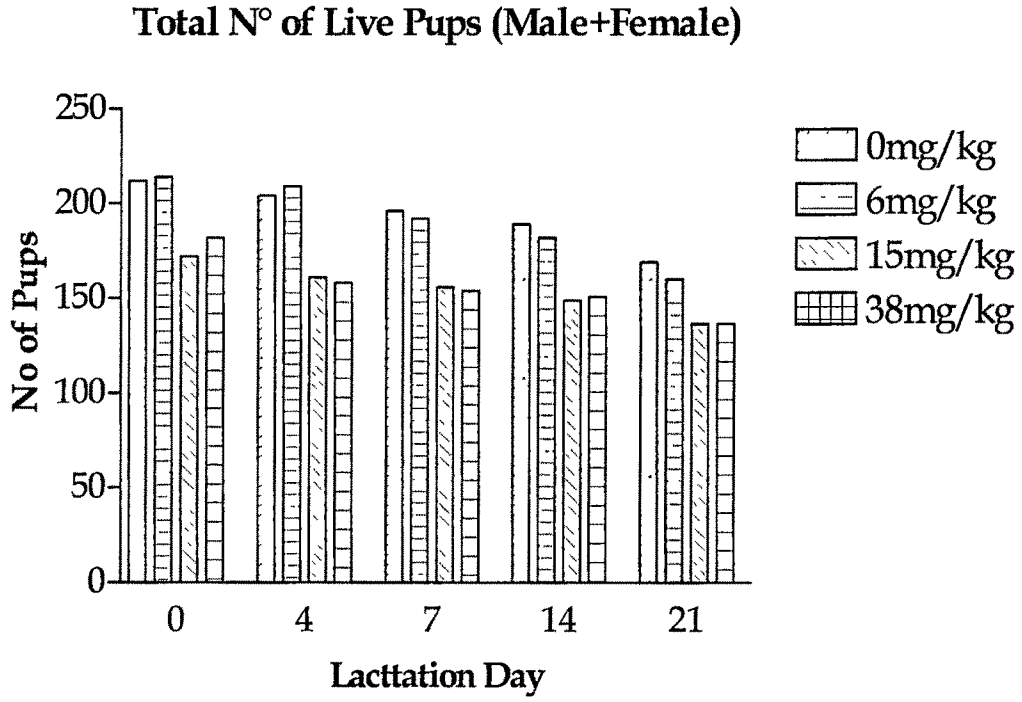
Table-4.6

Effect of Insecticide Combination on Total Number of Live Pups (Male + Female)

Lactation Day	Dose (mg/kg b. wt/day)			
	0 (N=20)	6 (N=19)	15 (N=17)	38 (N=18)
0	212	214	172	182
4	204	209	161	158
7	196	192	156	154
14	189	182	149	151
21	169	160	137	137

Note: Values are expressed as Mean ± SE

Figure 3.5



Developmental Neurotoxicity Study in Rats

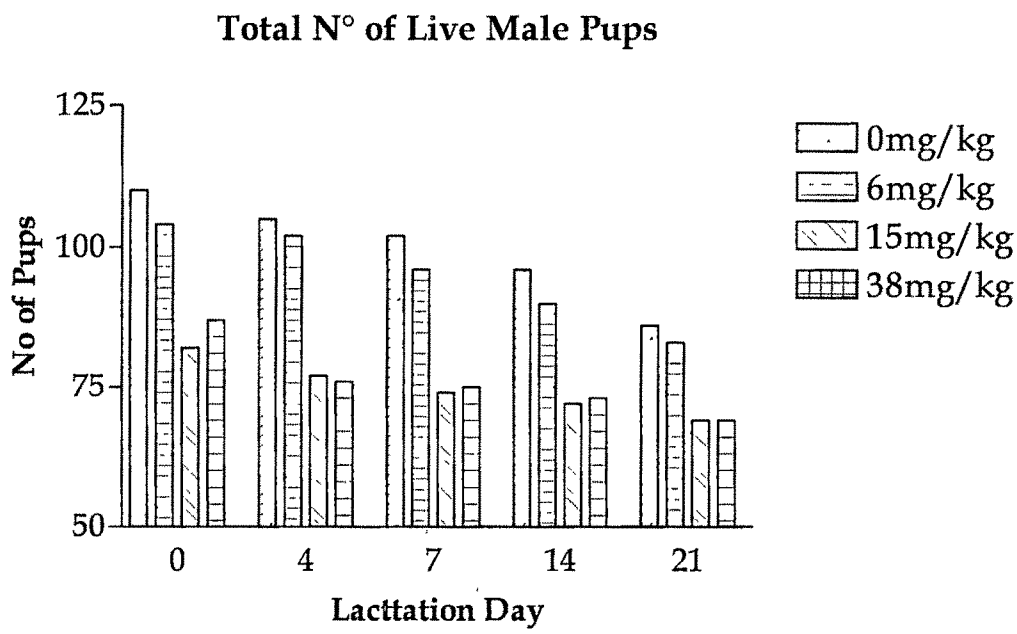
Table-4.7

Effect of Insecticide Combination on Number of Live Male Pups

Lactation Day	Dose (mg/kg b. wt/day)			
	0 (N=20)	6 (N=19)	15 (N=17)	38 (N=18)
0	110	104	82	87
4	105	102	77	76
7	102	96	74	75
14	96	90	72	73
21	86	83	69	69

Note: Values are expressed as Mean ± SE

Figure 3.6



Developmental Neurotoxicity Study in Rats

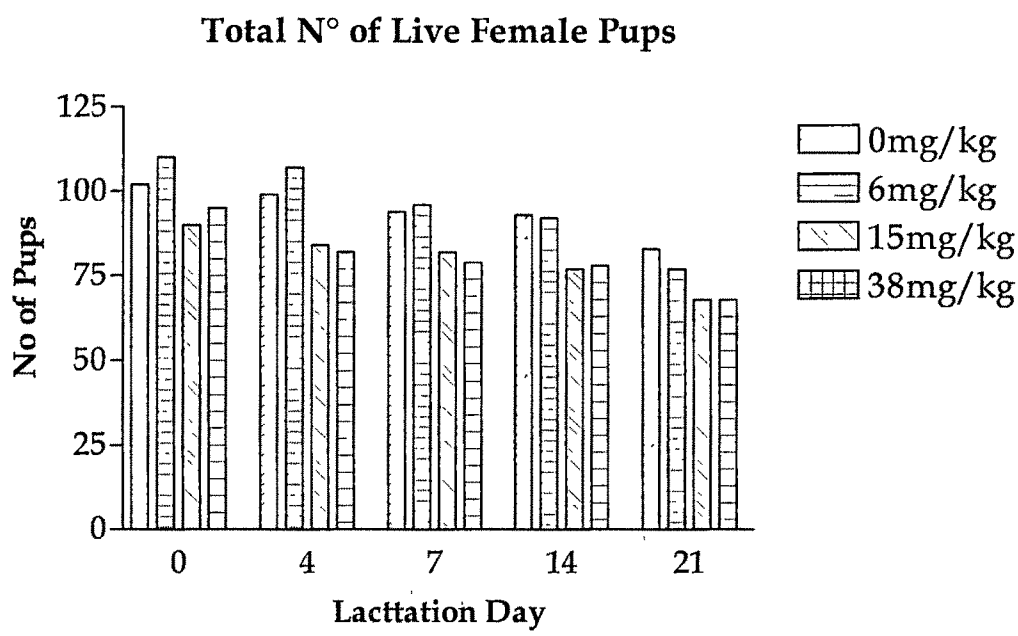
Table-4.8

Effect of Insecticide Combination on Number of Live Female Pups

Lactation Day	Dose (mg/kg b. wt/day)			
	0 (N=20)	6 (N=19)	15 (N=17)	38 (N=18)
0	102	110	90	95
4	99	107	84	82
7	94	96	82	79
14	93	92	77	78
21	83	77	68	68

Note: Values are expressed as Mean ± SE

Figure 3.7



Developmental Neurotoxicity Study in Rats

Table-4.9

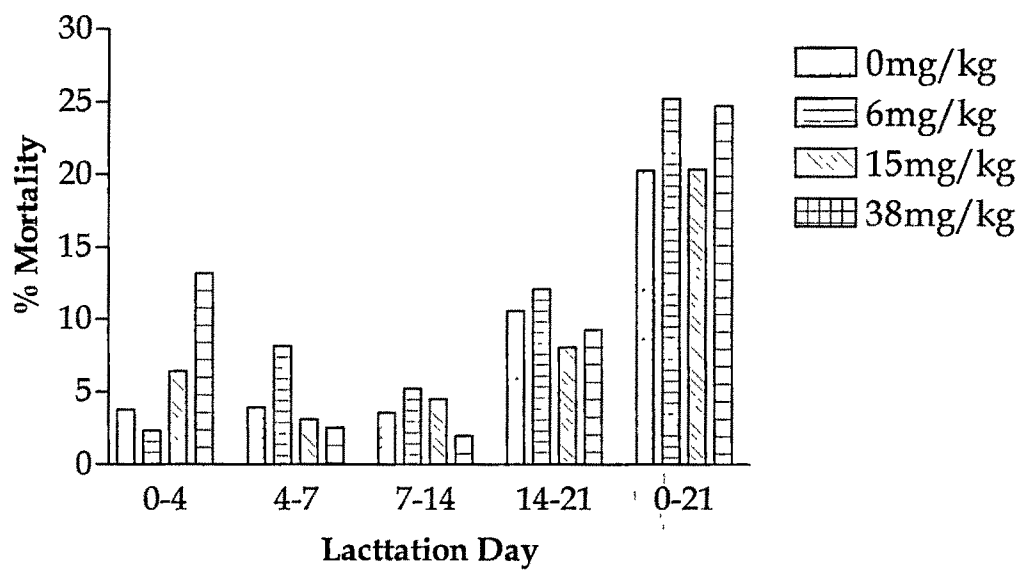
Effect of Insecticide Combination on Pup Mortality (Male+ Female)

Lactation Day	Dose (mg/kg b. wt/day)							
	0		6		15		38	
	N°	% Mortality	N°	% Mortality	N°	% Mortality	N°	% Mortality
0 - 4	8	3.77	5	2.34	11	6.4	24	13.19
4 - 7	8	3.92	17	8.13	5	3.11	4	2.53
7 - 14	7	3.57	10	5.21	7	4.49	3	1.95
14 - 21	20	10 58	22	12.09	12	8.05	14	9.27
0 - 21	43	20.28	54	25.23	35	20.35	45	24.73

Note: Values are expressed as Mean ± SE

Figure 3.8

Pup Mortality (Male+Female pups)



Developmental Neurotoxicity Study in Rats

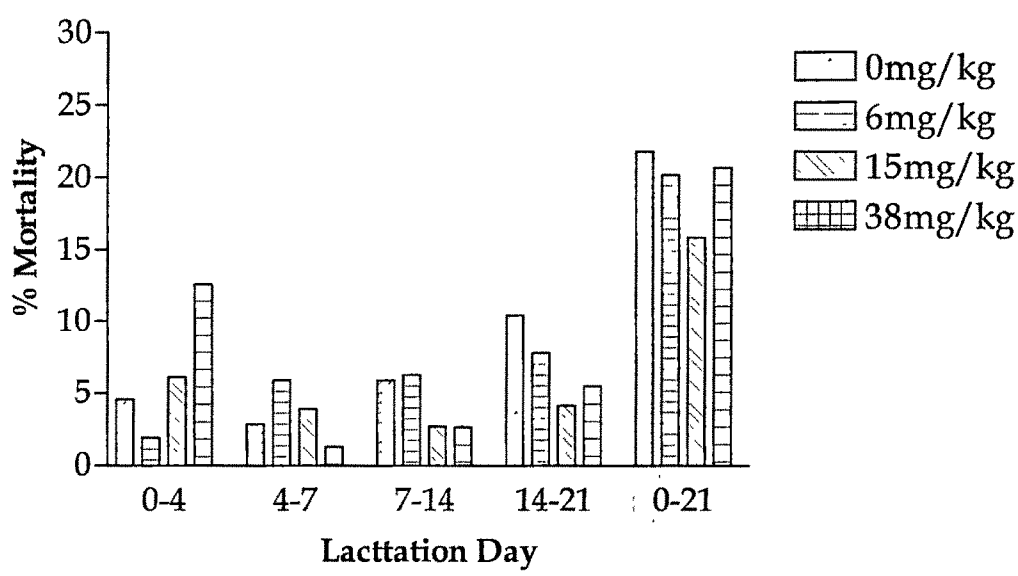
Table-4.10

Effect of Insecticide Combination on Pup Mortality - Male

Lactation Day	Dose (mg/kg b. wt/day)							
	0		6		15		38	
	N°	% Mortality	N°	% Mortality	N°	% Mortality	N°	% Mortality
0 - 4	5	4.55	2	1.92	5	6.1	11	12.64
4 - 7	3	2.86	6	5.88	3	3.9	1	1.32
7 - 14	6	5.88	6	6.25	2	2.7	2	2.67
14 - 21	10	10.42	7	7.78	3	4.17	4	5.48
0 - 21	24	21.82	21	20.19	13	15.85	18	20.69

