CHAPTER-4

COMBINATION EFFECT OF CHLORPYRIFOS AND LEAD ON NEUROBEHAVIOURAL ASPECTS BY REPEATED SUBCHRONIC DIETARY EXPOSURE

Introduction

Sub-chronic repeated dose studies have been widely used in safety assessment of chemicals by regulatory authorities. Some scientists have suggested that subchronic data may be sufficient to predict long-term, low-dose exposure to a particular compound. While this observation may be true for number of compounds where adequate structure-activity relationships exist, it is not true for compounds where little is known about them or where structure –activity relationships predict a potential adverse effect. For certain chemicals or chemical mixtures, results from a subchronic toxicity study may represent the most sophisticated toxicology available. It has been opined by many toxicologists that subchronic 90 day study is sufficient to find out target organ toxicity in many laboratory animals.

Single or repeated dose exposure to a chemical may produce adverse changes in structure or function of the nervous system. Since both single and repeated dose exposures are possible scenarios for human exposure, neurotoxicity studies must be performed in both situations. Further, adverse change in nervous system can be direct or indirect. Agents can act directly on the target sites of nervous system (direct effect) or outside the nervous system. It is difficult to differentiate between direct or indirect effects of chemicals based on results of single dose exposure studies (OECD, 2003).

Lead has been considered as a well known developmental neurotoxicant. Though animal studies reveal reduced or less action of lead on adult nervous system, severe central nervous system damage (encephalopathic and subencephalopathic symptoms) and peripheral nerve damage and peripheral nerve dysfunction have all been reported in adult human beings during occupational studies. Cognitive deficits have also been observed in lead workers. The overt clinical signs and brain damage and cognitive deficits depended on the exposure concentration.

Chlorpyrifos being an OP compound exerts its toxic action on nervous system by inhibiting cholinesterase enzymes. Repeated subchronic exposure studies conducted for neurotoxicity screening and systemic toxicity studies revealed mild behavioral changes at dose levels of 5 and 15 mg/kg/day (Mattson *et al.*, 1996; Yano *et al.*, 2000). No studies have documented their cumulative effects after long term exposure. Tolerance to CPF exposure has been reported by many workers. However, the action of a chemical changes dramatically when it interacts with other chemical(s). Therefore, the present study was undertaken to investigate the interactive effects of a combination of chlorpyrifos and lead acetate on neurobehaviour in Wistar rats upon oral administration relatively at lower concentrations via diet for a minimum period of 90 consecutive days.

Materials and Methods

The methods adopted in the present study have been based on international guidelines such as those of OECD N° 408 (1998), EU B.26 (2001), OPPTS 870.6200 (1996) and OECD N° 424 (1997) for investigating sub-chronic systemic toxicity and neurotoxic potential of chemicals.

Test Substances

Chlorpyrifos (technical 98.0% purity) was obtained from Enzymes, Pharmaceuticals & Industrial Chemicals Ltd (Factory: 32, Vithoba Industrial Complex, Village Lohop, Post Mazgaon, Tal.Khalapur, Dist. Raigad 410 206. Maharashtra). Lead acetate (99.103% purity), manufactured by s.d. fine CHEM Ltd., was used for the study.

Animals

Healthy Wistar rats consisting of 94 males and 94 females of approximately 4-5 weeks old were obtained from Animal Breeding Facility of Jai Research Foundation. Age of the animals at the time of start of treatment was approximately 5 to 6 weeks.

Acclimatization

Animals were acclimatized to environmental conditions for a minimum period of 5 days. During the acclimatization period, animals were observed for good health.

Environmental Conditions

Rats were maintained in an environment controlled room. The experimental room temperature was 22 ± 3°C. The relative humidity was 55 – 65%. In the experimental room, 12 hours of artificial fluorescent lighting and 12 hours of darkness were maintained. Light hours being 6:00 to 18:00. The experimental room was cleaned and mopped daily with a disinfectant (Suprasol L.C. 5%).

Housing, Diet and Water

The animals were housed in groups of two of same sex per cage in solid floor polypropylene rat cages. Each cage was fitted with stainless steel top grill

and having provision for keeping water bottles. Separate hoppers are attached to each cage. The bottom of the cage was layered with clean, sterile rice husk. The animals were provided with ad libitum laboratory rat powder feed and charcoal filtered UV sterlised water (Aquaguard water filter system).

Grouping

The animals were randomly allocated to 7 main groups and 4 recovery groups. Each main group comprised of 10 males and 10 females, and each recovery group consisted of 6 males and 6 females. Three recovery groups at high dose levels were included to study persistence or reversibility effects. At the start of treatment, the body weight variation among animals was within the ± 20% of mean body weight. The groups used in the study were as follows.

Main Groups

Group 1 (G1) – Vehicle control

Group 2 (G2) – Chlorpyrifos (low dose)

Group 3 (G3) – Lead acetate (low dose)

Group 4 (G4) – Chlorpyrifos (low dose) + Lead acetate (low dose)

Group 5 (G5) – Chlorpyrifos (high dose)

Group 6 (G6) – Lead acetate (high dose)

Group 7 (G7) – Chlorpyrifos (high dose) + Lead acetate (high dose)

Recovery Groups

Group 8 (G1R) – Vehicle control

Group 9 (G5R) – Chlorpyrifos (high dose)

Group 10 (G6R) – Lead acetate (high dose)

Group 11 (G7R) – Chlorpyrifos (high dose) + Lead acetate (high dose)

Animal Identification

Individual animal was identified with picric acid marking over the body coat, and colored cage label showing Group N° and Sex, Dose and Cage N°.

Dose Selection

Based on the available literature and considering the objective of the study, two doses were selected. Ten fold differences were maintained between low and high dose groups. The doses used in the study were as follows.

Main Groups

Group 1 (G1) – Vehicle control

Group 2 (G2) – Chlorpyrifos – 1 ppm

Group 3 (G3) – Lead acetate – 50 ppm

Group 4 (G4) – Chlorpyrifos -1 ppm + Lead acetate - 50 ppm

Group 5 (G5) – Chlorpyrifos -10 ppm

Group 6 (G6) – Lead acetate - 500 ppm

Group 7 (G7) – Chlorpyrifos -10 ppm + Lead acetate - 500 ppm

Recovery Groups

Group 8 (G1R) – Vehicle control

Group 9 (G5R) – Chlorpyrifos - 10 ppm

Group 10 (G6R) – Lead acetate - 500 ppm

Group 11 (G7R) – Chlorpyrifos - 10 ppm + Lead acetate - 500 ppm

Key: b.wt - body weight

Route of Administration and Experimental Diet Preparation

The route of test substance administration was oral through diet. According to group-wise required quantities of test substance and diet were weighed using calibrated balance. Each dose level was prepared separately and

maintained in the respective container. The chlorpyrifos was dissolved in acetone before premixing with the diet. Lead acetate was grounded with small amount of feed in mortar vessel and mixed with approximately 10% of untreated diet for 5 minutes to form a premix. The premix was then brought to the appropriate final concentration i.e., 1, 50, 1+50, 10, 500 and 10 + 500 ppm in diet for group 2, (Chlorpyrifos – 1 ppm), group 3 (Lead acetate – 50 ppm), group 4 (Chlorpyrifos -1 ppm + Lead acetate -50 ppm), group 5 (Chlorpyrifos -10 ppm), group 6 (Lead acetate -500 ppm) and group 7 (Chlorpyrifos -10 ppm + Lead acetate - 500 ppm) respectively with untreated diet, and mixed for 15 minutes in a blender. The experimental diet thus prepared was transferred to polythene bags and stored in labeled stainless steel containers inside the study room. Based on the results of stability test, experimental diet was prepared on weekly basis.

Duration of Treatment

Animals were fed *ad libitum* the test substance incorporated diet for a period of 90 days for 24 hours. Recovery group animals were observed post-treatment for a period of 28 days.

Method of Analysis of Chlorpyrifos Technical in the Test Diet

The stability and homogeneity of chlorpyrifos in the test diet was analysed using HPLC.

The representative samples of animal feed (25 g) were transferred to a blender jar containing 50-100 mL acetone and blended for 3 minutes with some amount of anhydrous sodium sulphate. The blended samples were shaken on orbital shaker for 30 minutes. The extract was filtered using Buchner funnel under vacuum. The filtered cake was re-extracted twice with 50 mL acetone and filtered through the same Buchner funnel. The container

was rinsed twice with 10 mL acetone and filtered through the same filtration assembly. The combined extract was concentrated to 25 mL using rotary vacuum evaporator at <50°C.

The concentrated extract was transferred to separatory funnels containing 25 mL saturated sodium chloride solution. The samples were partitioned with 50 mL n-hexane and the n-hexane layer was collected after passing through a bed of anhydrous sodium sulphate. The aqueous layer was re-extracted twice with 50 mL n-hexane and the n-hexane layer was passed through the same bed of anhydrous sodium sulphate. The extracts were pooled and concentrated to 2-3 mL using rotary vacuum evaporator at <40°C. The concentrated samples were processed for column chromatography.

The chromatographic column was packed with 10g Florisil and 2-3 cm layers of anhydrous sodium sulphate on both the end of the column to absorb moisture present in the sample. The column was pre-washed with 50 mL n-hexane. The concentrated extract was quantitatively transferred to the column and eluted with 150 mL of 5% ethyl acetate in n-hexane. The eluate was collected and concentrated to dryness using rotary vacuum evaporator at <40°C. The concentrated extract was re-dissolved in 10 mL mobile phase and filtered through Whatman N° 42 filter paper. The samples were analysed by HPLC for quantitation.

Calibration

A quantity 0.01g of chlorpyrifos was weighed in 10 mL volumetric flask and the volume was made up to the mark with mobile phase. The 0.5, 1.0, 2.0 5.0, and 10.0 ppm serial dilutions were made using stock solution (1000

ppm). The diluted sample was injected in duplicate onto HPLC and was found linear for 0.5 to 10.0 ppm level.

Accuracy and Precision

For accuracy, the quantity of 1.0 ppm and 10.0 ppm was fortified with the substrate (feed) in duplicate and extracted by using procedure as mentioned above. Following the same validated procedure, the precision was carried out in single fortification level (1.0 ppm) with five replications. The accuracy and precision were 91.90% and 92.00% respectively.

HPLC features

Instrument	: Shimadzu LC-10 and SPD-10A detector with CLASS LC-10 software
Column	: ODS [25 cm x 4.6 mm (i.d.); 5 um particle size]
Mobile Phase	: Methanol (90): Water (10)
Wavelength	: 290 nm
Flow rate	: 1 mL/min
Injection volume	: 20 µL
Retention time	: 5.4 minutes

Calculation

$$\text{Active Ingredient (ppm)} = \frac{(Y - a) \times V}{b \times W} \times F$$

Y = Peak area of the sample

a = Constant

b = Regression Co-efficient for Y on X

V = Volume of the extract (mL)

W = Weight of the sample (g)

F = Recovery factor = 100/Percent mean recovery

The stability study was performed at 0, 4th and 7th day. The percent recovery at 0, 4th and 7th day was 96.5, 94.25 and 89.7 for 1 ppm and 97.05, 93.95 and 89.55 for 10 ppm of chlorpyrifos in diet. The test substance was homogeneous with the experimental diet (Table 9).

Method of Analysis of Lead Acetate in the Test Diet

The homogeneity of lead acetate in the test diet was analyzed using atomic absorption spectrophotometer. The representative samples of animal feed were grounded in a blender jar, mixed well, and sieved through 1 mm sieve. One gram sample was taken in a beaker (250- 500 mL capacity) and 10-25 mL concentrated nitric acid (HNO_3) was added. The beaker was covered with a watch glass and boiled gently on a hot plate for 30-45 minutes to oxidize the entire matrix. The sample was cooled and 10 mL perchloric acid ($HCLO_4$) was added. The sample was cooled, filtered, diluted to 100 mL with distilled water and analyzed by atomic absorption spectrophotometer (AAS).

