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# EFFECTS OF CHLORPYRIFOS TOXICITY ON ORGAN BODY WEIGHT AND HAEMATOLOGICAL CONTENTS IN COMMON MALE BROWN RAT, *RATTUS NORVEGICUS*

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Chlorpyrifos is moderately toxic to mice and rat. Pesticide exposures cause disorders varying from straight forward topical irritant reactions, to complex systemic illness. These esters have the potential to produce several forms of toxicity. Chlorpyrifos, an organophosphate insecticide of phosphorothioate group was orally administered to male rats at the doses leval of 3, 6 and 9 mg $kg^{-1d-1}$ for 90 days. Animals exposed to high dose (9 mg $kg^{-1d-1}$) showed signs of toxicity including piloerection, diarrhoea, nose and eye bleeding, reduced body weight and till death of animals. Organ weight ratio of different vital organs did not show any change except increase in adrenal weight and decrease in the weight of testes in animals of high dose (9 mg $kg^{-1d\ 1}$). Microscopic examination of different tissues of male rats showed minor histo-pathological changes in liver, Kidney and Spleen. There was decrease in red blood cell (RBC) counts and levels of hemoglobin (Hb) and hematocrit (HCT) with increase in white blood cell counts (WBC). From the above mentioned findings, it is suggested that chlorpyrifos at 9mg/$kg^{/d}$ dose for 90 days has caused toxicological changes on physiology in albino rats.

**Key words :** Chlorpyrifos, Haematology, Toxicological studies, Rattus norvegicus.

## INTRODUCTION

Chlorpyrifos pesticides are chemical substances that are used to kill, repel, or regulate the growth of biological organisms. Pesticides are a diverse group of chemical compounds and consist of insecticides, fungicides, herbicides, and rodenticides. Problem of pesticide contamination appears considerable not only in India but in many other countries. Their persistence and ubiquitous nature coupled with a tendency to concentrate in organism as they move up the food chain may increase their toxicity to man and cause other harmful effects on man's health and well-being. Environmental pollution from pesticides is an important issue that attracts wide spread public concern. Among them, some organochlorine and organophosphate pesticides are routinely used in agriculture (Forget, 1991).

Chlorpyrifos (O-O-diethyl-O- {3, 5, 6 trichloro-2-pyridyl}-phosphorothioate) is one of the most heavily used organophosphate pesticides in domestic and agricultural applications throughout the world (Asperlin, 1994). It is evident that chlorpyrifos causes changes in some hematological and biochemical parameters in experimental animals (Kazmi *et al.,* 2003; Jacobson *et al,* 2004). This study is, therefore, undertaken to investigate the magnitude of toxicity of chlorpyrifos (an organophosphorus) on certain organ and haematology in rat, *Rattus norvegicus.*

## MATERIALS AND METHODS

***Animals husbandry and treatment*** : Male albino rats (*Rattus norvegicus*, Wistar strain) were maintained at temperature (21±3°C) and humidity (25-65%) in controlled animal house with 12:12 hr light: dark cycle. Animals were given pellet diet procured from Shri Raj Chakki Atta, Sai Industries, Shivajinagar, Darbhanga, Bihar, India and freshwater *ad libitum*. Rats were acclimatized for one week prior to the experiment. Forty male rats were divided into four groups having ten animals in each. The initial body weight of animals ranged between 150-180g. The animals of experimental groups were orally administered with doses (3, 6 and 9 mg $kg^{-1}d^{-1}$) of chlorpyrifos for 90 days. However, the animals of control group were orally given corn oil (0.4 ml $rat^{-1}$). The sign of toxicity and mortality during dosing were recorded. Body weight of each animal was recorded weekly. After 90 day treatment, animals were sacrificed and blood collected directly from jugular vein in 10% Ethylene diamine tetra acetic acid (EDTA) solution and non-oxalated tubes for the estimation of haematological parameters, respectively.

***Organ body weight ratio*** : The liver, kidney, spleen, and testes were removed, weighed individually, and calculated for organ weight ratio (organ weight divided by body weight and quotient multiplied with 100).

***Hematological studies*** : Blood collected in EDTA tubes was analyzed for white blood cells (WBC), red blood cells (RBC), hemoglobin (Hb), hematocrit (HCT) and differential leucocytes counts (DLC) using automated cell counter Melet Schloesing MS9-3, France.

## RESULTS AND DISCUSSION

### Morbidity and mortality

Male rats orally administered to chlorpyrifos (3, 6 and 9 mg $kg^{-1}d^{-1}$) for 90 days have shown signs of toxicity such as piloerection, diarrhea, nose and eye bleeding, tremor and death at highest dose. The mortality of animals during days 0-30, 31-60 and 61-90 are shown in Table I. Mortality pattern was not in a dose dependent manner, but was more in rats exposed to high dose (9 mg $kg^{-1}d^{-1}$) of chlorpyrifos.

### Relative organ body weight ratio

The absolute body weights of male rats on initial day, 30, 60 and 90 days are shown in Table I. No change in body weight gain was observed in male rats treated with 3 and 6 mg $kg^{-1}d^{-1}$ doses of chlorpyrifos as compared to controls. However, a significant decrease in the body weight gain was observed at high dose (9 mg $kg^{-1}d^{-1}$) of chlorpyrifos.