Figure 3.9

Mortality of Male pups



Developmental Neurotoxicity Study in Rats

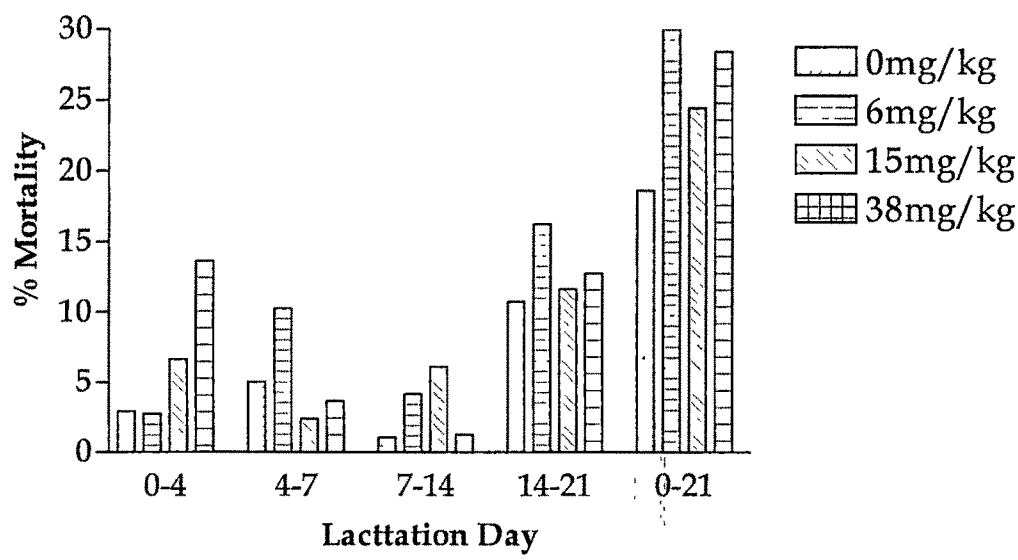
Table-4.11

Effect of Insecticide Combination on Pup Mortality - Female

Lactation Day	Dose (mg/kg b. wt/day)							
	0		6		15		38	
	N°	% Mortality	N°	% Mortality	N°	% Mortality	N°	% Mortality
0 - 4	3	2.94	3	2.73	6	6.67	13	13.68
4 - 7	5	5.05	11	10.28	2	2 38	3	3.66
7 - 14	1	1.06	4	4.17	5	6.1	1	1.27
14 - 21	10	10.75	15	16.3	9	11.69	10	12.82
0 - 21	19	18.63	33	30	22	24.44	27	28.42

Figure 3.10

Mortality of Female pups



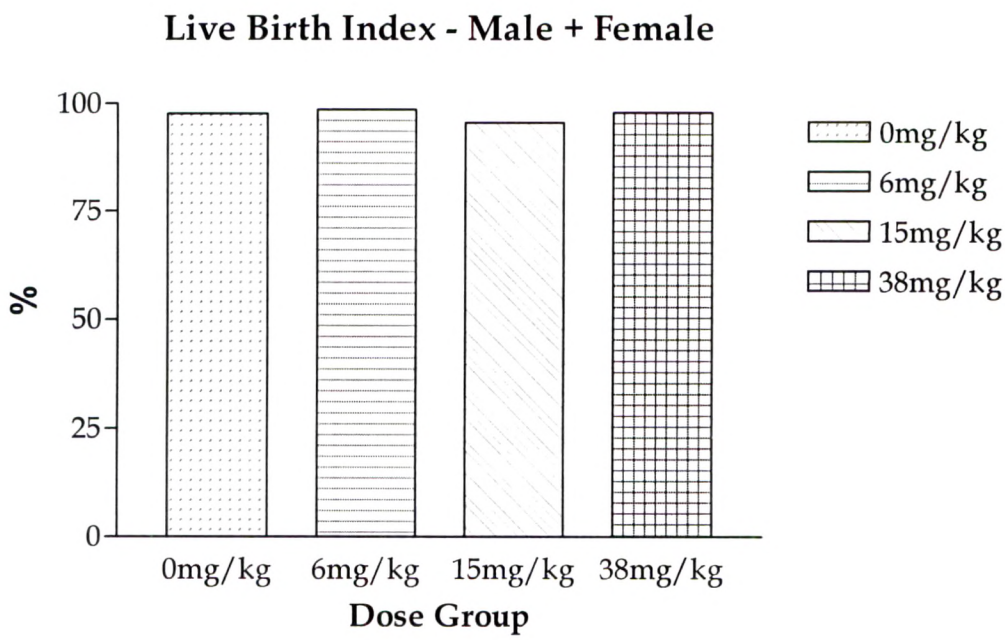
Developmental Neurotoxicity Study in Rats

Table-4.12

Effect of Insecticide Combination on Live Birth Index – (Male + Female)

Parameter	Dose group (mg/kg b. wt/day)			
	0	6	15	38
Live Birth Index	97.7 %	98.62 %	95.56 %	97.85 %

Figure 3.11



Developmental Neurotoxicity Study in Rats

Table-4.13

Effect of Insecticide Combination on Total Litter Size (number) and Sex Ratio

Lactation Day	Sex	Dose (mg/kg b. wt/day)			
		0 (N=20)	6 (N=19)	15 (N=17)	38 (N=18)
Day 0	M	110	104	82	87
	F	102	110	90	95
	M+F	212	214	172	182
	M: F	1.08	0.95	0.91	0.92
Day 4	M	105	102	77	76
	F	99	107	84	82
	M+F	204	209	161	158
	M: F	1.06	0.95	0.92	0.93
Day 7	M	102	96	74	75
	F	94	96	82	79
	M+F	196	192	156	154
	M: F	1.09	1.00	0.90	0.95
Day 14	M	96	90	72	73
	F	93	92	77	78
	M+F	189	182	149	151
	M: F	1.03	0.98	0.94	0.94
Day 21	M	86	83	69	69
	F	83	77	68	68
	M+F	169	160	137	137
	M: F	1.04	1.08	1.01	1.01

Developmental Neurotoxicity Study in Rats

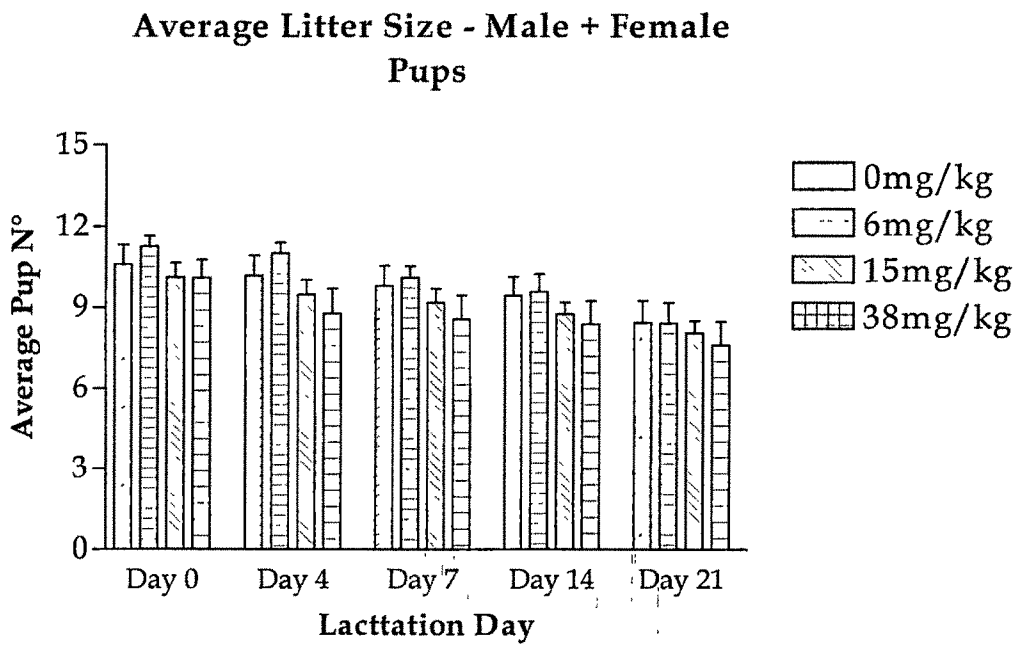
Table-4.14

Effect of Insecticide Combination on Average Litter Size (number) - (Male + Female)

Lactation Day	Dose (mg/kg b. wt/day)			
	0 (N=20)	6 (N=19)	15 (N=17)	38 (N=18)
Day 0	10.60 ± 0.73	11.26 ± 0.40	10.12 ± 0.55	10.11 ± 0.66
Day 4	10.20 ± 0.73	11.00 ± 0.41	9.47 ± 0.56	8.78 ± 0.93
Day 7	9.80 ± 0.76	10.11 ± 0.43	9.18 ± 0.52	8.56 ± 0.90
Day 14	9.45 ± 0.71	9.58 ± 0.68	8.76 ± 0.44	8.39 ± 0.86
Day 21	8.45 ± 0.82	8.42 ± 0.76	8.06 ± 0.44	7.61 ± 0.87

Note: Values are expressed as Mean ± SE

Figure 3.12



Developmental Neurotoxicity Study in Rats

Table-4.15

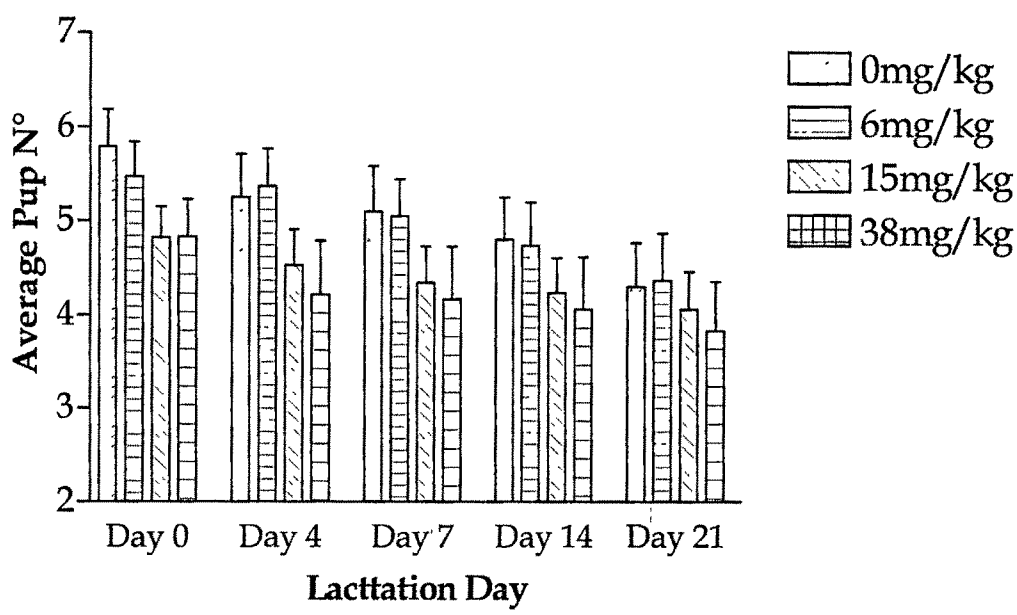
Effect of Insecticide Combination on Average Litter Size (number) – Male Pups

Lactation Day	Dose (mg/kg b. wt/day)			
	0 (N=20)	6 (N=19)	15 (N=17)	38 (N=18)
Day 0	5.79 ± 0.40	5.47 ± 0.37	4.82 ± 0.33	4.83 ± 0.40
Day 4	5.25 ± 0.46	5.37 ± 0.40	4.53 ± 0.38	4.22 ± 0.57
Day 7	5.10 ± 0.48	5.05 ± 0.39	4.35 ± 0.38	4.17 ± 0.56
Day 14	4.80 ± 0.45	4.74 ± 0.46	4.24 ± 0.37	4.06 ± 0.56
Day 21	4.30 ± 0.47	4.37 ± 0.50	4.06 ± 0.40	3.83 ± 0.53

Note: Values are expressed as Mean ± SE

Figure 3.13

Average Litter Size - Male Pups



Developmental Neurotoxicity Study in Rats

Table-4.16

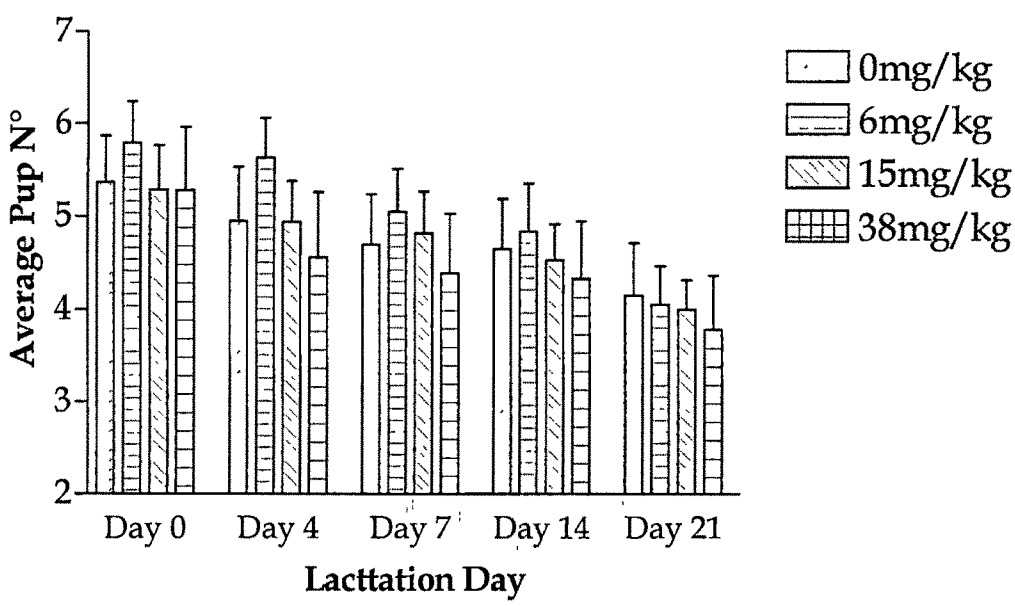
Effect of Insecticide Combination on Average Litter Size (number) - Female Pups