Homogeneity tests were detected in three random samples. The homogeneity results indicated that lead acetate was homogeneous with the feed and, the percent recovery for 500 ppm of lead acetate in experimental diets was 92.4, 92.5 and 92.3% for samples 1, 2 and 3 respectively (Table 9).

OBSERVATIONS

Clinical Signs

Animals were observed for mortality and morbidity twice a day. Observations were made daily for visible signs and symptoms such as skin and fur changes, eye and mucous membrane changes, respiratory, circulatory, autonomic and central nervous system disturbances as well as, somatomotor activity, behavioral pattern and general changes.

Neurobehavioral Tests (Functional Observational Battery)

Functional observational tests were conducted to assess the behavioral and neurological status of each animal. FOB was performed at the end of 4th, 13th and 17th weeks. The procedure for neurobehavioral tests was same as that for single dose study (Chapter 1).

Body Weight

Individual animal was weighed on the day of commencement of treatment and at weekly intervals during the experimental period.

Food Consumption

The weekly food consumption of the animals was calculated on weekly basis using the following formula.

$$\text{Food consumption (g/rat/week)} = \frac{\text{Feed input in the week} - \text{feed left over in the week}}{\text{Number of rats in the cage}}$$

Chemical Intake

The achieved test material intake by the animal was calculated using the formula mentioned below.

$$\text{Chemical Intake (mg/kg b. wt. /day)} = \frac{\text{Inclusion level of chlorpyrifos/lead acetate in diet (ppm)} \times \text{Diet consumed (g/rat/day)}}{\text{Average initial b.wt. in the cage} + \frac{[\text{Average b. wt. gain}]}{2}}$$

Evaluation of Data

Statistical evaluations were performed using validated statistical software (Developed by Jai Research Foundation). All the parameters characterized by continuous data were subjected to Bartlett's test to meet the homogeneity of variance before conducting Analysis of Variance (ANOVA) and Dunnett' s t-test. Where the data did not meet the homogeneity of variance, Student's t-test was performed to calculate significance. The significance was calculated at 5% ($P \leq 0.05$) and 1% ($P \leq 0.01$) level.

RESULTS

Subchronic exposure to chlorpyrifos, lead acetate and a combination of the two for 90 days was carried out in Wistar rats to investigate the effects on neurobehavioral changes. The results obtained from the study are as follows.

Clinical Observations

No mortality was observed during the entire course of experiment in any of the groups. Treatment related clinical signs were observed in group 5 (CP-10 ppm) and group 7 (CP-10 ppm + LA-500 ppm) animals of either sex. Treatment related clinical signs such as perennial staining of nose, chromorhinorrhoea, lacrimation and chromodacryorrhoea were observed in some of the animals belonging to groups 5 and 7. These treatment related clinical signs were observed predominantly during the early phase of the experimentation i.e., weeks 2 and 3 as represented in Figures 1A—1F. During later part of the exposure period, few animals from treatment groups (one animal each in high dose groups) showed perennial staining of nose and chromodacryorrhoea. Further, one male each belonging to groups 1 (week 1), 2 (week 4), 6 (week 6), 7 (week 9), group 6R (week 5) and group 7R (week 16) showed nasal discharge. One female belonging to group 2 (week 7) showed snuffles. Snuffles and nasal discharge were considered to be incidental findings and not related with the treatment.

No marked variations were observed in clinical signs associated with treatment between group 5 and group 7 animals i.e., between chlorpyrifos alone and in combination with lead acetate.

Neurobehavioral Observations/Functional Observational Batteries

Activity Measures (Posture, Rearing and Motor Activity)

Posture

Posture observation during home cage observation did not reveal any treatment related posture change in either sex.

Rearing

Vertical movements in the open field were slightly reduced but not statistically significant, in groups 4, 5 and 7 of male animals at the end of 4th week. With reference to females, in addition to groups 4, 5 and 7, group 6 females also showed slight reduction in rearing count after exposure to the test substance for a period of 4 weeks. The percent reduction in the mean values of rearing counts at the end of 4 weeks in males was 5.5, 9.0 and 9.0 % in groups 4, 5 and 7 respectively. In females, percent reduction was 12.2, 16.3, 13.8, and 17.8% in groups 4, 5, 6 and 7, respectively. At the end of 13 weeks, mean values of rearing counts for treatment group were comparable to the control group of animals (Table 1; Figure 2).

Motor Activity

All kinds of motor activity i.e., total activity, ambulatory activity and stereotypic activity of treatment groups, were comparable to the control group of animals at the end of weeks 4, 13 and 17 for either sex (Tables 3A – 3E).

Convulsive Domain (Tremor, Clonic and Tonic convulsion)

No animal from either sex showed any kind of clonic or tonic type of convulsions during the entire course of experimental period.

Excitability Measures (Ease of Removal and Handling Reactivity, Arousal, Vocalisation, Circling and Stereotypic and Bizarre behaviors)

Ease of removal from the cage was observed to be easy for majority of rats, though few rats across the groups reacted as very easy, moderately difficult or difficult without any consistent difference between the groups. The observed trend was a normal occurrence during the study of behavioral tests. Majority of the animals, either male or female, from all the groups reacted easily while animals were being held.

Arousal

Arousal rate in majority of the animals of either sex was high. Few animals across the groups showed very low and low arousal rates, which are common findings during neurobehavioral observational tests.

Vocalizations, Stereotypy and Bizarre Behaviors

No animal of either sex revealed vocalizations, stereotypy and bizarre behaviors during the course of scheduled behavioral observation tests.

Autonomic Measures (Lacrimation, Salivation, Pupil response, Palpebral closure, Piloerection, Eye examination, Skin examination. Urination and Defecation counts)

During neurobehavioral observations, no animal had shown either lacrimation or salivation. During handling, eye lids were examined for their opening condition. All animals belonging to either sex showed wide opening of eyelids. Piloerecton was absent in all the animals belonging to any group. Skin of the animals of both sexes of all groups appeared normal. No eye abnormalities were observed in either sex of animals treated with test substances.

Pupil Response

Majority of animals from both sexes sex showed normal response to light stimulus. However, one each from control and low dose (during week 4), two (one male + one female) from group 6 (during week 13) and one male from group 7 (during week 13) did not respond to light stimulus. These changes are considered to be incidental and commonly seen during behavioral observations.

Urination and Defecation Counts

No significant variations were observed in mean values of urination and defecation counts of treatment group animals as compared to control group animals of either sex (Tables 2A and 2B).

Neuromuscular Measures (Grip Strength, Hind Limb Foot Splay, Gait, Air Righting Response)

Grip Strength

The forelimb and hind limb grip strength measurements performed at the end of weeks 4, 13 and 17 did not reveal any treatment related statistically significant changes (Tables 4A and 4B).

Hind Limb Foot Splay

The mean hind limb foot splay values of treatment group of animals were comparable to the control group of animals at all the intervals (Table 5).

Air Righting Response

All the animals belonging to either sex had normal gait during the scheduled behavioral tests. When the animals were dropped from a height of approximately 30 cm to measure the air righting reflex, the ease of

uprightness of the landing was normal in all the animals. All the animals landed squarely on their legs.

Sensorimotor Measurements (Approach response, Touch response, Click response and Tail flinch response)

Almost all animals belonging to control and treatment groups showed fast approach response when the object was held in front at all intervals. Few animals of either sex across the groups showed low or no response when approached with the object. These findings are usual during behavioral observations. Normal touch response was shown by all animals from all groups at all intervals. There was no observable difference between the groups to sound stimuli at all intervals of sensory reactivity measurements. However, during week 4, one male each from groups 3, 6 and 7 showed no response to click stimuli. No treatment related changes were observed in any of the groups when tail tip was squeezed with forceps at all intervals of study. At the end of week 4, all the animals showed flinch response to stimulus. However, during weeks 13 and 17, few animals showed only slight reaction to flinch stimulus.

Other Measures

Body Weight

Males

No statistically significant differences were observed in weekly mean body weights of treatment group animals as compared to respective control groups throughout the exposure period. However, close observation of mean body weight data revealed slight reduction in mean body weight of group 7 males from week 2 to week 13 as compared to control group males. The percent decrease was 3.8, 2.4, 4.9, 5.6, 5.5, 7.1, 8.6, 6.9, 7.0, 6.2 and 5.1

respectively in weeks 2 – 13, as compared to control group animals (Tables 6A and 6C; Figure 3).

Females

Weekly mean body weights of treatment group animals were comparable to control group animals (Tables 6B and 6D; Figure 4).

Food Consumption

No significant differences were observed in mean food consumption of treatment group animals as compared to control group animals (Tables 7A - 7D).

Chemical Intake

Males

The average achieved mean chemical intake for 13 weeks of exposure in groups 2, 3, 4, 5, 6 and 7 were 0.09 (range :0.12 - 0.07), 4.2 (range:5.9 - 3.44), 0.08 (range:0.13 - 0.06) + 4.1 (range:6.42 - 3.33), 0.86 (range:1.26 - 0.66), 42.5 (range:62.68 - 35.45) and 0.85 (range:1.3 - 0.71) + 42.3 (range:64.94 - 34.28)mg/kg/body weight/day respectively. The weekly mean chemical intake data is represented in Tables 8A and 8C.

Females

The average achieved mean chemical intake for 13 weeks of exposure in groups 2, 3, 4, 5, 6 and 7 were 0.10 (range :0.12 - 0.08), 4.55 (range:5.99 - 3.98), 0.09 (range:0.12 - 0.08) + 4.54 (range:5.96 - 4.2), 0.1 (range:11.28 - 0.86), 44.5 (range:60.5 - 40.47) and 0.88 (range:1.22 - 0.74)+ 44.1 (range:61.1 - 37.2) mg/kg/body weight/day respectively. The weekly mean chemical intake data is represented in Table 8B and 8C.

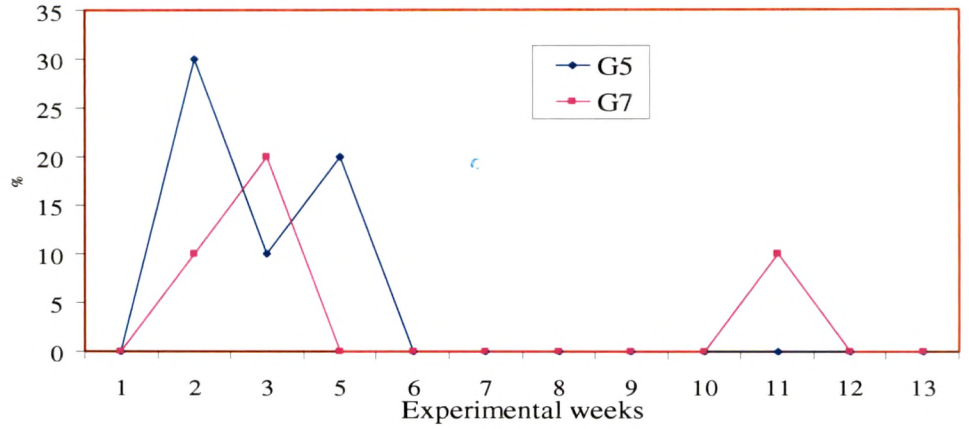

Figure 1A. Percent of G5 (chlorpyrifos -10 ppm) and G7 males (chlorpyrifos – 10 ppm plus lead acetate – 500 ppm) showing clinical sign – **chromorhinorrhoea** –Main group males.

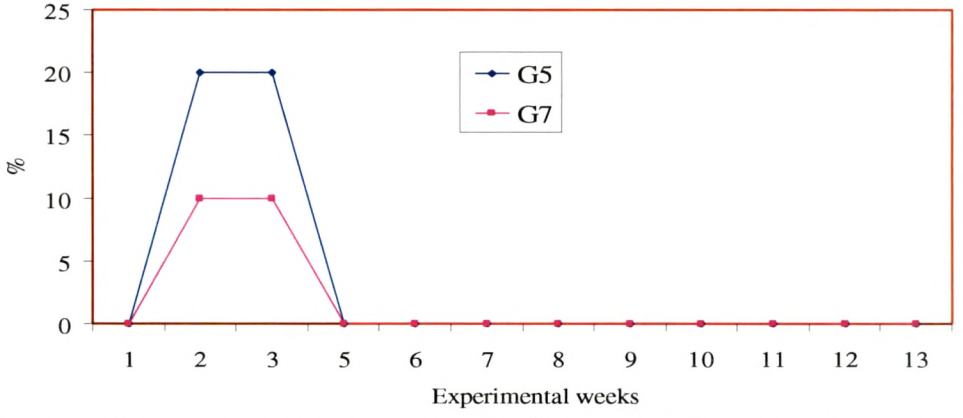

Figure 1B. Percent of G5 (chlorpyrifos -10 ppm) and G7 animals (chlorpyrifos – 10 ppm plus lead acetate – 500 ppm) showing clinical sign – **chromorhinorrhoea** – Main group females

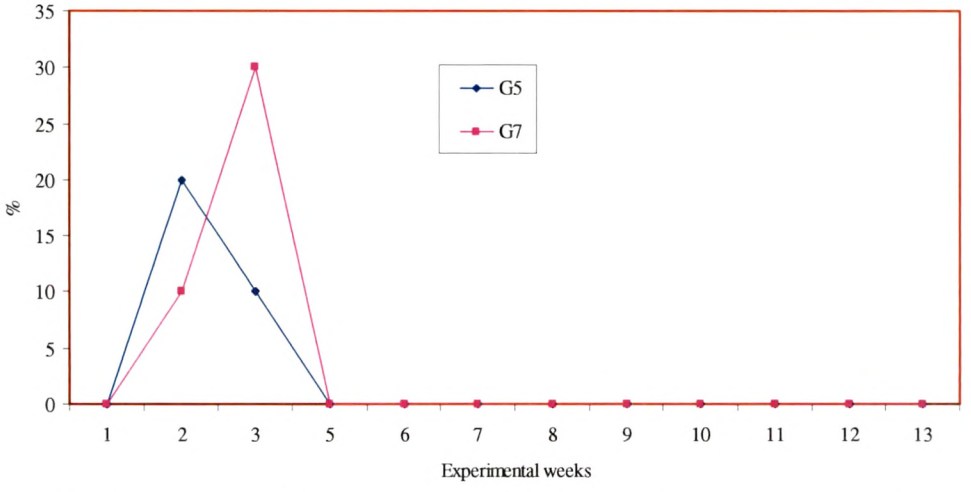

Figure 1C. Percent of group 5 (chlorpyrifos -10 ppm) and group 7 animals (chlorpyrifos – 10 ppm plus lead acetate – 500 ppm) showing clinical sign –**lacrimation –** Main group males

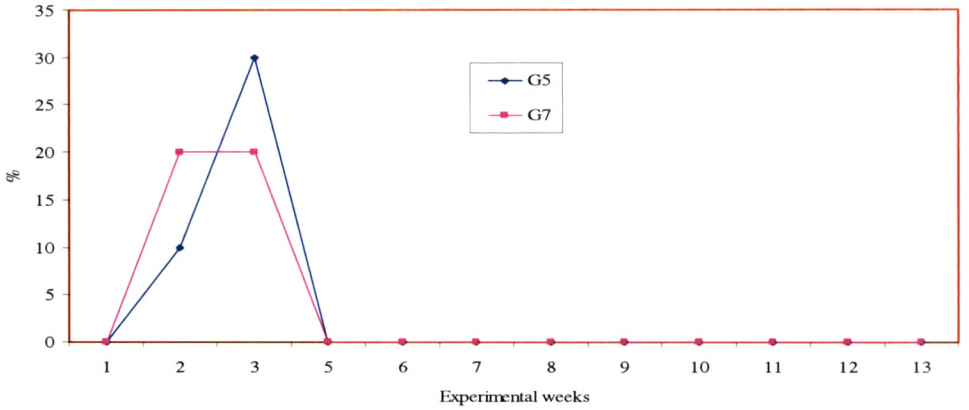

Figure 1D. Percent of group 5 (chlorpyrifos -10 ppm) and group 7 animals (chlorpyrifos – 10 ppm plus lead acetate – 500 ppm) showing clinical sign – **lacrimation –** Main group females

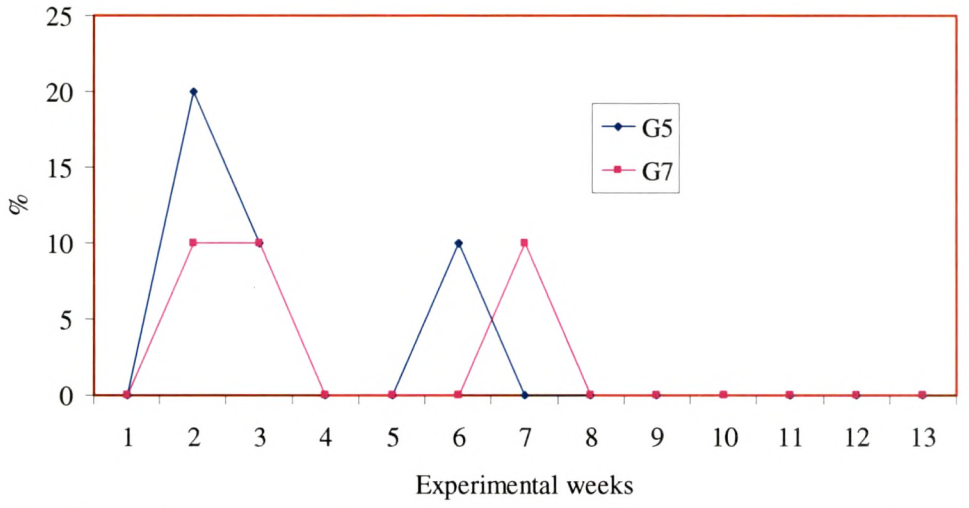

Figure 1E. Percent of group 5 (chlorpyrifos -10 ppm) and group 7 animals (chlorpyrifos – 10 ppm plus lead acetate – 500 ppm) showing clinical sign – **chromodacryorrhoea** – Main group males

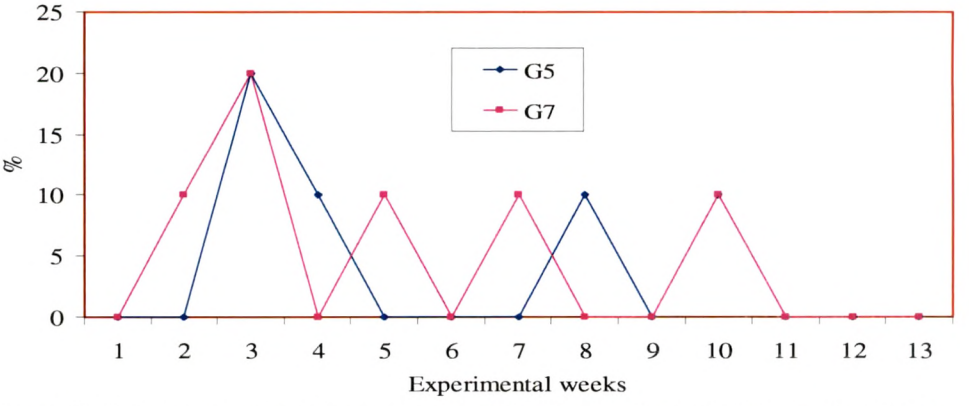

Figure 1F. Percent of group 5 (chlorpyrifos -10 ppm) and group 7 animals (chlorpyrifos – 10 ppm plus lead acetate – 500 ppm) showing clinical sign **– chromodacryorrhoea** – Main group females

Table 1

Rearing Count - Group Mean Values

Dose: G1- 0; G2 (CPF) - 1; G3 (LA) - 50; G4 (CPF+LA) - 1+50; G5 (CPF) - 10; G6 (LA) - 500; G7 (CPF+LA) - 10+500 ppm in diet

Parameter : Rearing count	Experimental Period							
	Male				Female			
Group N°	5th week		13th week		5th week		13th week	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
G1(N=8)	11.1	3.4	8.5	2.87	12.3	2.38	11.9	3.64
G2 (N=8)	13.5	1.93	9.5	3.02	11.5	2.67	12.1	1.13
G3 (N=8)	12.9	3.72	9.9	2.36	11.3	2.76	11.1	2.47
G4 (N=8)	10.5	4.78	7.3	1.67	10.8	2.19	13.4	2.92
G5 (N=8)	10.1	2.36	8.3	1.83	10.3	1.83	12.8	1.91
G6 (N=8)	12.6	1.69	8.8	2.43	10.6	2.13	9.5	4.5
G7 (N=8)	10.1	2.7	8.5	2.2	10.1	2.47	13.0	2.27

Parameter : Rearing count	Experimental Period			
	17th week			
Group N°	Male		Female	
	Mean	SD	Mean	SD
G1R (N=6)	12.2	3.92	15.0	3.79
G5R (N=6)	12.7	3.27	12.3	3.44
G6R (N=6)	12.7	2.14	11.7	1.63
G7R (N=6)	9.5	3.27	12.8	1.94

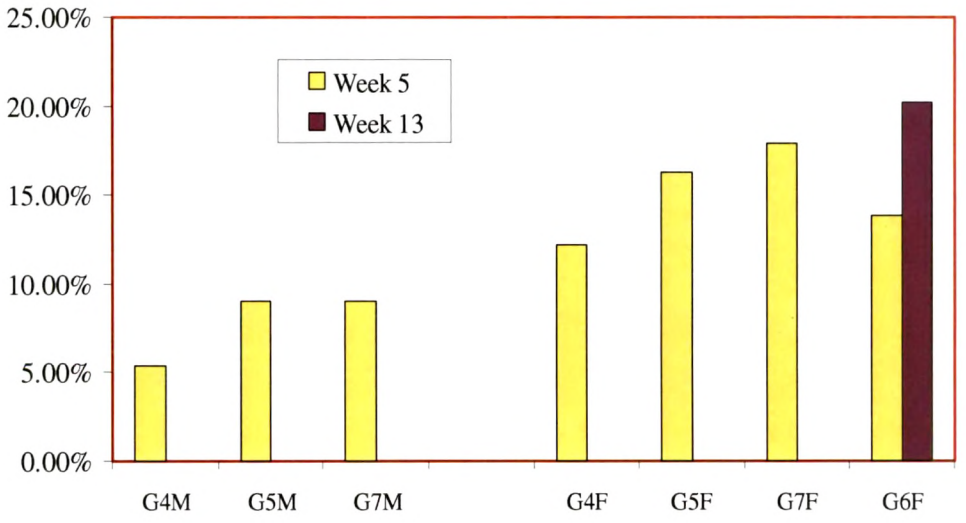

Figure 2. Percent decrease of mean rearing counts in open field on weeks 5 and 13 in animals belonging to G4 (chlorpyrifos – 1 ppm + lead acetate 50 ppm), G5 (chlorpyrifos – 10 ppm), G6 (lead acetate – 500 ppm) and G7 (chlorpyrifos – 10 ppm + lead acetate 500 ppm) as compared to control.