Lactation Day	Dose (mg/kg b. wt/day)			
	0 (N=20)	6 (N=19)	15 (N=17)	38 (N=18)
Day 0	5.37 ± 0.50	5.79 ± 0.45	5.29 ± 0.48	5.28 ± 0.68
Day 4	4.95 ± 0.58	5.63 ± 0.43	4.94 ± 0.44	4.56 ± 0.70
Day 7	4.70 ± 0.54	5.05 ± 0.46	4.82 ± 0.45	4.39 ± 0.64
Day 14	4.65 ± 0.54	4.84 ± 0.51	4.53 ± 0.39	4.33 ± 0.62
Day 21	4.15 ± 0.57	4.05 ± 0.42	4.00 ± 0.32	3.78 ± 0.59

Note: Values are expressed as Mean ± SE

Figure 3.14

Average Litter Size - Female Pups



Developmental Neurotoxicity Study in Rats

Table-4.17

Effect of Insecticide Combination on Average Litter Size (number) and Sex Ratio – (Male + Female)

Lactation Day		Dose (mg/kg b. wt/day)			
		0 (N=20)	6 (N=19)	15 (N=17)	38 (N=18)
Day 0	M	5.79 ± 0.40	5.47 ± 0.37	4.82 ± 0 33	4.83 ± 0.40
	F	5.37 ± 0.50	5.79 ± 0.45	5.29 ± 0.48	5.28 ± 0.68
	M+F	10.60 ± 0.73	11 26 ± 0.40	10.12 ± 0.55	10.11 ± 0.66
	Sex Ratio	1.33 ± 0.21	1.10 ± 0.13	1.11 ± 0.18	1.43 ± 0.26
Day4	M	5.25 ± 0.46	5.37 ± 0.40	4.53 ± 0.38	4.22 ± 0.57
	F	4.95 ± 0.58	5.63 ± 0.43	4.94 ± 0.44	4.56 ± 0.70
	M+F	10.20 ± 0.73	11.00 ± 0.41	9.47 ± 0.56	8.78 ± 0.93
	Sex Ratio	1.11 ± 0 17	1.12 ± 0.15	1.09 ± 0.18	1.28 ± 0.25
Day 7	M	5.10 ± 0.48	5.05 ± 0.39	4.35 ± 0.38	4.17 ± 0.56
	F	4.70 ± 0.54	5.05 ± 0.46	4.82 ± 0.45	4.39 ± 0.64
	M+F	9.80 ± 0.76	10.11 ± 0.43	9.18 ± 0.52	8.56 ± 0.90
	Sex Ratio	1.12 ± 0.16	1.29 ± 0.22	1.10 ± 0.19	1.26 ± 0.24
Day 14	M	4.80 ± 0.45	4.74 ± 0.46	4.24 ± 0.37	4.06 ± 0.56
	F	4.65 ± 0.54	4.84 ± 0.51	4.53 ± 0.39	4.33 ± 0.62
	M+F	9.45 ± 0.71	9.58 ± 0.68	8.76 ± 0.44	8.39 ± 0.86
	Sex Ratio	1.08 ± 0.16	1.18 ± 0.22	1.13 ± 0.19	1.25 ± 0.24
Day 21	M	4.30 ± 0.47	4.37 ± 0.50	4.06 ± 0.40	3.83 ± 0.53
	F	4.15 ± 0.57	4.05 ± 0.42	4.00 ± 0.32	3.78 ± 0.59
	M+F	8.45 ± 0.82	8.42 ± 0.76	8.06 ± 0.44	7.61 ± 0.87
	Sex Ratio	1.00 ± 0.15	1.12 ± 0.20	1.17 ± 0.18	1.24 ± 0.22

Note: Values are expressed as Mean ± SE

Developmental Neurotoxicity Study in Rats

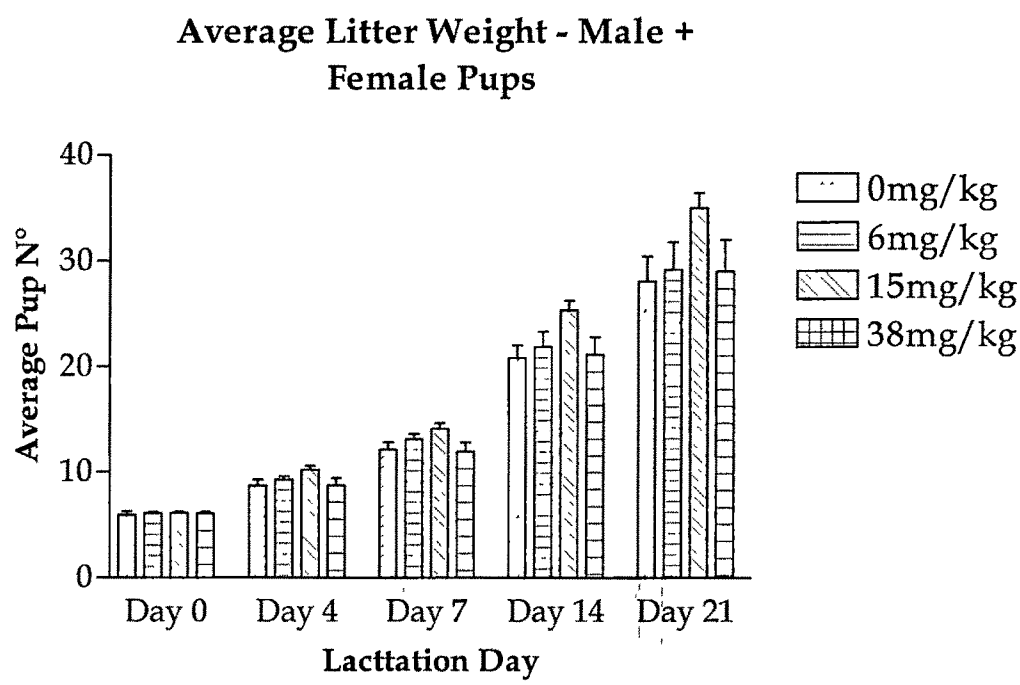
Table-4.18

Effect of Insecticide Combination on Average Litter Weight (g) - (Male + Female)

Lactation Day	Dose (mg/kg b. wt/day)			
	0 (N=20)	6 (N=19)	15 (N=17)	38 (N=18)
Day 0	5.92 ± 0.33	6.07 ± 0.15	6.11 ± 0.12	6.06 ± 0.17
Day 4	8.70 ± 0.54	9.25 ± 0.29	10.23 ± 0.35	8.72 ± 0.67
Day 7	12.09 ± 0.74	13.13 ± 0.52	14.13 ± 0.51	11.91 ± 0.91
Day 14	20.85 ± 1.20	21.89 ± 1.44	25.34 ± 0.95	21.15 ± 1.67
Day 21	28.11 ± 2.39	29.21 ± 2 63	35.06 ± 1.43	29.06 ± 3.02

Note. Values are expressed as Mean ± SE

Figure 3.15



Developmental Neurotoxicity Study in Rats

Table-4.19

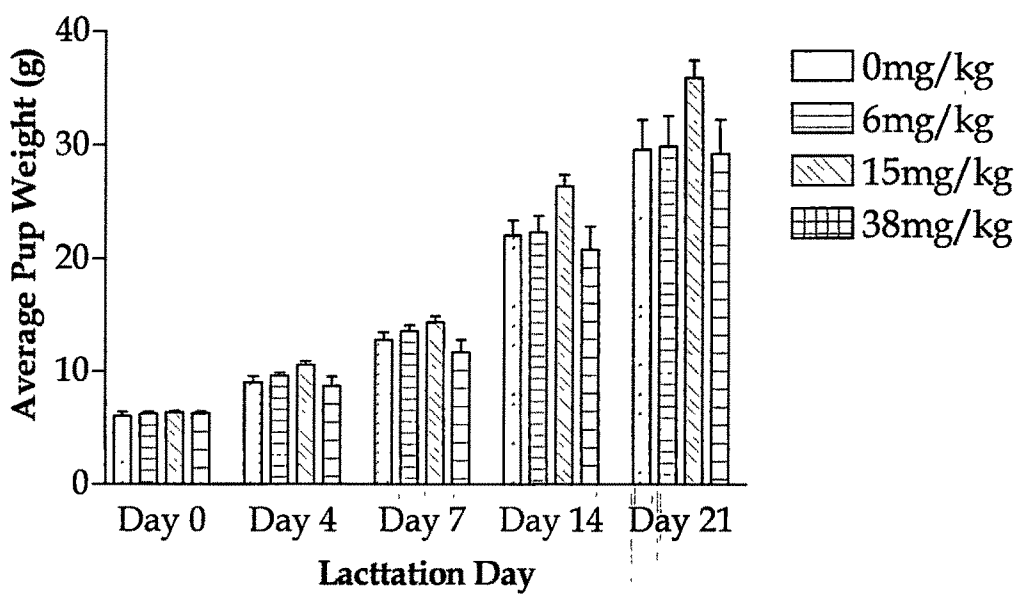
Effect of Insecticide Combination on Average Litter Weight (g) – Male Pups

Lactation Day	Dose (mg/kg b. wt/day)			
	0 (N=20)	6 (N=19)	15 (N=17)	38 (N=18)
Day 0	6.02 ± 0.36	6.23 ± 0.14	6.32 ± 0.13	6.24 ± 0.17
Day 4	9.02 ± 0.52	9.60 ± 0.29	10.51 ± 0.40	8.67 ± 0.81
Day 7	12.75 ± 0.74	13.54 ± 0.57	14.38 ± 0.55	11.70 ± 1.09
Day 14	22.09 ± 1.34	22.35 ± 1.48	26.41 ± 1.00	20.86 ± 2.01
Day 21	29.60 ± 2.60	29.90 ± 2.69	35.92 ± 1.54	29.25 ± 2.99

Note: Values are expressed as Mean ± SE

Figure 3.16

Average Litter Weight - Male Pups



Developmental Neurotoxicity Study in Rats

Table-4.20

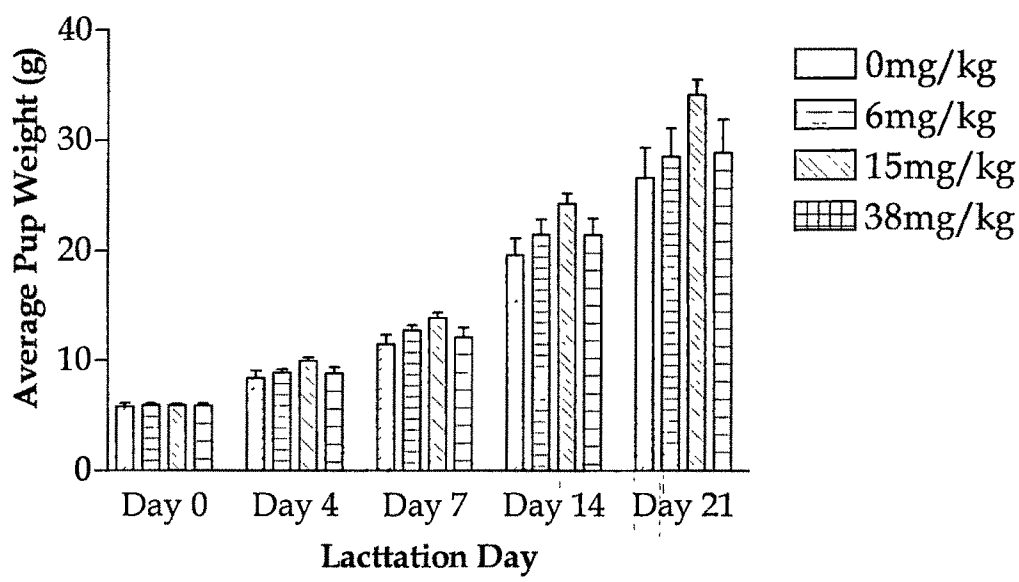
Effect of Insecticide Combination on Average Litter Weight (g) - Female Pups