Table 2A

Urination Count - Group Mean Values

Dose: G1- 0; G2 (CPF) - 1; G3 (LA) - 50; G4 (CPF+LA) - 1+50; G5 (CPF) - 10; G6 (LA) - 500; G7 (CPF+LA) - 10+500, ppm in diet

Open Field Observations	Experimental Period							
Parameter : Urination Count	Male				Female			
Group N°	5th week		13th week		5th week		13th week	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
G1 (N=8)	2.3	1.91	3.00	2.07	2.4	2.77	0.9	1.25
G2 (N=8)	2.4	2.67	5.4	2.5	2.5	1.85	2.0	2.83
G3 (N=5)	2.7	2.7	4.5	3.25	1.3	1.49	3.3	2.25
G4 (N=8)	3.6	2.4	1.8	1.16	1.4	2.6	1.7	2.00
G5 (N=8)	0.9	1.36	2.6	1.19	2.9	4.52	3.4	4.10
G6 (N=5)	1.4	1.59	2.4	2.07	2.8	4.23	0.9	1.81
G7 (N=8)	1.4	1.41	2.0	1.93	2.8	3.15	3.0	4.38

Recovery groups

Dose: G1R - 0; G5R (CPF) - 10; G6R (LA) - 500; G7R (CPF+LA) - 10+500 ppm in diet

Open Field Observations	Experimental Period			
Parameter : Urination Count	17th week			
Group N°	Male		Female	
	Mean	SD	Mean	SD
G1R (N=6)	3.4	1.17	3.2	1.6
G5R (N=6)	1.2	1.60	2.2	2.86
G6R (N=6)	2.0	1.10	0.5	0.55
G7R (N=6)	1.8	1.47	2.8	4.31

PE = Pre-exposure, N = Number of animals

Table 2B

Defecation Count - Group Mean Values

Dose: G1- 0; G2 (CPF) - 1; G3 (LA) - 50; G4 (CPF+LA) - 1+50; G5 (CPF) - 10; G6 (LA) - 500; G7 (CPF+LA) - 10+500 ppm in diet

Open Field Observations	Experimental Period							
Parameter : Defecation Count	Male				Female			
Group N°	5th week		13th week		5th week		13th week	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
G1 (N=8)	1.6	2.0	2.4	2.00	0.8	2.12	0.0	0.00
G2 (N=5)	2.3	1.75	3.3	1.4	0.9	1.81	0.3	0.46
G3 (N=8)	1.6	1.99	2.3	1.91	1.4	2.5	0.8	1.16
G4 (N=8)	2.6	2.83	1.3	1.91	0.0	0.0	0.0	0.00
G5 (N=8)	1.9	2.58	1.6	1.30	0.1	0.35	1.4	2.26
G6 (N=8)	1.9	2.95	1.3	1.28	1.1	2.23	0.6	1.41
G7 (N=8)	2.0	2.45	2.4	2.72	0.1	0.35	0.0	0.00

Recovery groups

Dose: G1R - 0; G5R (CPF) - 10; G6R (LA) - 500; G7R (CPF+LA) - 10+500 ppm in diet

Open Field Observations	Experimental Period			
Parameter : Defecation Count	17th week			
Group N°	Male		Female	
	Mean	SD	Mean	SD
G1R (N=6)	2.0	2.10	0.7	1.03
G5R (N=6)	1.2	1.83	0.0	0.0
G6R (N=6)	4.2	3.76	0.0	0.0
G7R (N=6)	1.2	2.86	0.5	1.22

PE = Pre-exposure, N = Number of animals

Table 3A

Motor Activity - Group Mean Values

Dose: G1- 0; G2 (CPF) - 1; G3 (LA) - 50; G4 (CPF+LA) - 1+50; G5 (CPF) - 10; G6 (LA) - 500; G7 – (CPF+LA) - 10+500 ppm in diet

Sex : Male **Period : 4th Week**

Group N° (N=8)		Time Interval and Parameter								
		0-10 minutes			11-20 minutes			21-30 minutes		
		Total	Ambu-latory	Stereo-typic	Total	Ambu-Latory	Stereo-typic	Total	Ambu-latory	Stereo-typic
G1	Mean	33.0	30.0	13.6	26.7	24.1	11.5	22.9	20.7	9.8
	SD	2.42	2.17	1.21	4.92	4.42	2.29	6.63	6.20	2.46
G2	Mean	34.8	32.0	13.6	28.3	25.8	11.7	23.6	21.2	10.3
	SD	2.69	2.82	0.76	3.31	3.28	1.02	4.76	4.67	1.50
G3	Mean	33.6	30.7	13.8	28.8	26.0	12.2	23.6	21.7	9.5
	SD	2.69	2.85	0.99	2.54	2.71	1.34	4.01	3.73	1.65
G4	Mean	34.5	31.5	13.9	28.8	26.2	11.8	22.7	20.5	9.5
	SD	2.23	2.31	0.90	2.01	2.21	0.75	4.06	4.06	1.34
G5	Mean	34.3	31.4	13.9	29.1	26.5	12.0	25.3	23.1	10.4
	SD	2.31	2.29	1.02	3.17	3.18	1.02	1.96	1.84	1.08
G6	Mean	34.8	31.8	14.0	27.2	24.6	11.6	22.4	20.1	9.7
	SD	2.28	2.48	0.90	3.33	3.64	0.62	6.16	6.11	2.20
G7	Mean	34.7	32.1	13.2	28.5	26.1	11.2	25.7	23.5	10.4
	SD	2.04	1.95	0.84	3.40	3.23	1.31	2.21	1.94	1.19

Key : N= Number of observations

Table 3B

Motor Activity - Group Mean Values

Dose: G1- 0; G2 (CPF) - 1; G3 (LA) - 50; G4 (CPF+LA) - 1+50; G5 (CPF) - 10; G6 (LA) - 500; G7 – (CPF+LA) - 10+500 ppm in diet

Sex : Female **Period : 4th Week**

Group N° (N=8)		Time Interval and Parameter								
		0-10 minutes			11-20 minutes			21-30 minutes		
		Total	Ambu-latory	Stereo-typic	Total	Ambu-latory	Stereo-typic	Total	Ambu-latory	Stereo -typic
G1	Mean	32.9	30.3	12.7	25.5	23.1	10.7	21.3	19.2	9.1
	SD	3.13	3.16	0.77	3.75	3.82	0.91	5.86	5.79	1.84
G2	Mean	33.9	31.1	13.6	27.9	25.7	11.0	24.7	22.5	10.1
	SD	1.55	1.41	0.77	2.34	2.15	1.23	3.11	2.86	1.42
G3	Mean	31.7	29.1	12.5	25.4	22.8	11.1	21.5	19.4	9.1
	SD	2.30	2.17	1.1	4.50	4.38	1.52	4.17	4.33	0.91
G4	Mean	32.9	30.4	12.7	26.8	24.4	11.2	22.4	20.3	9.6
	SD	3.57	3.42	1.2	4.32	4.15	1.39	5.49	5.22	2.03
G5	Mean	33.0	30.3	12.9	26.9	24.7	10.8	23.0	20.9	9.65
	SD	2.09	2.00	0.98	2.76	2.70	1.00	4.45	4.14	1.75
G6	Mean	32.0	29.5	12.3	26.8	24.6	10.8	22.2	20.3	9.0
	SD	1.49	1.65	0.27	3.43	3.29	1.16	4.43	4.17	1.62
G7	Mean	32.1	29.5	12.5	27.1	24.6	11.41	23.9	21.8	9.8
	SD	1.93	1.74	1.14	2.26	2.24	1.03	3.72	3.46	1.55

Key : N= Number of observations

Table 3C

Motor Activity - Group Mean Values

Dose: G1- 0; G2 (CPF) - 1; G3 (LA) - 50; G4 (CPF+LA) - 1+50; G5 (CPF) - 10; G6 (LA) - 500; G7 – (CPF+LA) - 10+500 ppm in diet

Sex : Male **Period : 13th Week**

Group N° (N=8)		Time Interval and Parameter								
		0-10 minutes			11-20 minutes			21-30 minutes		
		Total	Ambu-latory	Stereo-typic	Total	Ambu-latory	Stereo-typic	Total	Ambu-latory	Stereo-typic
G1	Mean	23.9	21.8	9.7	19.2	17.6	7.8	13.9	12.5	6.1
	SD	5.18	4.81	2.10	4.00	3.68	1.64	5.68	5.03	2.82
G2	Mean	23.7	21.2	10.4	19.2	17.2	8.4	18.5	16.7	8.0
	SD	4.17	4.42	1.28	3.87	3.82	1.44	2.80	2.60	1.25
G3	Mean	26.6	24.5	10.5	21.6	19.5	9.2	17.2	15.5	7.3
	SD	3.01	2.79	1.28	3.34	3.26	1.27	3.84	3.73	1.17
G4	Mean	28.4	25.9	11.5	22.8	20.7	9.6	20.0	18.1	8.6
	SD	2.56	2.56	0.73	4.77	4.48	1.74	4.12	4.06	1.42
G5	Mean	22.7	20.7	9.2	21.7	19.8	9.0	17.6	16.0	7.4
	SD	7.16	6.70	2.70	2.50	2.26	1.24	4.12	3.90	1.66
G6	Mean	25.8	23.5	10.5	25.8	23.6	10.3	18.7	17.2	7.2
	SD	5.49	5.79	0.99	3.96	4.02	1.37	4.72	4.73	1.30
G7	Mean	28.3	25.9	11.2	24.3	22.3	9.7	20.7	19.0	8.3
	SD	5.30	5.05	1.76	3.87	3.77	1.14	4.93	4.63	1.88

Key : N= Number of observations

Table 3D

Motor Activity - Group Mean Values

Dose: G1- 0; G2 (CPF) - 1; G3 (LA) - 50; G4 (CPF+LA) - 1+50; G5 (CPF) - 10; G6 (LA) - 500; G7 – (CPF+LA) - 10+500 ppm in diet

Sex : Female **Period : 13th Week**

Group N° (N=8)		Time Interval and Parameter								
		0-10 minutes			11-20 minutes			21-30 minutes		
		Total	Ambu-latory	Stereo-typic	Total	Ambu-latory	Stereo-typic	Total	Ambu-latory	Stereo-typic
G1	Mean	27.3	24.8	11.1	25.9	23.5	10.8	23.1	20.8	10.0
	SD	5.41	5.45	1.51	2.24	2.30	0.73	2.03	2.27	1.10
G2	Mean	30.5	27.8	12.5	24.6	22.2	10.4	20.6	18.8	8.5
	SD	2.00	1.84	0.90	3.76	3.64	1.37	3.80	3.64	1.40
G3	Mean	29.0	26.9	10.8	24.0	21.9	9.7	18.5	16.7	8.0
	SD	2.72	2.70	0.71	3.72	3.60	1.24	4.56	4.33	1.69
G4	Mean	32.3↑	29.7	12.6	26.6	24.3	10.7	24.4	22.3	9.85
	SD	3.75	3.48	1.74	3.31	3.18	1.10	5.40	5.13	1.77
G5	Mean	30.4	27.9	11.98	25.4	23.4	10.0	22.2	20.1	9.32
	SD	2.71	2.58	1.33	2.92	2.67	1.13	3.41	3.30	1.12
G6	Mean	29.8	27.4	11.8	25.0	22.8	10.1	23.1	21.1	9.3
	Mean	3.86	3.66	1.43	4.10	3.65	2.14	4.68	4.45	1.60
G7	Mean	30.1	27.6	12.0	26.1	23.9	10.6	23.3	21.3	9.5
	SD	1.46	1.24	1.02	3.58	3.36	1.42	3.62	3.34	1.54

Key : N= Number of observations

Table 3E

Motor Activity - Group Mean Values

Dose: G1R- 0; G5R (CPF) - 10; G6 (LA) - 500; G7 – (CPF+LA) - 10+500 ppm in diet

Sex : Male **Period : 17th Week**

Group N° (N=6)		Time Interval and Parameter								
		0-10 minutes			11-20 minutes			21-30 minutes		
		Total	Ambu-latory	Stereo-typic	Total	Ambu-latory	Stereo-typic	Total	Ambu-latory	Stereo-typic
G1R	Mean	23.0	21.0	9.3	19.2	12.6	6.4	12.9	11.4	5.9
	SD	10.01	9.52	3.32	9.65	8.78	4.07	7.31	6.83	3.11
G5R (N=6)	Mean	32.0	30.0	12.1	24.7	22.6	10.0	19.8	18.0	8.2
	SD	3.24	2.90	1.73	5.72	5.43	1.97	2.85	2.71	1.16
G6R (N=6)	Mean	30.8	28.4	11.9	25.0	22.9	10.0	20.6	18.5	8.9
	SD	3.64	3.42	1.77	4.94	4.62	1.97	7.57	7.03	3.11
G7R (N=6)	Mean	30.6	28.2	11.8	23.3	21.1	9.7	19.3	17.4	8.3
	SD	1.85	1.83	0.91	2.80	2.86	0.63	3.24	3.42	0.89

Sex : Female **Period : 17th Week**

Group N°		Time Interval and Parameter								
		0-10 minutes			11-20 minutes			21-30 minutes		
		Total	Ambu-latory	Stereo-typic	Total	Ambu-latory	Stereo-typic	Total	Ambu-latory	Stereo-typic
G1R (N=6)	Mean	30.3	28.0	11.4	24.4	22.2	9.8	22.2	20.4	8.8
	SD	4.70	4.53	1.34	7.56	7.61	1.86	3.44	3.21	1.33
G5R (N=6)	Mean	33.6	31.1	12.7	27.9	25.5	11.2	22.1	20.1	9.0
	SD	3.15	2.94	1.50	3.14	3.03	1.46	5.78	5.54	1.87
G6R (N=6)	Mean	34.4	32.0	12.5	28.3	26.1	11.0	24.5	22.5	9.7
	SD	2.62	2.49	1.24	3.38	3.26	1.07	3.71	3.36	1.8
G7R (N=6)	Mean	33.3	30.7	12.9	25.6	23.5	10.0	20.2	18.1	8.8
	SD	2.34	2.17	0.99	5.03	4.78	1.89	5.44	5.21	1.90

Key : N= Number of observations

Table 4A

Grip Strength (g) - Group Mean Values

Dose: G1- 0; G2 (CPF) - 1; G3 (LA) - 50; G4 (CPF+LA) - 1+50; G5 (CPF) - 10; G6 (LA) - 500; G7 - (CPF+LA) - 10+500 ppm in diet

Sex : Male **Period : 4th Week**

Group	Forelimb		Hind Limb	
	Mean	SD	Mean	SD
G1 (N=8)	1118.3	170.07	474.3	103.95
G2 (N=8)	932.0	125.08	471.6	45.92
G3 (N=8)	984.1	81.46	399.5	56.61
G4 (N=8)	906.6	60.84	372.3	41.40
G5 (N=8)	991.5	83.58	384.0	62.67
G6 (N=8)	1042.3	121.31	412.9	63.98
G7 (N=8)	960.0	64.75	415.9	54.62

Sex : Male **Period : 13th Week**

Group	Forelimb		Hind Limb	
	Mean	SD	Mean	SD
G1 (N=8)	1982.1	189.88	653.1	53.52
G2 (N=8)	2023.5	258.77	644.5	97.12
G3 (N=8)	1881.3	298.17	626.1	68.33
G4 (N=8)	1855.0	263.94	587.0	71.48
G5 (N=8)	1952.8	248.83	557.8	75.94
G6 (N=8)	1830.5	189.40	521.5	53.37
G7 (N=8)	1756.4	258.93	567.9	83.57

Sex : Male **Period : 17th Week**

Group	Forelimb		Hind Limb	
	Mean	SD	Mean	SD
G1R (N=6)	1493.5	293.90	760.83	147.33
G5R (N=6)	1764.7	312.05	858.0	79.81
G6R (N=6)	1811.5	190.59	770.0	88.73
G7R (N=6)	1589.8	360.42	727.7	146.62

Key : N= Number of observations

Table 4B

Grip Strength (g) - Group Mean Values

Dose: G1- 0; G2 (CPF) - 1; G3 (LA) - 50; G4 (CPF+LA) - 1+50; G5 (CPF) - 10; G6 (LA) - 500; G7 – (CPF+LA) - 10+500 ppm in diet

Sex : Female **Period : 4th Week**

Group	Forelimb		Hind Limb	
	Mean	SD	Mean	SD
G1 (N=8)	839.9	68.63	389.0	39.14
G2 (N=8)	816.4	83.60	428.3	103.79
G3 (N=8)	846.8	80.40	379.3	98.51
G4 (N=8)	845.6	72.13	358.3	39.74
G5 (N=8)	835.6	55.87	357.0	64.36
G6 (N=8)	932.0	119.75	416.8	113.02
G7 (N=8)	789.6	87.56	342.4	67.82

Sex : Female **Period : 13th Week**

Group	Forelimb		Hind Limb	
	Mean	SD	Mean	SD
G1 (N=8)	1415.5	119.03	480.9	37.81
G2 (N=8)	1490.9	175.73	524.0	36.82
G3 (N=8)	1500.0	153.46	543.3	81.45
G4 (N=8)	1380.6	259.05	606.8	108.30
G5 (N=8)	1405.6	98.65	547.6	63.87
G6 (N=8)	1438.0	167.77	533.9	61.56
G7 (N=8)	1351.4	200.32	553.0	61.86

Sex : Female **Period : 17th Week**

Group	Forelimb		Hind Limb	
	Mean	SD	Mean	SD
G1R (N=6)	1431.8	94.63	716.0	87.21
G5R (N=6)	1421.3	167.34	664.5	33.96
G6R (N=6)	1497.2	230.96	702.0	39.93
G7R (N=6)	1654.8	162.0	722.7	64.46

Key : N= Number of observations

Table 5

Hind Limb Foot Splay (mm) - Group Mean Values

Dose: G1- 0; G2 (CPF) - 1; G3 (LA) - 50; G4 (CPF+LA) - 1+50; G5 (CPF) - 10; G6 (LA) - 500; G7 – (CPF+LA) - 10+500 ppm in diet

Period : 4th Week

Group	Male		Female	
	Mean	SD	Mean	SD
G1 (N=8)	94.4	11.44	74.0	16.28
G2 (N=8)	85.6	15.36	73.8	12.58
G3 (N=8)	87.5	18.24	73.6	12.79
G4 (N=8)	78.5	14.39	83.6	13.20
G5 (N=8)	82.1	15.34	75.0	7.41
G6 (N=8)	91.9	10.33	84.5	17.21
G7 (N=8)	78.6	12.85	74.1	15.57

Period : 13th Week

Group	Male		Female	
	Mean	SD	Mean	SD
G1 (N=8)	119.3	14.10	92.0	18.99
G2 (N=8)	103.8	11.51	83.9	14.26
G3 (N=8)	96.4	19.14	89.8	10.05
G4 (N=8)	100.8	17.22	95.6	21.00
G5 (N=8)	106.1	18.46	88.3	14.85
G6 (N=8)	104.0	13.09	99.3	13.26
G7 (N=8)	99.4	14.77	84.1	13.99

Period : 17th Week

Group	Male		Female	
	Mean	SD	Mean	SD
G1R (N=6)	116.3	22.65	85.8	15.37
G5R (N=6)	102.0	20.04	88.3	20.04
G6R (N=6)	105.0	20.81	87.0	9.67
G7R (N=6)	108.0	26.34	73.8	16.50

Key : N= Number of observations

Table 6A

Body Weight – Group Mean Values

Dose: G1- 0; G2 (CPF) - 1; G3 (LA) - 50; G4 (CPF+LA) - 1+50; G5 (CPF) - 10; G6 (LA) - 500; G7 – (CPF+LA) - 10+500 ppm in diet

Sex : Male

Week N°		Group N° (N=10)						
		G1	G2	G3	G4	G5	G6	G7
#	Mean	71.7	71.2	70.1	71.7	71.8	71.4	70.1
	SD	8.07	7.79	7.23	6.43	6.53	7.63	7.55
1	Mean	115.9	114.3	112.2	114.2	116.2	117.4	112.3
	SD	12.16	10.73	13.18	9.41	11.40	11.27	13.52
2	Mean	164.2	161.6	156.9	163.2	165.2	166.8	157.9
	SD	18.58	15.17	16.77	11.02	14.37	14.90	14.53
3	Mean	205.5	205.9	195.6	203.8	212.1	205.3	200.6
	SD	24.87	21.18	16.22	17.69	16.89	16.49	20.94
4	Mean	243.0	246.8	237.4	246.4	252.5	246.3	231.2
	SD	26.93	26.27	14.74	18.62	20.05	20.36	19.21
5	Mean	285.3	286.2	279.4	287.5	292.0	286.5	269.2
	SD	34.37	30.69	20.20	17.68	22.32	18.46	18.04
6	Mean	322.2	319.6	305.4	325.1	329.7	324.2	304.6
	SD	37.98	34.15	23.66	23.30	28.05	19.05	26.97
7	Mean	353.4	342.7	339.3	349.9	359.3	354.7	328.2
	SD	42.41	37.54	29.66	23.54	33.58	22.08	27.14
8	Mean	377.3	366.8	361.2	371.1	380.9	381.8	344.9
	SD	46.39	37.45	30.63	19.59	32.69	25.45	22.96
9	Mean	399.0	389.7	378.7	396.1	401.0	399.9	366.7
	SD	45.06	41.65	32.73	25.52	39.32	27.19	24.54
10	Mean	412.6	408.0	400.4	420.2	420.1	419.0	384.1
	SD	46.19	49.22	37.47	26.99	40.26	30.28	24.27
11	Mean	430.3	425.0	414.7	438.6	434.5	432.6	400.2
	SD	48.70	51.23	39.02	26.70	41.52	31.93	27.49
12	Mean	444.6	434.9	431.4	453.6	450.2	447.0	417.0
	SD	46.35	50.31	40.19	29.48	43.40	35.61	29.87
13	Mean	441.0	438.0	426.3	441.2	451.4	449.4	419.7
	SD	41.80	48.09	40.88	27.30	47.93	36.45	27.95

Key : N= Number of observations; # = Day of commencement of treatment

Table 6B

Body Weight – Group Mean Values

Dose: G1- 0; G2 (CPF) - 1; G3 (LA) - 50; G4 (CPF+LA) - 1+50; G5 (CPF) - 10; G6 (LA) - 500; G7 - (CPF+LA) - 10+500 ppm in diet

Sex : Female

Week N°		Group N° (N=10)						
		G1	G2	G3	G4	G5	G6	G7
#	Mean	71.3	69.7	72.9	70.6	71.5	71.2	71.6
	SD	7.97	6.98	7.98	9.00	7.00	8.11	5.95
1	Mean	109.9	106.0	111.8	104.5	111.4	105.3	109.3
	SD	8.32	7.26	11.04	10.65	9.43	10.61	8.54
2	Mean	144.4	136.9	148.1	139.7	144.1	137.9	145.4
	SD	7.71	7.42	14.93	13.3	13.10	12.56	8.37
3	Mean	168.3	159.6	170.2	165.2	163.0	162.8	170.0
	SD	9.12	10.47	16.54	14.16	15.04	12.82	8.79
4	Mean	189.9	178.0	191.2	182.2	178.8	181.3	190.4
	SD	14.07	11.03	17.67	13.21	13.73	11.95	11.05
5	Mean	213.5	199.7	214.9	202.0	198.1	202.2	210.6
	SD	17.27	13.70	20.65	16.32	16.39	17.05	14.15
6	Mean	232.1	217.0	231.8	215.2	213.0	219.9	226.0
	SD	20.17	15.04	24.33	17.34	18.70	17.84	13.73
7	Mean	243.2	228.5	245.5	230.9	226.3	230.4	241.7
	SD	24.78	17.04	24.07	17.60	21.44	17.28	15.66
8	Mean	252.9	236.8	256.7	240.3	234.3	239.1	250.8
	SD	25.84	16.64	24.44	19.89	21.88	17.11	16.90
9	Mean	261.8	244.9	265.4	248.1	240.2	246.6	256.6
	SD	26.68	19.24	25.32	20.93	22.61	15.49	18.23
10	Mean	268.8	251.5	274.4	256.0	247.6	254.9	260.2
	SD	31.18	22.03	26.63	21.35	23.65	19.20	18.75
11	Mean	274.2	259.9	280.8	261.6	252.6	258.8	266.3
	SD	29.75	24.88	25.42	22.12	22.12	17.25	20.30
12	Mean	279.4	264.9	285.3	267.9	260.3	265.9	270.6
	SD	30.93	24.05	25.69	21.81	25.06	14.79	23.01
13	Mean	282.4	268.5	291.5	267.4	258.8	263.1	273.3
	SD	34.55	24.42	26.04	24.66	22.21	16.96	22.15