Lactation Day	Dose (mg/kg b. wt/day)			
	0 (N=20)	6 (N=19)	15 (N=17)	38 (N=18)
Day 0	5.81 ± 0 32	5.91 ± 0.16	5.90 ± 0.14	5.89 ± 0.19
Day 4	8.39 ± 0.68	8.89 ± 0.30	9.95 ± 0.31	8.77 ± 0.60
Day 7	11.44 ± 0.91	12.73 ± 0.50	13.89 ± 0.48	12.12 ± 0.85
Day 14	19.60 ± 1.56	21.44 ± 1.40	24.28 ± 0.95	21.43 ± 1.53
Day 21	26.62 ± 2.72	28.53 ± 2.58	34.19 ± 1.38	28.87 ± 3.07

Note: Values are Mean ± SE

Figure 3.17

Average Litter Weight - Female Pups



Developmental Neurotoxicity Study in Rats

Table-4.21

Effect of Insecticide Combination on Absolute Organ Weights (g) - Parent Males

Organ	Dose (mg/kg b. wt/day)			
	0 (N=10)	6 (N=10)	15 (N=10)	38 (N=10)
Body Weights	469.8 ± 44 24	408.5 ± 49.18*↓	446.8 ± 35.25	475.3 ± 42.78
Adrenals	0.07 ± 0.01	0.07 ± 0.02	0.07 ± 0.01	0.07 ± 0.01
Testes	3.47 ± 0.33	3.37 ± 0.54	3.47 ± 0.28	3.49 ± 0.45
Epididymis	1.39 ± 0.16	1 31 ± 0.22	1.38 ± 0.10	1.40 ± 0.14
Seminal vesicle & Coagulation gland	2.13 ± 0.47	2.03 ± 0.47	2.07 ± 0.22	2.14 ± 0.42
Kidneys	3.21 ± 0.56	2.97 ± 0.31	3.31 ± 0.27	3.34 ± 0.33
Brain	2.19 ± 0.16	2.04 ± 0.12	2.10 ± 0.13	2.11 ± 0.13
Heart	1.52 ± 0.23	1.42 ± 0.15	1.42 ± 0.14	1.54 ± 0.11
Spleen	1.21 ± 0.17	1.24 ± 0.41	1.33 ± 0.18	1.26 ± 0.35
Liver	15.54 ± 1.97	13.08 ± 1.81	14.64 ± 1.00	15.65 ± 1.62
Lungs	2.31 ± 0.50	2.43 ± 0.36	2.30 ± 0.47	2.46 ± 0.37
Thymus	0.33 ± 0.12	0.29 ± 0.06	0.33 ± 0.08	0.30 ± 0.10

Note: Values are expressed as Mean ± SE

*↓ = Significantly lower than control ($P \leq 0.05$)

Developmental Neurotoxicity Study in Rats

Table-4.22

Effect of Insecticide Combination on Relative Organ Weights (%)- Parent Males

Organ	Dose (mg/kg b. wt/day)			
	0 (10)	6 (N=10)	15 (N=10)	38 (N=10)
Adrenals	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Testes	0 74 ± 0.02	0.83 ± 0.05	0.78 ± 0.03	0.74 ± 0.04
Epididymis	0.30 ± 0.01	0.32 ± 0.02	0.31 ± 0.01	0.30 ± 0.01
Seminal vesicle & Coagulation gland	0 45 ± 0.03	0.50 ± 0.03	0.47 ± 0.02	0.45 ± 0.03
Kidneys	0.68 ± 0.03	0.73 ± 0.02	0 74 ± 0.01	0.70 ± 0.02
Brain	0.47 ± 0.01	0.51 ± 0.02	0.47 ± 0.01	0.45 ± 0.01
Heart	0.32 ± 0.01	0.35 ± 0.01	0.32 ± 0.01	0.32 ± 0.01
Spleen	0.26 ± 0 01	0.30 ± 0.03	0.30 ± 0.02	0.27 ± 0.02
Liver	3.32 ± 0.12	3.20 ± 0.07	3.30 ± 0.12	3.31 ± 0.11
Lungs	0.49 ± 0.03	0.61 ± 0.05	0.52 ± 0.04	0.52 ± 0.03
Thymus	0.07 ± 0.01	0.07 ± 0.00	0.07 ± 0.01	0.06 ± 0.01

Note: Values are expressed as Mean ± SE

Developmental Neurotoxicity Study in Rats

Table-4.23

Effect of insecticide combination on Relative Brain Weights- Parent Males

Organ	Dose (mg/kg b. wt/day)			
	0 (N=10)	6 (N=10)	15 (N=10)	38 (N=10)
Adrenals	3.19 ± 0.14	3.55 ± 0.30	3.17 ± 0.23	3.34 ± 0.18
Testes	158.84 ± 4.43	164.62 ± 7.49	165.81 ± 3.55	165.95 ± 6.63
Epididymis	63.61 ± 2.68	64.30 ± 3.23	66.06 ± 2.12	66.33 ± 2.22
Seminal vesicle & Coagulation gland	97.77 ± 7.19	99.19 ± 6.66	99.05 ± 4.10	102.03 ± 6.76
Kidneys	146.75 ± 7.82	145.75 ± 4.37	158.24 ± 2.95	158.35 ± 2.85
Heart	69.42 ± 3.35	69.51 ± 2.00	67.86 ± 2.17	73.04 ± 1.66
Spleen	55.56 ± 2.67	61.02 ± 6.51	63.97 ± 3.83	59.79 ± 5.01
Liver	708.68 ± 19.76	641.99 ± 28.79	702.37 ± 26.13	743.48 ± 23.76
Lungs	105.11 ± 5.92	119.03 ± 5.58	109.85 ± 6.87	116.76 ± 5.60
Thymus	14.97 ± 1 659	14.4 ± 1.122	15.80 ± 0.987	14.40 ± 1.459

Note: Values are expressed as Mean ± SE

Developmental Neurotoxicity Study in Rats

Table-4.24

Effect of insecticide combination on Absolute Organ Weights (g) – Parent Females

Organ	Dose (mg/kg b. wt/day)			
	0 (N=17)	6 (N=19)	15 (N=18)	38 (N=18)
Body weight	273.76 ± 8.77	271.47 ± 8.93	273.89 ± 4.90	269.17 ± 7.45
Adrenals	0.10 ± 0.00	0.14 ± 0.04	0.10 ± 0.01	0.11 ± 0.01
Ovaries	0.12 ± 0.01	0.13 ± 0.00	0.14 ± 0.01	0.14 ± 0.01
Uterus	0.52 ± 0.05	0.47 ± 0.02	0.58 ± 0.11	0.61 ± 0.07
Kidneys	2.37 ± 0.05	2.33 ± 0.06	2.27 ± 0.07	2.33 ± 0.04
Brain	1.99 ± 0.06	1.97 ± 0.02	1.92 ± 0.02	1.97 ± 0.02
Heart	1.12 ± 0.03	1.09 ± 0.04	1.18 ± 0.04	1.21 ± 0.06
Spleen	0.95 ± 0.05	0.81 ± 0.04	0.98 ± 0.10	0.85 ± 0.03
Liver	12.90 ± 0.44	12.51 ± 0.40	11.98 ± 0.26	12.60 ± 0.27
Lung	1.68 ± 0.05	1.71 ± 0.07	1.85 ± 0.05	1.74 ± 0.05
Thymus	0.17 ± 0.02	0.19 ± 0.02	0.23 ± 0.02	0.18 ± 0.02

Note: Values are expressed as Mean ± SE

Developmental Neurotoxicity Study in Rats

Table-4.25

Effect of insecticide combination on Relative Organ weights (%) - Parent Females

Organ	Dose (mg/kg b. wt/day)			
	0 (N=17)	6 (N=19)	15 (N=18)	38 (N=18)
Adrenals	0.04 ± 0 00	0.05 ± 0.02	0.04 ± 0.00	0.04 ± 0.00
Ovary	0.04 ± 0.00	0.05 ± 0.00	0.05 ± 0.01	0.05 ± 0.00
Uterus	0.19 ± 0.02	0.17 ± 0.01	0.21 ± 0.04	0.23 ± 0.02
Kidneys	0.87 ± 0.02	0.87 ± 0.03	0.83 ± 0.02	0.87 ± 0.03
Brain	0.74 ± 0.02	0.74 ± 0.02	0.71 ± 0.01	0.74 ± 0.02
Heart	0.41 ± 0.02	0.40 ± 0.01	0.43 ± 0.01	0.46 ± 0.03
Spleen	0.35 ± 0.01	0.30 ± 0.02	0.36 ± 0.03	0.32 ± 0.01
Liver	4.73 ± 0.12	4.65 ± 0.16	4.38 ± 0.09	4.73 ± 0.14
Lung	0.62 ± 0.02	0.64 ± 0.02	0.68 ± 0.02	0.65 ± 0.03
Thymus	0.06 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.07 ± 0.01

Note: Values are expressed as Mean ± SE

Developmental Neurotoxicity Wistar Rats

Table-4.26

Effect of Insecticide Combination on Relative Brain Weights (%) – Parent Females

Organ	Dose (mg/kg b. wt/day)			
	0 (N=17)	6 (N=19)	15 (N=18)	38 (N=18)
Adrenal	4.86 ± 0.25	7.10 ± 2.41	5.23 ± 0.33	5.49 ± 0.37
Ovary	5.91 ± 0.51	6.44 ± 0.21	7.53 ± 0.64	6.93 ± 0.71
Uterus	26.00 ± 2.25	117.85 ± 2.57	30.19 ± 5.46	30.72 ± 3.37
Kidneys	119.44 ± 2.81	117.85 ± 2 57	117.81 ± 3.22	117.97 ± 2.10
Heart	56.30 ± 1.70	54.94 ± 1.70	61.56 ± 1.89	61.18 ± 3.06
Spleen	47.75 ± 1.91	41.14 ± 2.14	50.54 ± 4.89	42.85 ± 1.38
Liver	647.53 ± 16.43	633.91 ± 19.19	623.31 ± 13.08	638.76 ± 12.78
Lung	84.61 ± 2.71	86.64 ± 3.03	96.46 ± 2.59	88.21 ± 2.54
Thymus	8.43 ± 0.69	9.38 ± 0.84	12.19 ± 1.13	9.40 ± 0.87

Note: Values are expressed as Mean ± SE

Developmental Neurotoxicity Study in Rats

Table-4.27

Effect of Insecticide Combination on Absolute Organ Weights (g) - Male Pups

Organ	Dose (mg/kg b. wt/day)			
	0 (N=17)	6 (N=18)	15 (N=16)	38 (N=17)
Body weight	33.41 ± 1.23	33.06 ± 1.16	35.06 ± 1.58	33.00 ± 1.94
Kidneys	0.47 ± 0.02	0.49 ± 0.02	0.54 ± 0.02	0.55 ± 0.02
Brain	1.47 ± 0.03	1.44 ± 0.03	1.75 ± 0.27	1.46 ± 0.03
Spleen	0.15 ± 0.01	0.14 ± 0.01	0.19 ± 0.02	0.20 ± 0.01*↑
Liver	1.67 ± 0.11	1.65 ± 0.08	2.01 ± 0.13	2.11 ± 0.10*↑
Thymus	0.13 ± 0.01	0.18 ± 0.06	0.18 ± 0.02	0.13 ± 0.01

Note: Values are expressed as Mean ± SE

*↑ = Significantly higher than control ($P \leq 0.05$)

Developmental Neurotoxicity Study in Rats

Table-4.28

Effect of Insecticide Combination on Absolute Organ Weights (g) – Female Pups

Organ	Dose (mg/kg b. wt/day)			
	0 (N=15)	6 (N=18)	15 (N=16)	38 (N=17)
Body weight	31.44 ± 1.67	31 94 ± 1.08	35.19 ± 1.82	31.53 ± 1.92
Kidneys	0.48 ± 0 02	0.52 ± 0.02	0.56 ± 0.02	0.55 ± 0.02
Brain	1 43 ± 0.02	1.43 ± 0.03	1.43 ± 0.03	1.41 ± 0.03
Spleen	0.19 ± 0.06	0.16 ± 0.01	0.18 ± 0.01	0.15 ± 0.01
Liver	1.57 ± 0.10	1.65 ± 0.08	1.93 ± 0.10*↑	1.94 ± 0.08*↑
Thymus	0.14 ± 0 01	0.15 ± 0.01	0.16 ± 0.01	0.16 ± 0.01

Note: Values are expressed as Mean ± SE
*↑ = Significantly higher than control (P ≤ 0.05)

Table-4.29

Effect of Insecticide Combination on Relative Organ Weights (%) – Male pups

Organ	Dose (mg/kg b. wt/day)			
	0 (N=17)	6 (N=18)	15 (N=16)	38 (N=17)
Kidneys	1.42 ± 0.05	1.50 ± 0.06	1.56 ± 0.04	1.69 ± 0.04*↑
Brain	4.46 ± 0.11	4.43 ± 0 13	5.14 ± 0.78	4.60 ± 0.19
Spleen	0.44 ± 0.03	0.43 ± 0.02	0.55 ± 0.05	0.62 ± 0.05*↑
Liver	4.98 ± 0.22	4.98 ± 0.15	5.77 ± 0.33	6.59 ± 0.39*↑
Thymus	0.39 ± 0.03	0.55 ± 0.17	0.53 ± 0.07	0.40 ± 0.03

Note: Values are expressed as Mean ± SE
*↑ = Significantly higher than control (P ≤ 0.05)

Developmental Neurotoxicity Study in Rats

Table-4.30

Effect of Insecticide Combination on Relative Organ Weights (%) - Female Pups

Organ	Dose (mg/kg b. wt/day)			
	0 (N=15)	6 (N=18)	15 (N=16)	38 (N=17)
Kidneys	1.52 ± 0.04	1.64 ± 0.05	1.63 ± 0.08	1.78 ± 0.07*↑
Brain	4.69 ± 0.18	4.56 ± 0.17	4.23 ± 0.26	4.69 ± 0.25
Spleen	0.63 ± 0.19	0.49 ± 0.04	0.51 ± 0.03	0.49 ± 0.03
Liver	5.00 ± 0.10	5.19 ± 0.19	5.56 ± 0.20	6.40 ± 0.38*↑
Thymus	0.45 ± 0.02	0.47 ± 0.04	0.47 ± 0.03	0.53 ± 0.07

Note: Values are expressed as Mean ± SE

*↑ = Significantly higher than control ($P \leq 0.05$)

Table-4.31

Effect of Insecticide Combination on Relative Brain Weights (%) - Male Pups

Organ	Dose (mg/kg b. wt/day)			
	0 (N=17)	6 (N=18)	15 (N=16)	38 (N=17)
Kidneys	32.02 ± 1.40	34.18 ± 1.55	35.27 ± 2.51	37.35 ± 1.11
Spleen	9.86 ± 0.63	9.79 ± 0.59	12.54 ± 1.29	13.42 ± 0.91*↑
Liver	112.67 ± 6.05	113.89 ± 4.54	126.98 ± 9.70	144.66 ± 6.70*↑
Thymus	8.78 ± 0.66	12.78 ± 3.98	10.85 ± 0.72	8.99 ± 0.84

Note: Values are expressed as Mean ± SE

*↑ = Significantly higher than control ($P \leq 0.05$)

Table-4.32

Effect of Insecticide Combination on Relative Brain Weights (%)– Female Pup

Organ	Dose (mg/kg b. wt/day)			
	0 (N=15)	6 (N=18)	15 (N=16)	38 (N=17)
Kidneys	33.03 ± 1.37	36.46 ± 1.32	39.22 ± 1.46*↑	38.78 ± 1.39*↑
Spleen	13.62 ± 4.00	10.97 ± 0.95	12.58 ± 1 00	10.78 ± 0.80
Liver	109.05 ± 5.51	115.12 ± 4.49	135.52 ± 6.32*↑	138.04 ± 5.53*↑
Thymus	9.86 ± 0.77	10.81 ± 1.25	11.41 ± 0.87	11.41 ± 1.07

Note: Values are expressed as Mean ± SE

*↑ = Significantly higher than control ($P \leq 0.05$)

Developmental Neurotoxicity Study in Rats

Table-4.33

Effect of Insecticide Combination on Gross Findings – Parent Males

Organ	Observation	Dose (mg/kg b. wt. /day)			
		0	6	15	38
		N=10	N=10	N=10	N=10
Liver	Hyperemic/Congestion/Consolidation	2	1	2	2
	Mottled	1	0	0	1
	Pin point/petechial hemorrhages	0	1	2	3
Total		**3**	**2**	**4**	**5**
Lungs	Congested/Consolidation	2	0	0	2
	Diffused pnuemonic foci	1	1	0	1
Total		**3**	**1**	**0**	**3**
Spleen	Diffused white foci	1	0	2	1
	Congestion	2	0	1	2
Total		**3**	**0**	**3**	**3**
Kidneys	Pallor	1	2	0	1
	Congestion	0	0	1	3
Total		**1**	**2**	**1**	**4**

Developmental Neurotoxicity Study in Rats

Table-4.34

Effect of Insecticide Combination on Gross Findings- Parent Females

Organ	Observation	Dose (mg/kg b. wt./day)			
		0	6	15	38
		N=20	N=20	N=20	N=20
Liver	Hyperemic/Congestion/Consolidation	2	2	7	9
	Mottled	1	0	2	0
	Pin point/petechial hemorrhages	0	2	3	2
Total		**3**	**4**	**12**	**11**
Lungs	Congested/Consolidation	4	8	7	5
	Diffused pneumonic foci	3	6	6	6
	Edematous	2	0	1	2
	Hemorrhages	0	0	0	1
Total		**9**	**14**	**14**	**13**
Spleen	Diffused white foci	1	0	0	2
	Congestion	1	1	1	4
	Atrophy	0	0	1	0
	Enlarged	0	0	1	0
Total		**2**	**1**	**3**	**6**
Kidneys	Pallor	0	2	2	1
	Congestion	0	0	1	1
Total		**0**	**2**	**3**	**2**
Uterus	Thickened wall with abscess formation of pyometra	1	0	1	1
	Hydrometra	0	0	0	1
Total		**1**	**0**	**1**	**2**
Ovaries	Atrophic ovary	0	1	0	0
Intestine	Hyperemic	0	0	1	0

Developmental Neurotoxicity Study in Rats

Table-4.35

Effect of Insecticide Combination on Histopathological Findings - Parent Males

Organ	Observation	Dose (mg/kg b. wt. /day)			
		0	6	15	38
		N=10	N=10	N=10	N=10
Lung	Multiple Abscess	1	0	1	0
	Peribronchial/ perivascular MNC infiltration	1	0	0	0
	Alveolar histocytosis	1	1	0	0
	Increase in BALT	0	0	0	1
	Centribular hepatocellular vacuolation	0	1	1	4
	Congestion/fatty change/focal minimal necrosis	0	0	0	2
Liver	Congestion	2	1	1	0
Oesophagus	Acanthosis and hyperkeratosis	1	0	0	1
Urinary bladder	MNC Infiltration	1	0	0	1
Epididymides	Spermatocoele	1	0	1	0
Prostate	Focal minimal hyperplasia with distruction of cells	1	1	0	1
Kidneys	Cortical tubular dilatation with flattened epithelium/Congestion	0	0	0	6
	Multifocal regenerating tubules	0	0	0	1
Nerve	Demyelination / vacuolation	0	0	0	4
	Axonal degeneration	0	0	0	1
Tongue	Inflammation	1	0	1	3
Lymph nodes	Depleted germinal center	0	0	0	1
Pituitary	Focal increased acidophils	0	0	0	1

Developmental Neurotoxicity Study in Rats

Table-4.36

Effect of Insecticide Combination on Histopathological Findings – Parent Females

Organ	Observation	Dose (mg/kg b.wt./day)			
		0	6	15	38
		N=20	N=20	N=20	N=20
Adrenal	Congestion	0	0	0	1
	vacuolation	0	0	1	2
Brain	Inflammatory cell infiltration	0	0	0	1
	Gliosis	0	0	0	3
Oesophagus	Acanthosis and hyperkeratosis	2	0	1	1
Kidneys	Cortical tubular dilatation with flattened epithelium/Congestion	0	1	3	8
Lung	Multiple Abscess	1	1	0	0
	Peribronchial/perivascular MNC infiltration/ Edema	2	0	0	1
	Foam cell accumulation/emphysema	0	0	0	2
	Congestion/fatty change/focal minimal necrosis	1	0	3	0
Liver	Congestion	4	2	1	3
	Minimal fatty changes	0	0	1	2
	centribular hypertrophy/multifocal degeneration/ necrotic foci	0	0	3	5
Lymph nodes	Depleted germinal center	0	0	1	0
	Focal cyst	1	0	0	0
Ovary	Atretic follicle	0	0	1	6
Pituitary	Congestion	1	0	0	0
Spleen	Congested	2	0	1	3
	Lymphoid hyperplasia	1	0	1	0
Stomach (Non glandular)	Acanthosis and hyperkeratosis	1	0	0	0
	Cystic dilatation	0	0	0	1
Thymus	Atrophy/epithelisation	0	0	0	4
Uterus	Pigmented cells	1	0	0	0
	Inflammatory cell infiltration in myometrium / cell debris in lumen	1	0	1	0

Developmental Neurotoxicity Study in Rats

Table-4.37

Effect of Insecticide Combination on Histopathological Findings – Male and Female Pups

Brain:

Multiple pale acellular areas, gliosis, demyelination,

Liver:

Cystic tubules, extramedullary hematopoisis, sinusoidal dilation, vacoulation and congestion, sinusoidal dilation

Thymus:

Atrophy characterized by epithiolisation

Kidney:

Tubular degeneration with flattened epithelium

Spleen:

Extramedullary hematopoisis, megalokaryocytosis

Parents

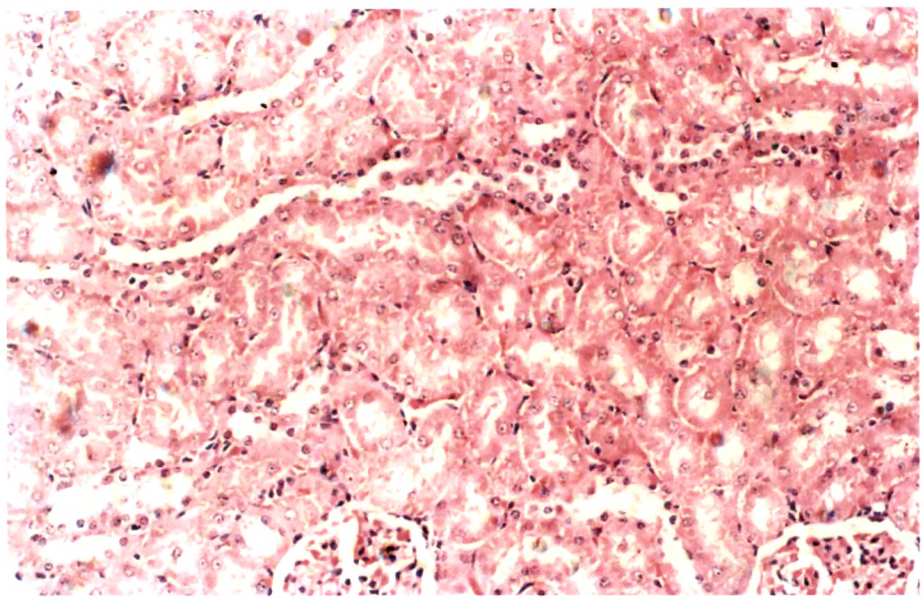


Figure 1: Kidney normal

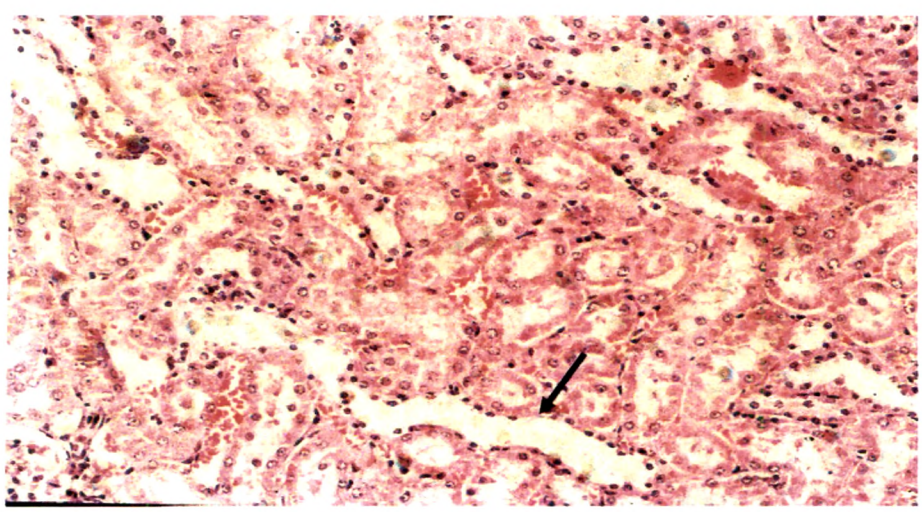


Figure2 : Kidney: Showing(⟵) minimal tubular dilatation with flattening of epithelium (Higher Magnification)-100X