Key : N= Number of observations; # = Day of commencement of treatment

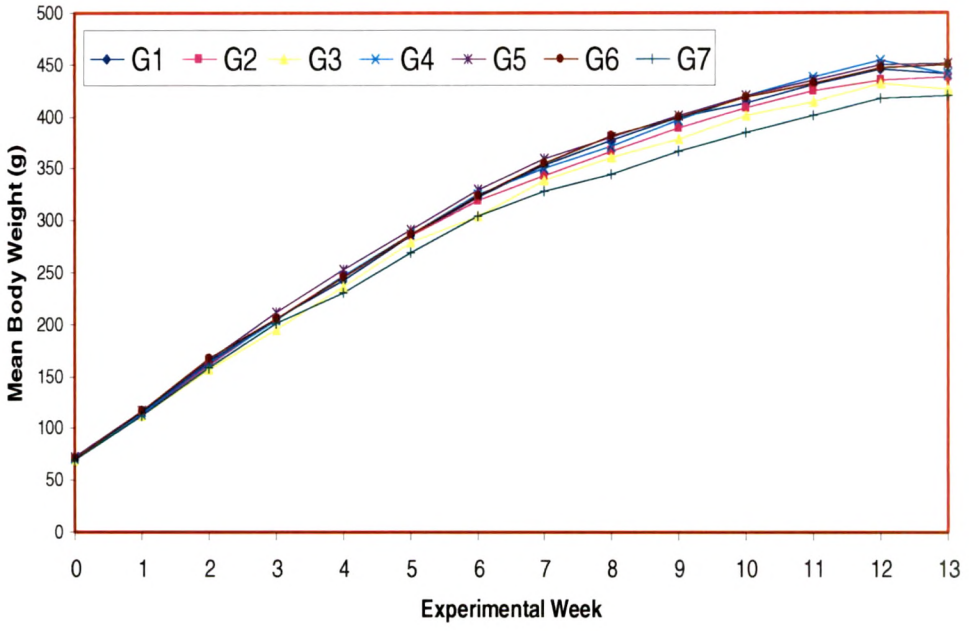

Figure 3. Growth Curve (Group Mean Body Weight) – **Males** -Main Group. Key: 0 = experiment commencement day

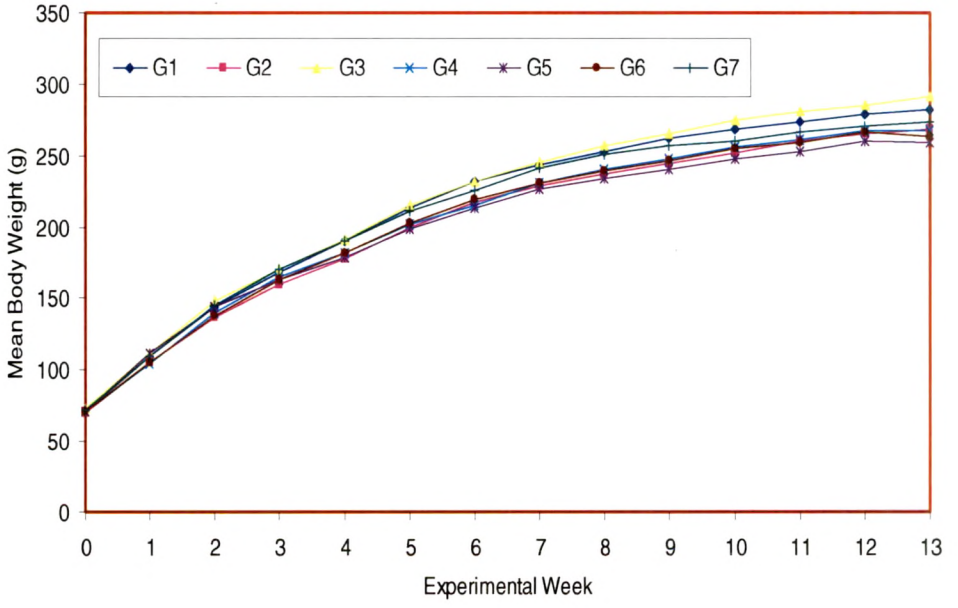

Figure 4. Growth Curve (Group Mean Body Weight) – **Females** -Main Group. Key: 0 = experiment commencement day

Table 6C

Body Weight – Group Mean Values

Dose: G1R - 0; G5R (CPF) - 10; G6R (LA) - 500; G7R (CPF+LA) - 10+500 ppm in diet

Sex : Male

Week N°	Group N°							
	G1R (N=6)		G5R (N=6)		G6R (N=6)		G7R (N=6)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
#	70.2	22.28	75.7	22.66	76.0	23.14	71.0	24.58
1	113.7	28.27	119.5	30.90	121.7	32.23	109.2	26.14
2	155.0	33.78	167.5	34.72	163.7	34.22	157.3	31.49
3	197.3	37.30	211.7	39.59	202.8	33.93	201.5	38.18
4	244.2	35.2	259.5	38.24	246.5	32.56	239.0	39.14
5	279.2	32.49	294.2	34.04	278.5	29.27	282.8	40.28
6	302.8	37.87	329.0	35.56	312.5	30.18	319.3	41.56
7	325.2	42.23	359.7	42.11	336.0	31.75	338.5	37.84
8	341.2	40.17	383.7	42.61	357.5	39.01	364.0	40.76
9	365.5	54.93	401.8	45.21	384.5	45.50	388.3	44.30
10	382.3	59.77	417.2	48.5	399.3	47.61	406.2	48.06
11	394.3	63.97	433.8	50.29	414.7	49.84	425.0	52.56
12	406.7	65.89	451.3	55.06	431.5	53.65	438.7	53.04
13	415.3	65.43	463.7	59.11	451.2	61.32	453.8	57.10
14	419.8	68.41	469.0	60.91	459.3	61.18	459.5	58.66
15	421.5	69.51	477.2	65.24	465.3	64.86	462.0	57.81
16	429.8	71.44	487.8	66.73	476.0	65.16	467.7	60.28
17	436.8	73.03	469.5	66.59	481.7	70.13	477.0	58.68

Key : N= Number of observations; # = Day of commencement of treatment

Table 6D

Body Weight – Group Mean Values

Dose: G1R - 0; G5R (CPF) - 10; G6R (LA) - 500; G7R (CPF+LA) - 10+500 ppm in diet

Sex : Female

Week N°	Group N°							
	G1R (N=6)		G5R (N=6)		G6R (N=6)		G7R (N=6)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
#	70.5	16.01	72.0	16.26	72.7	16.06	72.0	16.52
1	106.0	23.92	108.5	24.59	110.0	20.86	109.0	19.46
2	140.3	26.99	140.2	31.77	145.3	25.78	143.2	15.69
3	166.8	31.04	164.5	36.38	165.3	23.72	167.5	15.02
4	191.3	33.89	186.3	41.59	188.2	25.71	193.5	16.38
5	210.8	36.01	204.2	38.81	207.3	27.61	215.3	13.71
6	224.8	31.66	219.2	37.13	223.5	32.79	230.2	14.46
7	239.2	35.56	233.5	37.68	234.3	33.79	243.0	12.43
8	251.8	39.10	244.5	38.91	248.3	36.97	252.8	13.45
9	257.2	38.46	251.7	38.16	256.2	40.32	263.0	13.91
10	263.5	40.48	262.0	43.62	261.8	44.68	271.8	15.54
11	270.0	43.89	267.3	40.94	265.3	47.09	280.0	14.41
12	280.0	48.11	273.5	39.59	274.7	48.78	287.0	16.43
13	282.5	45.65	284.0	46.73	279.2	50.11	291.3	18.13
14	293.2	61.02	286.7	45.33	280.0	53.64	292.8	13.63
15	291.5	58.14	287.0	47.32	282.2	57.16	293.5	13.84
16	301.7	64.03	291.8	47.73	287.3	54.88	299.0	19.82
17	309.67	70.85	297.2	54.06	289.0	56.67	301.3	17.67

Key : N= Number of observations; # = Day of commencement of treatment

Table 7A

Food Consumption (g/rat/week) – Group Mean Values

Dose: G1- 0; G2 (CPF) - 1; G3 (LA) - 50; G4 (CPF+LA) - 1+50; G5 (CPF) - 10; G6 (LA) - 500; G7 - (CPF+LA) - 10+500 ppm in diet

Sex : Male

Week N°		Group N°						
		G1 (N=10)	G2 (N=10)	G3 (N=10)	G4 (N=10)	G5 (N=10)	G6 (N=10)	G7 (N=10)
1	Mean	14.0	17.2	13	14.6	14.6	14.7	14.6
	SD	1.83	3.96	1.23	0.44	1.27	1.41	1.80
2	Mean	19.4	19.2	18.5	18.7	17.2	18.7	17.7
	SD	1.83	1.03	1.76	0.56	2.44	2.39	1.78
3	Mean	21.2	21.9	20.4	20.74	19.9	20.1	19.8
	SD	2.64	2.52	1.21	2.54	2.18	1.97	1.48
4	Mean	21.7	22.7	21.7	21.9	21.8	21.2	19.8
	SD	2.20	2.13	1.38	0.82	1.21	1.63	1.45
5	Mean	25.1	25.4	24.8	23.2	23.6	23.9	22.2
	SD	2.03	3.25	1.69	3.88	1.84	1.49	1.25
6	Mean	25.76	26.0	24.4	23.3	24.3	24.5	24.1
	SD	2.76	3.22	2.20	3.88	1.11	1.82	1.31
7	Mean	26.8	26.2	25.8	24.4	24.6	25.0	26.0
	SD	2.39	2.37	2.25	3.56	1.73	1.16	1.84
8	Mean	26.9	27.3	26.6	25.6	26.6	26.4	26.50
	SD	2.10	1.96	1.85	2.96	1.90	1.42	1.87
9	Mean	28.5	28.4	27.4	26.6	28.2	27.7	27.60
	SD	2.0	1.35	1.55	2.69	1.05	2.10	1.75
10	Mean	29.8	29.2	27.9	28.4	28.3	28.0	28.0
	SD	1.61	0.93	0.98	0.64	1.22	2.29	1.30
11	Mean	30.0	30.3	29.1	29.0	29.7	28.9	29.2
	SD	1.73	1.36	1.47	1.71	1.09	1.78	1.28
12	Mean	29.5	29.5	30.1	29.6	29.8	29.3	29.6
	SD	2.02	1.41	1.34	1.27	1.39	1.09	1.01
13	Mean	29.4	29.6	29.3	29.4	29.8	29.1	29.5
	SD	1.75	1.43	1.78	1.65	1.42	1.20	1.10

Key : N= Number of observations

Table 7B

Food Consumption (g/rat/week) – Group Mean Values

Dose: G1- 0; G2 (CPF) - 1; G3 (LA) - 50; G4 (CPF+LA) - 1+50; G5 (CPF) - 10; G6 (LA) - 500; G7 - (CPF+LA) - 10+500 ppm in diet