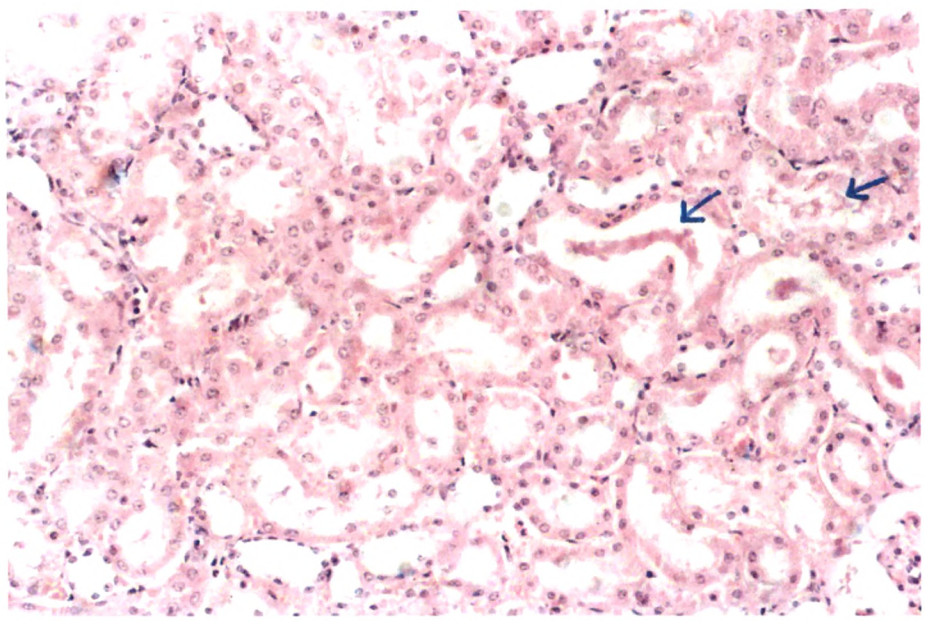


Figure 3: Kidney: Tubular dilatation with eosinophilic material (⟵) in lumen

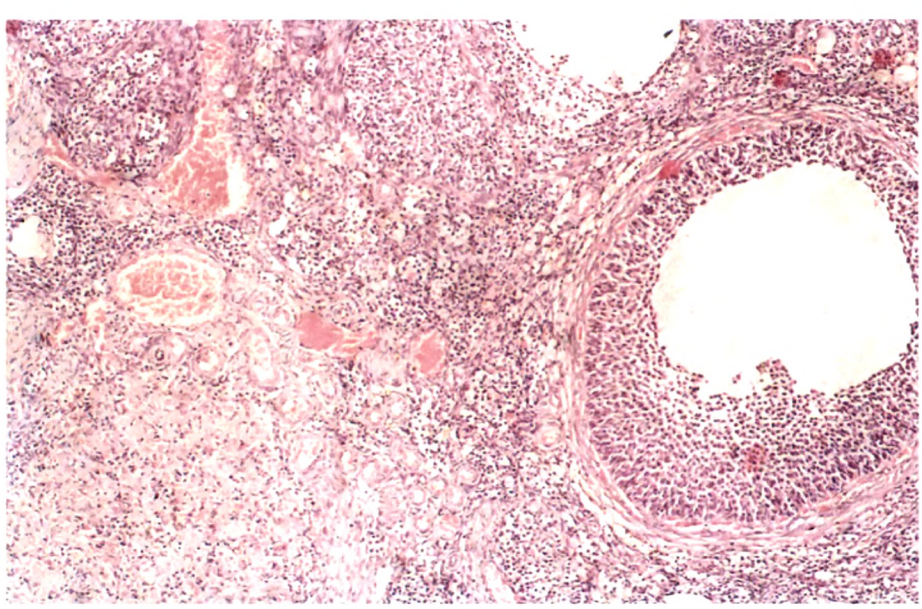


Figure 4: Ovary: Normal follicle

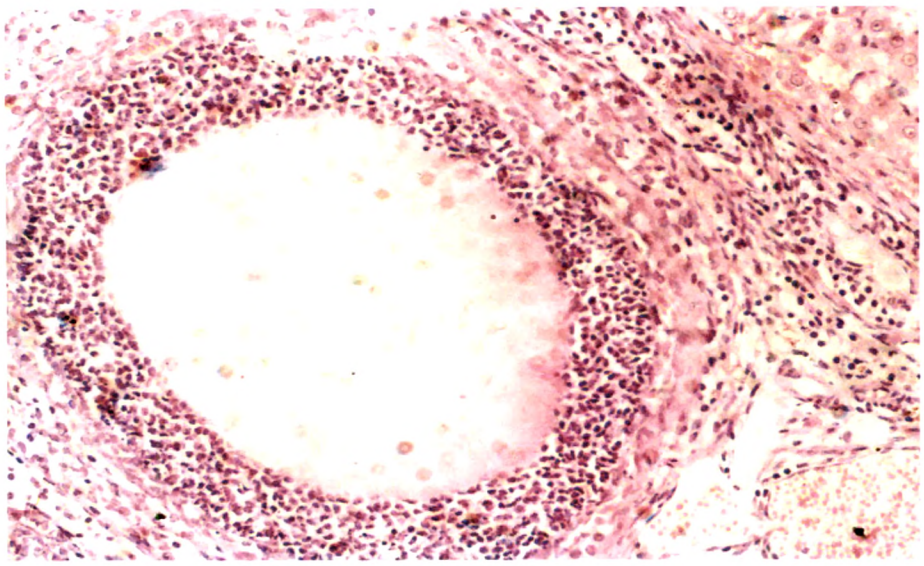


Figure 5: Ovary: Atretic Follicle

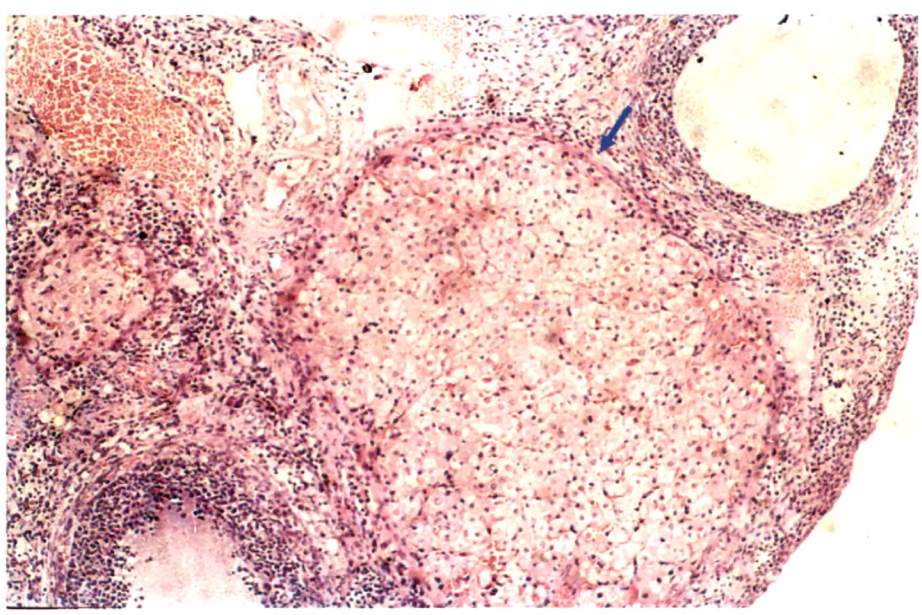


Figure 6: Ovary : Atretic Follicle with corpus leuteum (⟵)

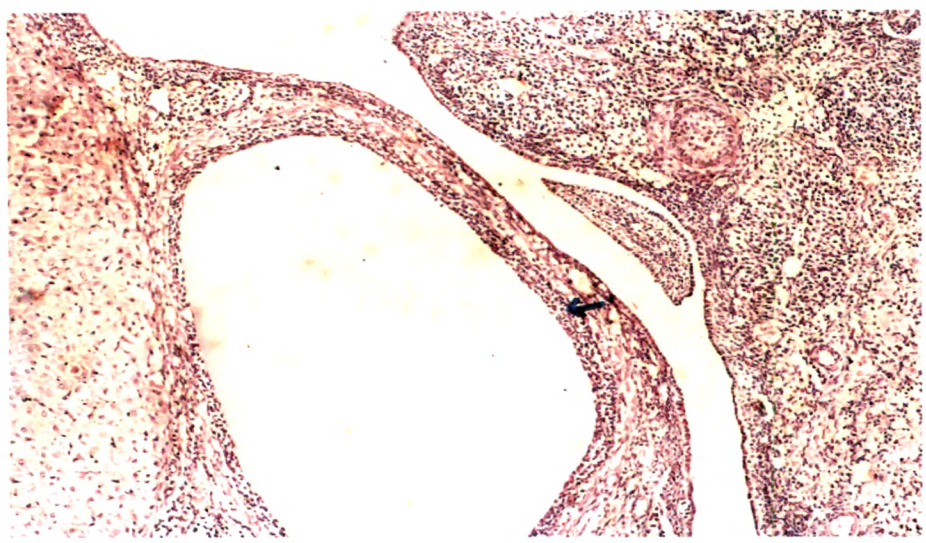


Figure 7: Ovary: Atretic follicle with flattened granulosa cell layer (⟵)

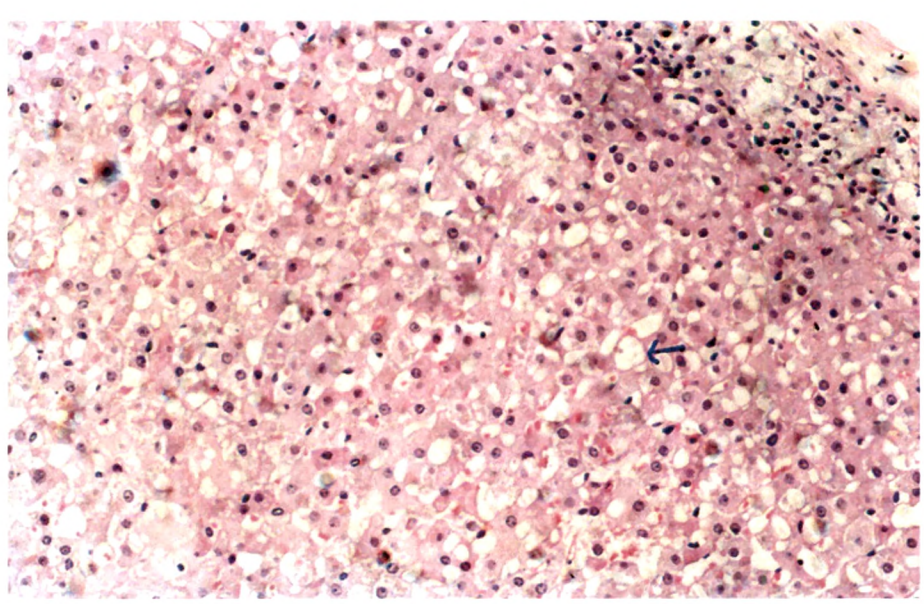


Figure 8: Adrenal : Presence of cytoplasmic vacuolation in zona fasciculate (⟵)

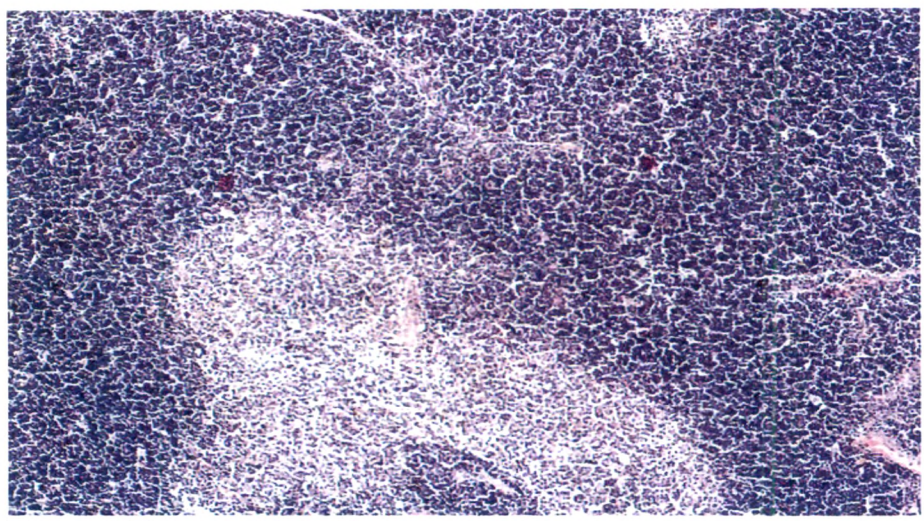


Figure 9: Thymus: Normal

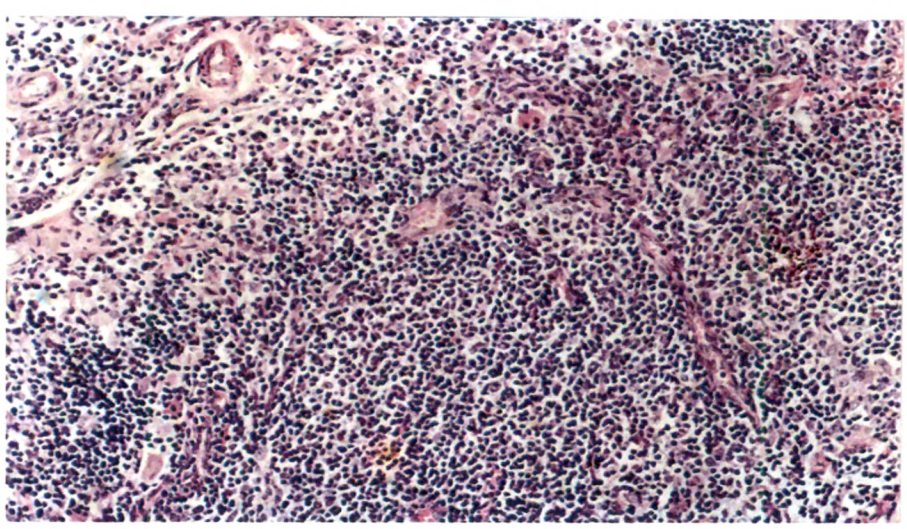


Figure 10: Thymus: Atrophy

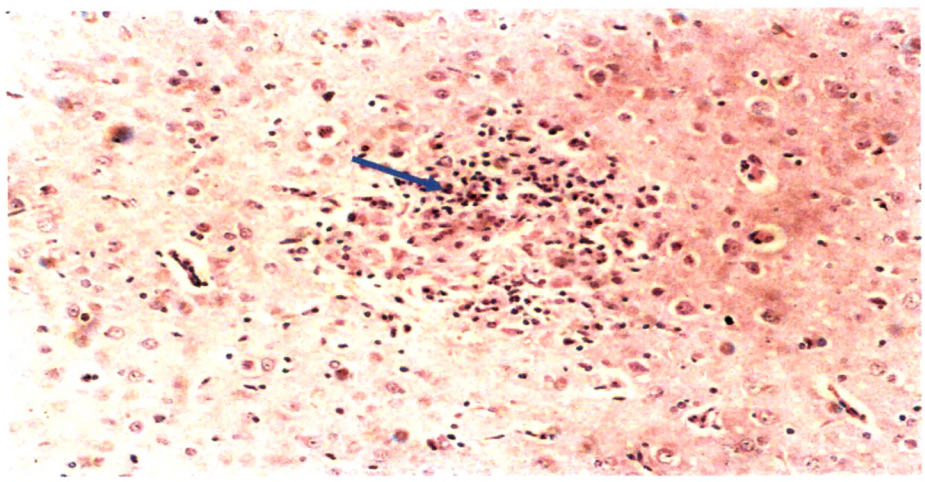


Figure 11:Brain Cortex: Inflammatory Cell Infiltration (⟵)