Sex : Female

Week N°		Group N°						
		G1 (N=10)	G2 (N=10)	G3 (N=10)	G4 (N=10)	G5 (N=10)	G6 (N=10)	G7 (N=10)
1	Mean	13.2	13.1	13.3	12.4	14.3	12.7	13.3
	SD	0.97	1.16	0.91	1.25	1.70	1.48	1.63
2	Mean	17.2	16.7	15.1	14.7	16.4	15.6	16.5
	SD	1.57	1.59	0.74	1.36	1.57	1.64	1.12
3	Mean	17.7	17.2	17.0	16.7	16.50	16.50	17.6
	SD	1.23	1.90	1.57	1.23	1.70	0.99	1.70
4	Mean	19.0	17.7	17.4	17.1	18.1	17.1	17.6
	SD	1.43	2.03	1.46	0.68	1.51	1.12	0.88
5	Mean	22.5	20.1	19.4	17.4	19.3	18.4	18.9
	SD	1.38	2.92	1.79	0.90	1.67	1.28	2.08
6	Mean	21.4	20.8	20.8	18.1	20.7	19.0	18.7
	SD	1.80	3.32	1.67	1.75	1.32	1.87	2.58
7	Mean	21.3	21.7	21.4	18.7	20.3	19.8	18.3
	SD	1.56	2.37	1.74	1.89	2.78	2.17	1.96
8	Mean	20.4	23.1	22.2	20.3	21.1	20.6	18.6
	SD	1.64	1.81	1.50	1.54	2.15	1.65	1.85
9	Mean	22.8	22.3	23.1	21.5	22.5	21.0	19.4
	SD	1.23	1.81	1.72	1.62	1.74	1.60	2.22
10	Mean	23.0	22.0	23.6	20.6	22.6	20.5	19.4
	SD	1.96	2.69	1.79	2.21	2.58	1.70	1.54
11	Mean	23.8	22.4	23.0	22.2	22.8	21.0	20.3
	SD	2.09	3.11	1.51	1.72	2.27	1.55	1.44
12	Mean	23.6	22.6	22.8	22.5	23.0	22.1	21.4
	SD	2.41	2.69	1.66	1.72	2.78	1.98	1.67
13	Mean	24.0	22.6	23.1	22.4	23.2	22.2	21.8
	SD	1.75	2.68	1.43	1.75	2.04	1.58	1.53

Key : N= Number of observations

Table 7C

Food Consumption (g/rat/week) – Group Mean Values

Dose: G1R - 0; G5R (CPF) - 10; G6R (LA) - 500; G7R (CPF+LA) - 10+500 ppm in diet

Sex : Male

Week N°	Group N°							
	G1R (N=6)		G5R (N=6)		G6R (N=6)		G7R (N=6)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	14.0	2.90	15.0	2.05	15.1	2.10	13.9	1.74
2	19.5	2.58	18.3	2.42	18.8	2.14	17.9	1.10
3	19.6	3.44	21.6	2.49	20.6	2.11	20.5	2.03
4	21.6	3.2	22.9	1.92	22.1	2.80	22.3	2.04
5	25.5	1.73	24.3	1.53	23.4	1.59	23.9	1.35
6	25.6	3.64	23.7	1.55	24.6	1.36	25.1	1.26
7	27.0	2.67	24.1	1.18	25.9	1.05	26.1	1.34
8	26.3	1.80	25.2	1.97	27.3	2.27	27.7	1.25
9	29.3	1.01	26.8	1.92	27.9	1.25	28.6	1.0
10	28.6	1.64	27.5	1.55	27.8	1.99	27.9	1.20
11	30.2	0.97	30.0	1.85	30.1	1.61	28.2	1.40
12	30.4	1.00	30.6	1.75	29.8	1.46	29.1	1.03
13	29.4	1.44	29.8	1.64	29.4	1.45	29.4	1.99
14	30.2	1.21	30.2	1.29	29.9	1.6	29.8	1.07
15	29.0	1.34	30.3	1.58	30.4	1.25	30.2	1.07
16	29.5	1.10	30	1.21	30.5	1.96	30.7	1.74
17	29.8	1.85	30.8	1.32	30.6	1.72	30.8	1.40

Key : N= Number of observations

Table 7D

Food Consumption (g/rat/week) – Group Mean Values

Dose: G1R - 0; G5R (CPF) - 10; G6R (LA) - 500; G7R (CPF+LA) - 10+500 ppm in diet

Sex : Female

Week N°	Group N°							
	G1R (N=6)		G5R (N=6)		G6R (N=6)		G7R (N=6)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	12.7	1.84	13.6	2.91	14.4	1.82	13.4	1.41
2	16.3	2.80	16.1	2.0	16.7	1.89	16.3	1.15
3	17.4	3.39	16.9	2.0	16.4	1.82	17.5	1.48
4	18.5	2.63	17.3	1.87	17.1	1.51	18.2	1.56
5	18.7	2.85	18.2	2.35	18.2	2.49	18.8	2.13
6	19.0	3.06	18.7	2.20	18.9	2.69	19.3	2.52
7	20.3	2.36	19.2	2.30	19.5	1.83	19.6	3.04
8	20.7	2.10	19.8	2.05	19.6	1.74	19.7	3.39
9	21.7	1.91	20.7	2.52	19.7	1.75	20.1	3.41
10	21.4	1.94	21.4	2.90	20.5	1.85	20.5	3.35
11	21.5	2.68	22.2	3.12	21.1	1.84	20.4	2.41
12	21.1	2.31	21.9	2.64	21.7	2.17	20.7	2.67
13	21.6	2.29	21.7	2.25	22.0	2.23	21.1	2.65
14	22.1	1.74	22.2	2.46	22.4	2.25	22.7	2.05
15	22.8	1.79	22.3	2.22	21.9	2.61	21.5	2.51
16	22.6	1.97	22.3	1.97	22.1	2.59	21.6	2.34
17	22.3	1.66	22.5	1.83	22.5	2.52	22.1	2.48

Key : N= Number of observations

Table 8A

Chemical Intake (g/rat/week) – Group Mean Values

Dose: G1- 0; G2 (CPF) - 1; G3 (LA) - 50; G4 (CPF+LA) - 1+50; G5 (CPF) - 10; G6 (LA) - 500; G7-(CPF+LA) - 10+500 ppm in diet

Sex : Male

Week N°		Group N°							
		G2-CPF	G3-LA	G4-CPF+LA		G5-CPF	G6-LA	G7-CPF+LA	
1	Mean	0.12	5.282	0.13	6.42	1.26	62.68	1.30	64.94
	SD	0.0	0.11	0.01	0.39	0.04	3.35	0.05	2.25
2	Mean	0.12	5.90	0.11	5.72	1.04	55.78	1.12	55.97
	SD	0.0	0.21	0.01	0.23	0.10	3.93	0.04	2.05
3	Mean	0.11	5.21	0.10	5.09	0.94	48.92	0.99	49.52
	SD	0.01	0.10	0.01	0.39	0.08	2.72	0.06	2.91
4	Mean	0.09	4.56	0.09	4.45	0.86	43.09	0.86	42.94
	SD	0.0	0.16	0.0	0.08	0.06	3.07	0.08	3.98
5	Mean	0.09	4.43	0.08	4.03	0.81	41.74	0.83	41.39
	SD	0.0	0.19	0.0	0.35	0.03	1.59	0.06	2.90
6	Mean	0.08	3.99	0.07	3.58	0.74	37.76	0.79	39.79
	SD	0.0	0.28	0.01	0.51	0.04	1.19	0.08	4.00
7	Mean	0.08	3.79	0.07	3.48	0.68	35.27	0.79	39.71
	SD	0.08	0.27	0.01	0.44	0.06	1.53	0.09	4.75
8	Mean	0.01	3.68	0.07	3.44	0.70	34.58	0.77	38.52
	SD	0.07	0.16	0.0	0.36	0.05	1.88	0.09	4.19
9	Mean	0.07	3.61	0.07	3.36	0.71	34.65	0.76	37.77
	SD	0.01	0.14	0.01	0.33	0.05	3.11	0.07	3.33
10	Mean	0.07	3.48	0.07	3.38	0.68	33.42	0.73	36.57
	SD	0.0	0.17	0.01	0.12	0.05	2.11	0.05	2.64
11	Mean	0.07	3.52	0.07	3.31	0.68	33.39	0.73	36.59
	SD	0.0	0.16	0.0	0.14	0.05	1.70	0.06	2.75
12	Mean	0.07	3.49	0.06	3.26	0.66	32.74	0.71	35.57
	SD	0.01	0.13	0.01	0.13	0.03	1.81	0.05	2.42
13	Mean	0.07	3.44	0.06	3.33	0.66	32.45	0.71	35.28
	SD	0.01	0.24	0.01	0.20	0.04	2.22	0.05	2.67

Key : N= Number of observations

Table 8B

Chemical Intake (g/rat/week) – Group Mean Values

Dose: G1- 0; G2 (CPF) - 1; G3 (LA) - 50; G4 (CPF+LA) - 1+50; G5 (CPF) - 10; G6 (LA) - 500; G7-(CPF+LA) - 10+500 ppm in diet

Sex : Female

Week N°		Group N°							
		G2- CPF	G3-LA	G4-CPF+LA		G5-CPF	G6-LA	G7- CPF+LA	
1	Mean	012	5.99	0.12	5.96	1.28	60.5	1.22	61.06
	SD	0.0	0.41	0.01	0.32	0.08	5.33	0.05	2.35
2	Mean	0.12	5.15	0.11	5.28	1.12	56.77	1.13	56.64
	SD	0.01	0.62	0.01	0.21	0..04	2.77	0.06	2.92
3	Mean	0.11	4.99	0.10	5.05	1.02	50.60	1.03	51.64
	SD	0.01	0.29	0.01	0.38	0.07	1.74	0.03	1.28
4	Mean	0.10	4.55	0.09	4.70	0.98	42.76	0.92	43.65
	SD	0.01	0.27	0.01	0.24	0.07	1.75	0.05	2.43
5	Mean	0.10	4.51	0.08	4.30	0.95	45.48	0.90	44.95
	SD	0.01	0.38	0.0	0.21	0.06	1.88	0.09	4.47
6	Mean	0.10	4.50	0.08	4.21	0.95	43.18	0.83	41.37
	SD	0.01	0.33	0.01	0.25	0.04	2.49	0.11	5.43
7	Mean	0.09	4.37	0.08	4.05	0.88	42.82	0.76	37.76
	SD	0.01	0.33	0.0	0.22	0.09	3.05	0.07	3.54
8	Mean	0.10	4.34	0.09	4.24	0.89	43.15	0.74	37.16
	SD	0.01	0.29	0.01	0.42	0.07	2.11	0.07	3.23
9	Mean	0.09	4.35	0.09	4.34	0.92	42.65	0.76	37.81
	SD	0.0	0.26	0.01	0.43	0.06	1.78	0.07	3.59
10	Mean	0.09	4.30	0.09	4.27	0.90	40.47	0.74	37.24
	SD	0.0	0.24	0.01	0.38	0.08	3.51	0.05	2.68
11	Mean	0.08	4.10	0.09	4.26	0.88	40.68	0.76	38.13
	SD	0.0	0.35	0.01	0.28	0.07	2.53	0.05	2.38
12	Mean	0.09	4.0	0.08	4.21	0.86	41.53	0.79	39.66
	SD	0.01	0.28	0.01	0.26	0.08	2.05	0.06	2.87
13	Mean	0.09	3.98	0.08	4.20	0.87	42.35	0.80	39.98
	SD	0.01	0.36	0.01	0.39	0.06	3.43	0.06	2.90

Key : N= Number of observations

Table 8C

Chemical Intake (g/rat/week) – Group Mean Values

Dose: G1R - 0; G5R (CPF) - 10; G6R (LA) - 500; G7R (CPF+LA) - 10+500 ppm in diet

Recovery Group

Week N°		Male				Female			
		Group N°				Group N°			
		G5R - CPF	G6R - LA	G7R -CPF+LA		G5R - CPF	G6R - LA	G7R - CPF+LA	
1	Mean	1.28	63.5	1.31	65.38	1.26	66.02	1.24	61.95
	SD	0.15	7.9	0.24	11.77	0.10	4.72	0.09	4.15
2	Mean	1.10	58.12	1.16	57.94	1.16	58.02	1.14	56.91
	SD	0.05	5.59	0.16	7.89	0.12	3.82	0.03	1.62
3	Mean	1.03	51.31	1.03	51.42	1.04	49.96	1.04	52.13
	SD	0.04	3.89	0.09	4.50	0.09	4.13	0.04	1.80
4	Mean	0.88	44.95	0.94	46.97	0.94	45.56	0.94	46.91
	SD	0.02	1.63	0.05	2.71	0.08	3.17	0.04	1.75
5	Mean	0.83	42.12	0.85	42.52	0.90	43.80	0.87	43.57
	SD	0.02	1.67	0.05	2.65	0.06	1.69	0.06	3.05
6	Mean	0.72	39.53	0.79	39.41	0.85	42.25	0.84	41.83
	SD	0.04	1.89	0.05	2.27	0.04	0.92	0.07	3.69
7	Mean	0.67	38.66	0.77	38.62	0.82	41.68	0.81	40.26
	SD	0.03	2.57	0.02	1.21	0.05	1.77	0.11	5.39
8	Mean	0.66	38.27	0.76	38.19	0.81	39.59	0.78	38.93
	SD	0.06	2.36	0.04	2.16	0.07	1.83	0.13	6.41
9	Mean	0.67	36.55	0.74	36.92	0.83	38.62	0.76	38.24
	SD	0.05	3.39	0.04	2.19	0.10	1.89	0.14	6.72
10	Mean	0.66	35.07	0.69	34.43	0.82	39.47	0.75	37.63
	SD	0.02	2.97	0.04	1.97	0.09	3.16	0.12	5.94
11	Mean	0.69	36.58	0.67	33.33	0.83	40.06	0.73	36.38
	SD	0.02	3.57	0.04	1.84	0.08	3.68	0.09	4.34
12	Mean	0.68	34.79	0.67	33.26	0.80	39.75	0.72	36.11
	SD	0.02	3.55	0.05	2.22	0.05	3.24	0.10	5.06
13	Mean	0.64	32.90	0.65	32.51	0.77	39.6	0.73	36.28
	SD	0.01	3.46	0.05	2.49	0.07	3.48	0.10	5.14