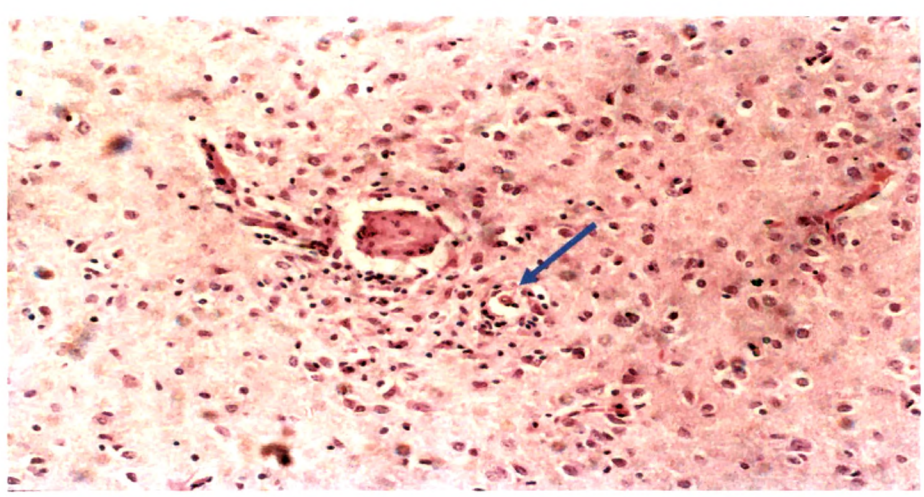


Figure 12:Brain: Perivascular cuffing and neuronal neurosis(⟵)

Pups

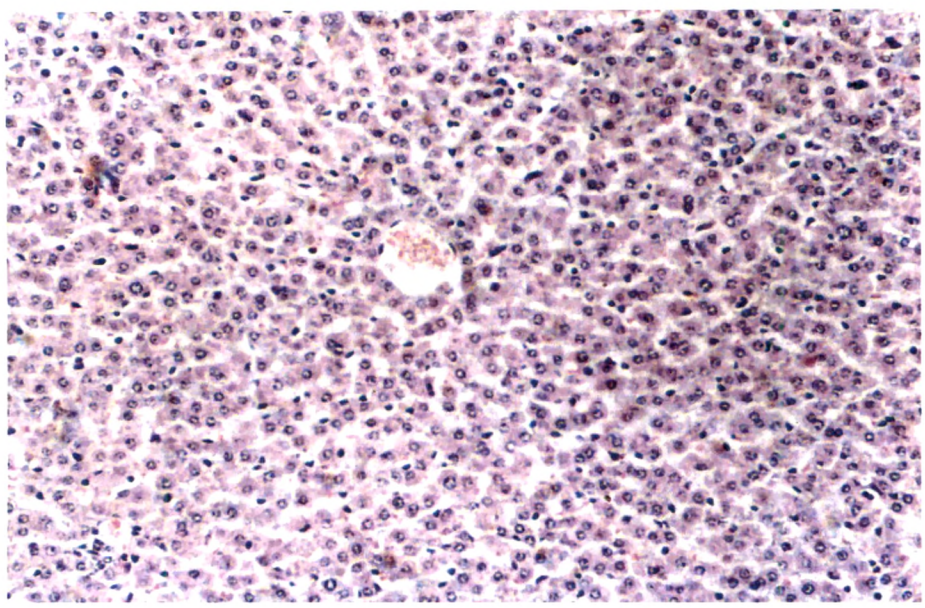


Figure 13: 14:Liver (Pup): Normal

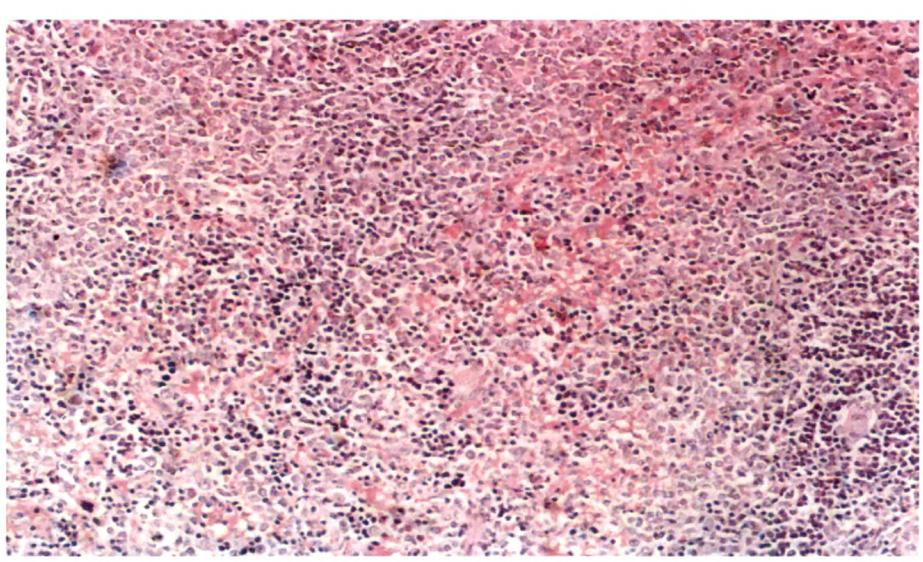


Figure 14: Liver (Pup): Moderate vacuolation and congestion

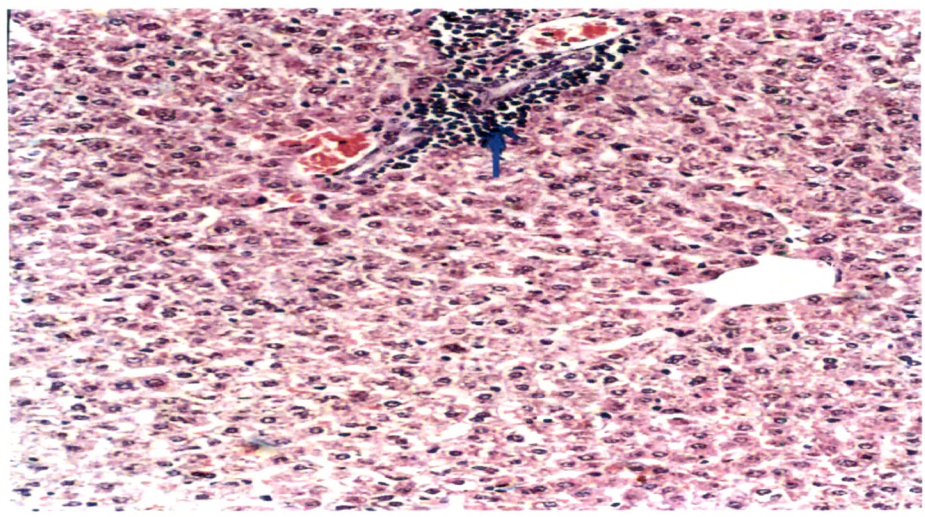


Figure 15: Liver (Pup): Extra medullary hematopoisis (⟷)

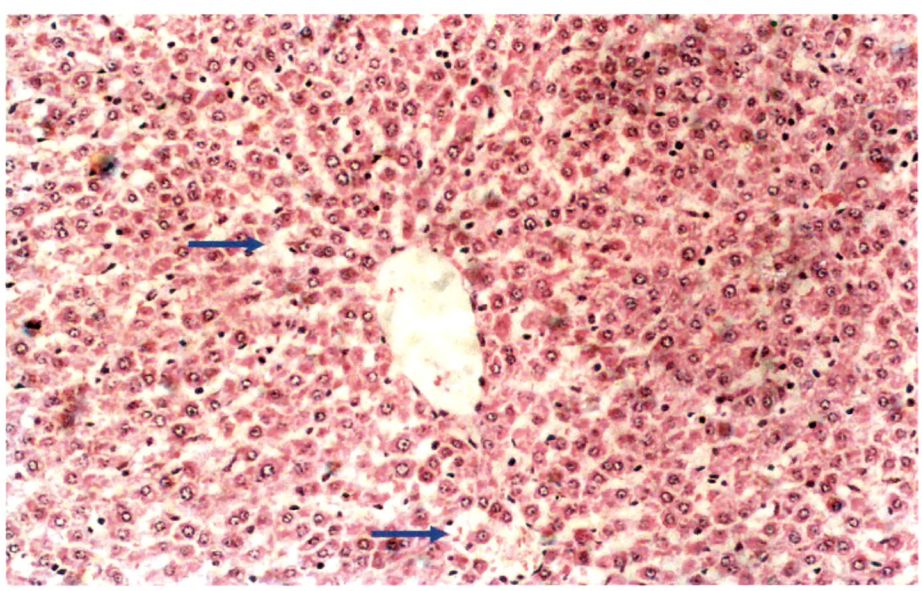


Figure 16: Liver (Pup): Sinusoidal Dilatation(⟷)

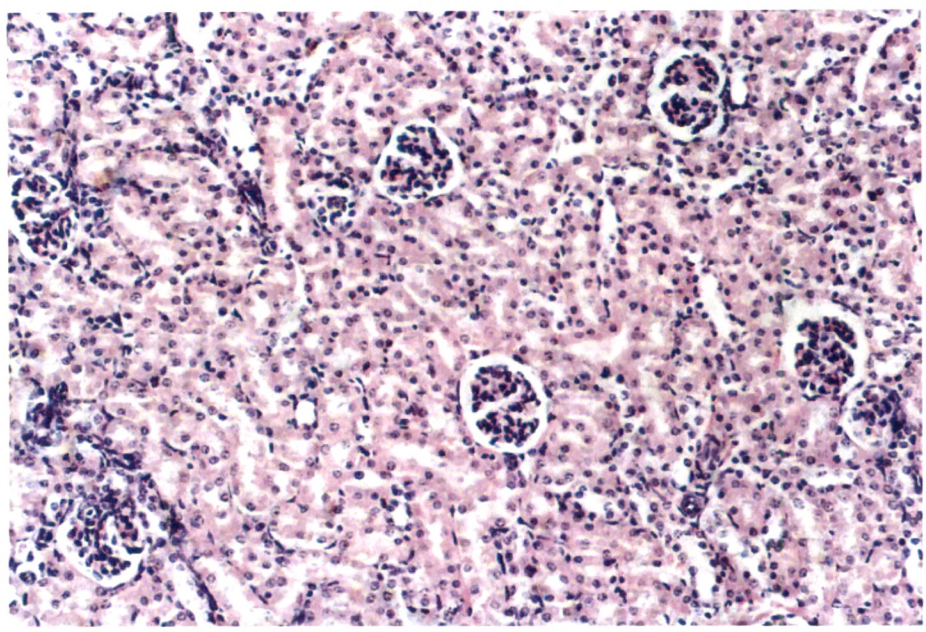


Figure 17: Kidney (Pup): Normal

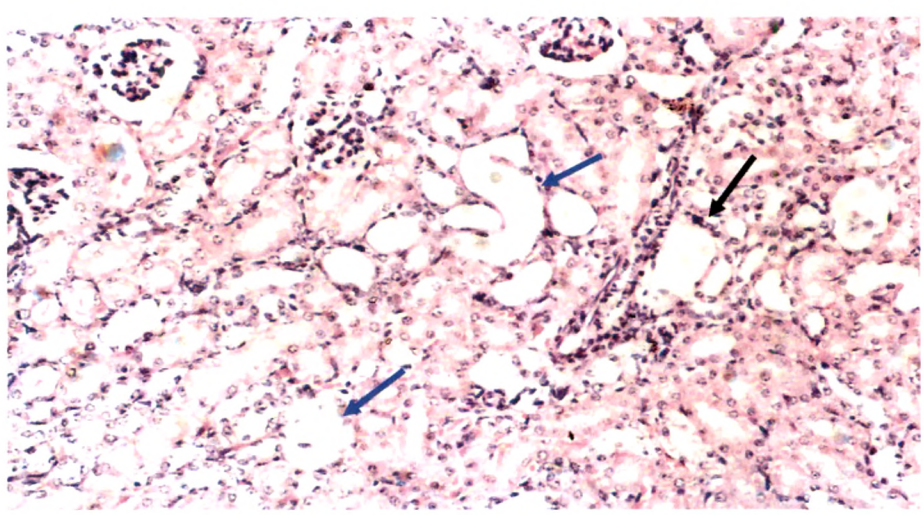


Figure 18: Kidney(pup): Tubular dilatation with flattened epithelium

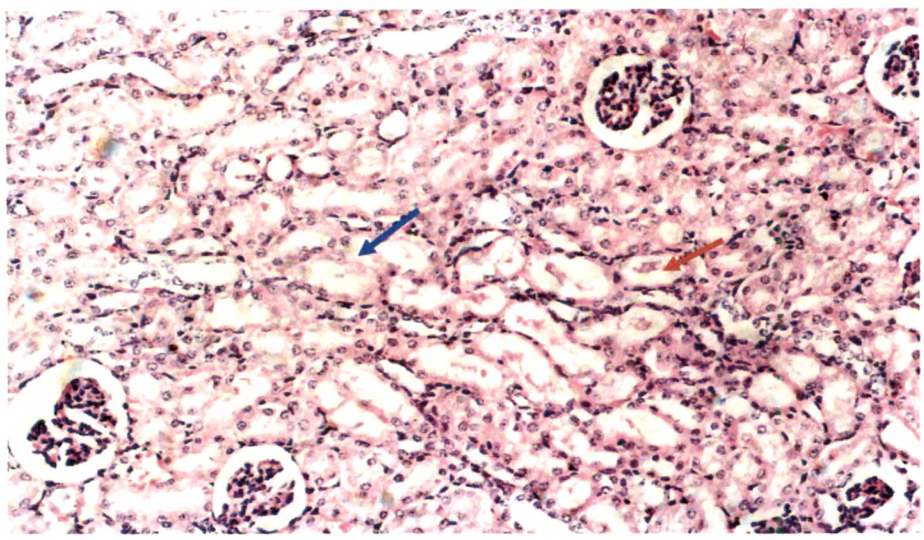


Figure 19: Kidney (Pup): Tubular degeneration, dilatation and eosinophilic material

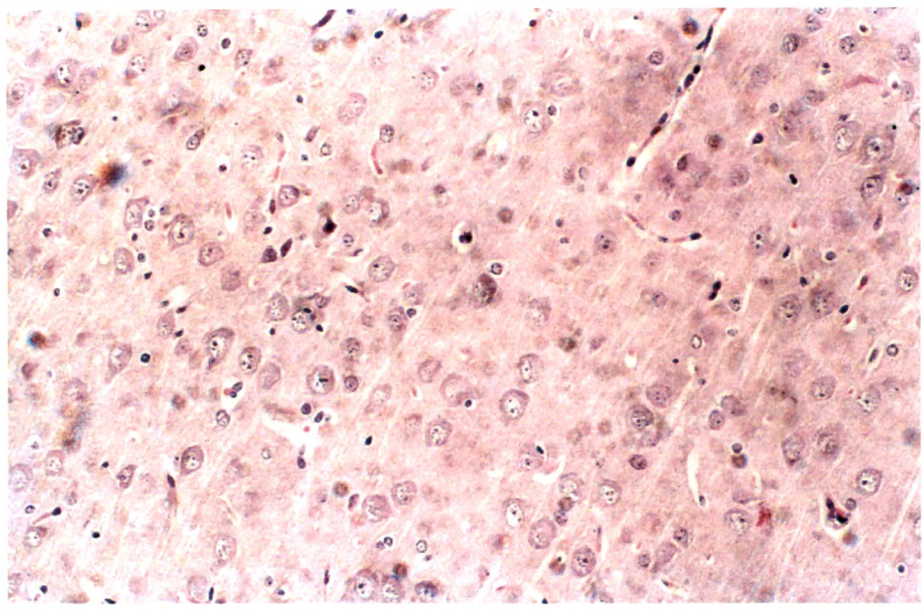


Figure 20: Brain (Pup): Normal

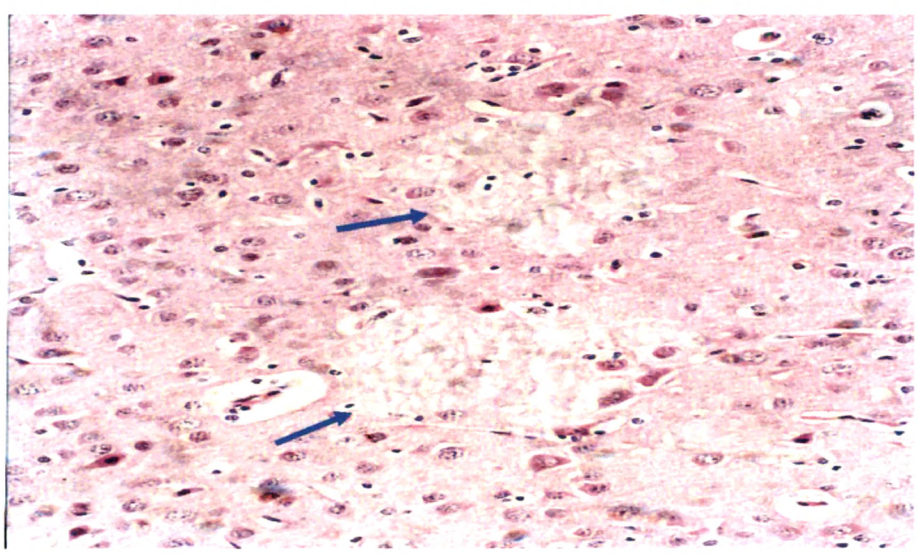


Figure 21: Brain (Pup): Pale and vacoular areas (⟵)

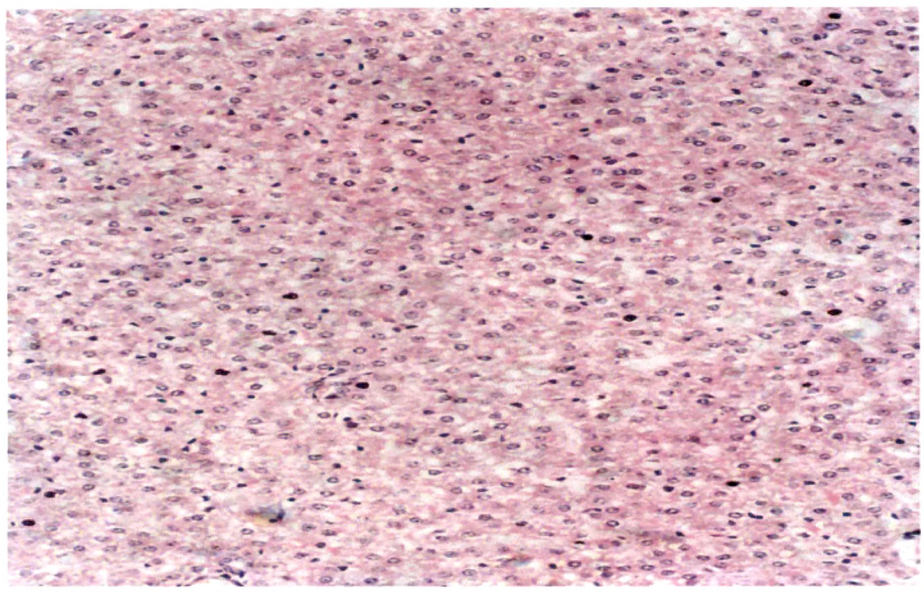


Figure 22: Spleen (Pup): Normal

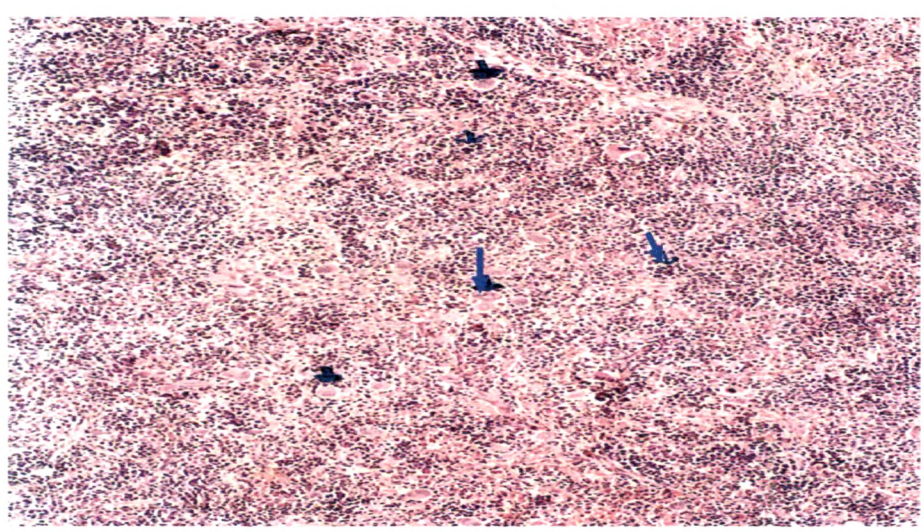


Figure 23: Spleen (Pup): Extra medullary hematopoisis characterized by increase in aggregates of erythroid precursors and megakaryocytes

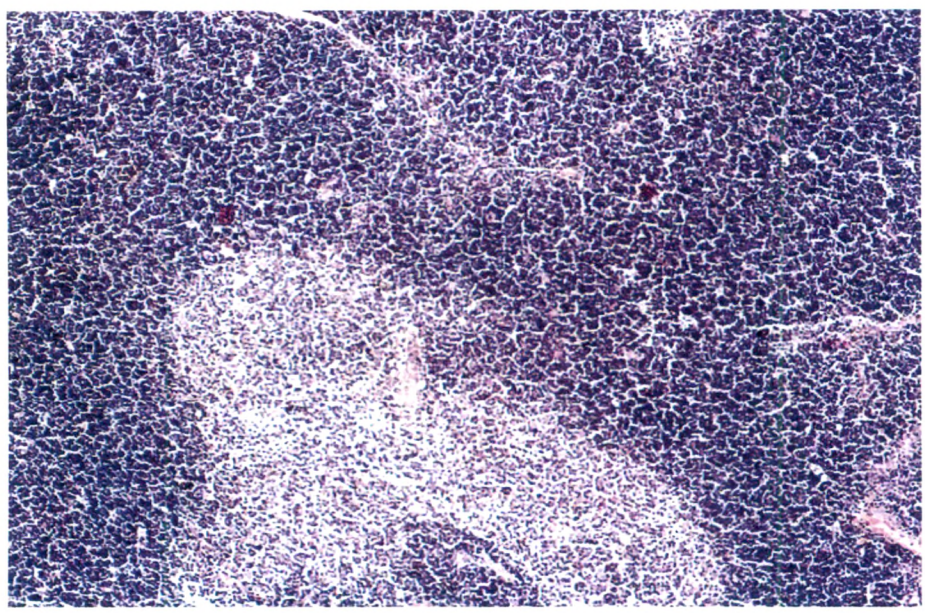


Figure 24: Thymus (Pup): Normal

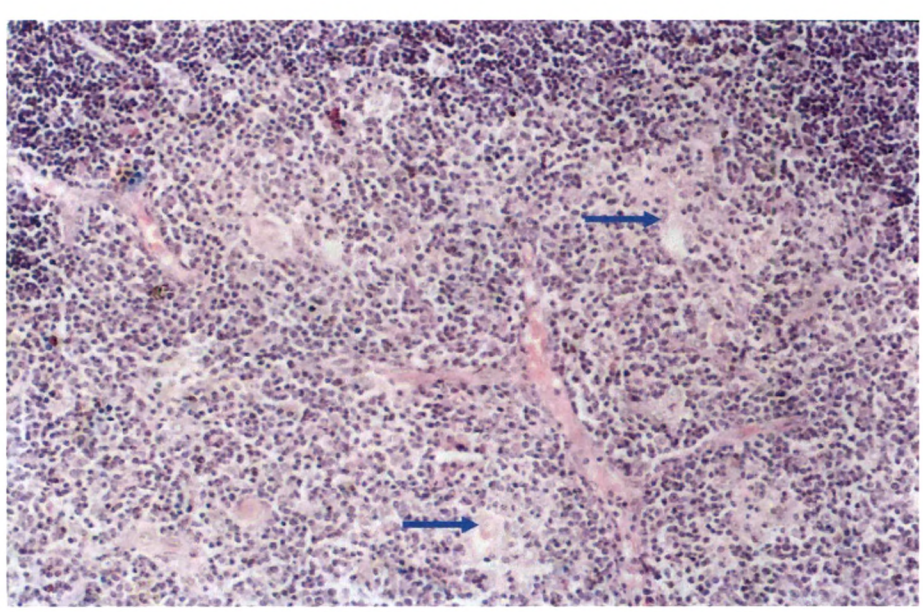


Figure 25: Thymus (Pup): Atrophy characterized by epithiolisation (⟶)