Key : N= Number of observations

Table 9

Stability, Homogeneity and Active Ingredient of chlorpyrifos in Experimental Diet

Theoretical Concentration		Active Ingredient (CPF) Content in Experimental Diet (ppm)					
		0 Day	% Recovery	4th Day	% Recovery	7th Day	% Recovery
1 ppm	Sample 1	0.89	97.0	0.87	94.5	0.824	89.8
	Sample 2	0.88	96.0	0.86	94.0	0.822	89.6
	Mean	**0.885**	**96.5**	**0865**	**94.25**	**0.823**	**89.7**
10 ppm	Sample 1	8.91	9.71	8.66	94.4	8.22	89.6
	Sample 2	8.90	97.0	8.58	93.5	8.21	89.5
	Mean	**8.905**	**97.05**	**8.62**	**93.95**	**8.215**	**89.55**

Homogeneity and Active Ingredient of Lead acetate in Experimental Diet

Theoretical Concentration	Sample N°	Detected Concentration of Lead in Experimental Diet (ppm)	% Recovery
500 ppm	Sample 1	462.2	92.4
	Sample 2	462.7	92.5
	Sample 3	461.6	92.3
	Mean	**462.2**	**92.4**

DISCUSSION

The present study has evaluated the effects of low level repeated 90-day exposure to chlorpyrifos, lead acetate and a combination of the two on neurobehavioral changes in Wistar rats. Since these chemicals exist in food commodities at low concentration as residues, the study was designed for low dose oral exposure through diet, the major route of entry of chemicals.

Dietary exposure to chlorpyrifos and lead acetate combination (10 ppm chlorpyrifos i.e., equivalent to 1 mg/kg body weight/day and 500 ppm lead acetate i.e., equivalent to 44.0 mg/kg body weight/day) in Wistar rats for a period of 13 weeks, did not reveal serious alterations in the studied neurobehavioral aspects. Clinical signs such as perennial staining of nose, chromorhinorrhoea, lacrimation and chromodacryorrhoea were observed in some of the animals belonging to groups 5 and 7. Though incidences of clinical signs observable in groups 5 and 7 were less, no animal from control group revealed any of these signs and, these cholinergic signs were also evident in single dose study. Hence, these clinical signs can be considered treatment related.

After 4 weeks of exposure, the rearing counts (vertical movements) in the open field were slightly reduced in either sex of groups 4, 5 and 7 (Table 1). Mattsson *et al.* (1996) observed decrease in motor activity at 15 mg/kg/body weight/day of chlorpyrifos at week 4. The rearing movements measured in the open field may be more indicative of exploratory behaviour and emotional tendencies than of motor activity per se (Meyer, 1998). This observation also indicates that vertical movement measurements in open field are more sensitive to gauge the effects of chemicals on motor activity and emotional tendencies. Locomotor activity of an animal is dependent not

only on the animal's motor system but also on the sensory and motivational factors (Meyer, 1998).

The occurrence of cholinergic clinical signs were more during weeks 2 and 3 as compared to remaining weeks of exposure. The rearing counts of treatment group animals were comparable to control group animals after 13 weeks of exposure. The reduction of vertical movements at week 4 and higher number of clinical signs before week 4 indicate lack of persistence or cumulative effects. This is indicative of tolerance to repeated exposure of chlorpyrifos. Many authors have reported tolerance to chlorpyrifos after repeated exposure. [Bushnell *et al.*, 1993; Bushnell *et al.*, 1994; Pope *et al.*, 1992; Mattsson *et al.*, 1996]. Tolerance to organophosphate cholinesterase inhibitors indicates that functional recovery accompanies neurochemical compensations for the inhibited enzyme. It is evident from several studies that a cholinesterase inhibiting organophosphate pesticide like chlorpyrifos induces tolerance to repeated exposure. Tolerance i.e., reduction in effects with continued treatment, in case of OP treatment, is generally characterized by decrease in the symptoms of cholinergic over stimulation (such as lacrimation, salivation and hypothermia) and, down regulation of muscarinic and N-methyl-D-aspartate (NMDA) receptors in various regions of the brain.

Bushnell *et al.* (1994) reported tolerance after repeated injection of chlorpyrifos in rats. Tremor and behavioral changes were observed in rats when blood cholinesterase was inhibited by 60% to 90% after 5 weeks on weekly injections up to a dose of 60 mg/kg body weight (0, 15, 30 or 60 mg/kg s.c.). They observed reduced blood cholinesterase inhibition (50% - 75% of control) and absence of behavioral effects, when CPF injection was

given every other week. Restart of weekly injections for 10 weeks reduced blood cholinesterase by 75% to 90%. Tremor did not recur while, behavioral changes like motor slowing and working memory impairment persisted throughout the dosing period in all treated groups. They also observed pharmacological evidence for tolerance to the muscarinic effects of CPF (i.e., CPF-treated rats were supersensitive to scopolamine and subsensitive to pilocarpine).

The density of various cholinergic receptors in different parts of brain markedly affects brain cholinergic signaling and subsequent metabolic events and pathological consequences. Repeated exposure to organophosphorus (OP) insecticides both in vivo and in vitro results in a decrease of muscarinic acetylcholine receptors (MRs) in the central nervous system, which is one of the characteristic features of tolerance. The in-vivo effects of disulfoton (OP) exposure on the mRNA levels of the three muscarinic acetylcholine receptor (mAChRs) for subtypes (M1, M2, and M3) were studied by Yagle and Costa (1996) in the brain tissue and, in peripheral mononuclear cells which express the m3 subtype. Sprague Dawley rats were exposed to doses of 2 mg/kg/day of disulfoton for 14 consecutive days, and the messenger ribonucleic acid (mRNA) levels of muscuranic receptor subtypes m1, m2 and m3 were assayed immediately after the cessation of exposure as well as, after a withdrawal period of 28-days. There was a significant decrease in the levels of muscarinic receptor subtypes in different brain regions immediately after exposure (m1 mRNA levels in hippocampus (23%); m2 subtype mRNA in both hippocampus (24%) and medulla (19%) and m3 mRNA levels in cortex (10%)). After the recovery period, no variation in reduction in m1 or m3 mRNA levels was observed in any of the brain regions examined while the m2 subtype mRNA levels (in the hippocampus)

remained decreased by 29%. This result indicates that m2 muscarinic receptors in the hippocampus may be more susceptible to OP induced alterations.

The role of NMDA receptor during OP intoxication has been studied using binding agents such as [^{3}H]MK-801. The NMDA receptor is one which is dependent on excitatory amino acids. NMDA receptor down regulation is a kind of tolerance mechanism to OP induced toxicity via excess excitatory amino acids. During NMDA receptor activation, permeability to Na^+, Ca^{2+}, K^+, and Mg^{2+} increases and the resultant overload of Na^+ and Ca^{2+} can prove detrimental to cells. Such down regulation of NMDA receptors has been demonstrated by Zhou *et al.* (2005). They studied the effect of an acute exposure to an OP compound, dichlorvos, on NMDA receptor density and, the protective efficacy of memantine on dichlorvos toxicity. Dichlorvos induced significant decrease in NMDA receptor density in rat brain.

No specific interactions could be noticed in the present study based on neurobehavioral changes through functional observational battery. However, a decrease in rearing counts of group 4 animals after week 4 was noticeable. The reduction in rearing counts after the 4th week, irrespective of sex, suggests that even at low dose levels, the combination of chlorpyrifos and lead produces behavioral changes in the nervous system. Though vertical movements in open field are more relevant to know the effects of chemicals on motor activity and emotional tendencies, the observed change with reference to a single parameter is not sufficient to interpret treatment related behavioral abnormality at low dose level (1 ppm Chlorpyrifos + 50 ppm lead acetate). Many higher levels of tests for detection of cognitive functions should also be considered.

The mean body weight of group 7 females was comparable to control group females throughout the exposure period. The group 7 males revealed consistent slight decrease in mean body weight from week 2 – 13 and the percent decrease ranged from minimum of 2.4 to maximum of 8.6, compared to control group males (Tables 6A and 6C; Figure 3). The decrease was never statistically significant but this statistically insignificant change may be biologically significant due to its consistent decrease from initial stage to final stage of exposure. The general decrease in mean body weight of animals exposed to lead is a common finding in both acute and chronic exposure and, same has been reported by several laboratories during neurobehavioral evaluations of lead (Moser *et al.*, 1997). The observed decrease in mean body weight is a systemic effect and not an abnormal behavioral finding. However, lack of same finding in group 6 males is not clear. Higher retention of lead in group 7 animals could be due to chelating complex formation properties of chlorpyrifos and lead.

SUMMARY

Organophosphorus insecticide, chlorpyrifos, and heavy metal, lead, were studied for their interactive effects on neurobehavior in Wistar rats when exposed for a period of 90 consecutive days through diet. The tests used for neurobehavior were functional observation battery (including grip strength and foot splay measurements) and motor activity. The study was designed using two different dose levels of chlorpyrifos and lead acetate and grouped into seven groups; control (Group 1), chlorpyrifos- 1 ppm (Group 2), lead acetate- 50 ppm (Group 3), chlorpyrifos-1 ppm + lead acetate- 50 ppm (Group 4), chlorpyrifos-10 ppm (group 5), lead acetate-1000 ppm (Group 6) and chlorpyrifos-10 ppm + lead acetate-500 ppm (Group 7). Neurobehavioral observations were performed at the end of weeks 4, 13 and 17. Repeated Dietary exposure to chlorpyrifos and lead acetate at dose levels of 10 ppm of chlorpyrifos (i.e., equivalent to 1mg/kg body weight/day) and in combination group of 10 ppm chlorpyrifos plus 500 ppm of lead acetate (i.e., equivalent to 44.0 mg/kg body weight/day) revealed mild cholinergic symptoms and decreased rearing counts before week 5. The lack of persistence and/or cumulative effects of these changes after 4 weeks of exposure is due to tolerance induced by chlorpyrifos. No specific interactions could be noticed in the present study based on neurobehavioral changes through functional observational battery. However, a decrease in rearing counts of group 4 animals after week 4 was noticeable, irrespective of sex, suggesting that even at low dose levels, the combination of chlorpyrifos and lead produces behavioral changes. Though vertical movements in open field are more relevant to know the effects of chemicals on motor activity and emotional tendencies, the observed change with reference to a single parameter is not sufficient to interpret treatment related behavioral

abnormality at low dose level i.e., 1 ppm of chlorpyrifos (i.e., 0.1 mg/kg body weight/day) plus 50 ppm of lead acetate (i.e., equivalent to 4.5 mg/kg body weight/day). Many higher levels of tests for detection of cognitive functions should also be considered.