The relative organ body weight ratios of different vital organs of male rats exposed to chlorpyrifos for 90 days are shown in Table II. The vital organs like liver, kidney, spleen, and testis of rats exposed to different doses of chlorpyrifos were comparable to control. However, testes showed a significant decrease and increase in their weights, respectively at high dose (9 mg $kg^{-1}d^{-1}$) level.

### Hematological changes

The results of hematological parameters in male rats exposed to different doses (3, 6 and 9 mg $kg^{-1}d^{-1}$) of chlorpyrifos are shown in Table III. The Hb and HCT contents were

**Table I :** Body weight and mortality pattern of male rats orally administered to chlorpyrifos for 90 days.

| Chlorpyrifos (mg $^{kg-1d-1}$) | Body weight (g) | | | | Final weight gain (%) | Mortality during specific periods (days) | | |
|---|---|---|---|---|---|---|---|---|
| | Initial | 30 days | 60 days | 90days | | 0-30 | 31-60 | 61-90 |
| Control | 171.6 ± 8.35 | 190.3 ± 3.25 | 221.7 ± 5.32 | 244.1 ± 7.22 | 42 | 0 | 0 | 0(0)* |
| 3 | 162.2 ± 3.05 | 196.5 ± 7.21 | 211.5 ± 4.27 | 238.2 ± 3.60 | 47 | 0 | 1 | 1(2) |
| 6 | 163.8 ± 6.90 | 189.0 ± 3.12 | 219.5 ± 2.14 | 229.6 ± 6.68 | 40 | 0 | 0 | 2(2) |
| 9 | 171.0 ± 2.42 | 199.5 ± 5.56 | 243.8 ± 3.80 | 231.4 ± 3.86 | 35# | 0 | 3 | 2(5) |

Value represents mean ± SE of 20 rats; * = Values in parenthesis indicates total mortality in each group; # = Significant by ANOVA at $p<0.05$ level.

**Table II :** Relative organ weight (g)* of male rats orally administered to chlorpyrifos for 90 days.

| Organs | Chlorpyrifos (mg kg-1d-1) | | | |
|---|---|---|---|---|
| | Control | 3 | 6 | 9 |
| Liver | 3.02 ± 0.317 | 3.28 ± 0.094 | 3.25 ± 0.028 | 3.14 ± 0.086 |
| Kidney | 0.625 ± 0.062 | 0.715 ± 0.016 | 0.646 ± 0.013 | 0.714 ± 0.043 |
| Spleen | 0.304 ± 0.015 | 0.300 ± 0.008 | 0.366 ± 0.025 | 0.394 ± 0.021 |
| Testes | 1.06 ± 0.046 | 0.958 ± 0.085 | 0.914 ± 0.052 | 0.908 ± 0.051# |

Value represents mean ± SE of 10 rats, # = Significant by Student't' test at $p<0.05$ level; * = Relative organ weight = Organ weight x 100 Body weight

**Table III :** Hematological profile of male rats orally administered to chlorpyrifos for 90 days.

| Parameters | Chlorpyrifos (mg kg-1d-1) | | | |
|---|---|---|---|---|
| | Control | 3 | 6 | 9 |
| R.B.C (M mm-3) | 7.96 ± 0.095 | 7.26 ± 0.248# | 7.02 ± 0.321# | 6.98 ± 0.291# |
| W.B.C (m mm-3) | 9.20 ± 2.40 | 9.26 ± 1.94 | 9.39 ± 1.31 | 15.10 ± 1.34# |
| Hb (g $^{dl-1}$) | 13.34 ± 0.291 | 12.52 ± 0.416 | 11.64 ± 0.391# | 11.02 ± 0.468# |
| HCT (%) | 41.43 ± 1.35 | 40.76 ± 2.42 | 36.24 ± 1.61# | 32.83 ± 2.72# |

Value represents mean ± SE of 5 rats; # = Significant by Student 't' test at $p<0.05$ level.

significantly decreased in animals exposed to chlorpyrifos at 6 and 9 mg $kg^{-1}d^{-1}$ doses. However, RBC showed significant decrease at all doses. There was no change in WBC count at 3 and 6 mg $kg^{-1}d^{-1}$ but showed a significant increase at 9 mg $kg^{-1}d^{-1}$ dose level. The present study has shown significant signs of toxicity in animals during long-term exposure of chlorpyrifos. These included piloerection, diarhoea, nose and eye bleeding and tremor. It has been noted that high dose of chlorpyrifos resulted more deaths than the lower doses indicating a dose dependency. Interestingly death of treated animals in the present study may be associated with intoxication and general weakness. Further chlorpyrifos at high dose (9 mg $kg^{-1}d^{-1}$) has produced loss in body weight gain. In contrast, no change in the body weight at 15 mg $kg^{-1}d^{-1}$ of chlorpyrifos has been reported (Anonymous, 1993). Similar findings were reported earlier on decrease of body weight gain (4-6%) in male rats given 15 mg $kg^{-1}d^{-1}$ dose of chlorpyrifos in a two years dietary study (Yano *et al.*, 2000). Anorexia and general weakness of the animals may be the reason for weight loss and death in animals exposed to high dose of chlorpyrifos (food intake not monitored).

The present study has indicated its interference with blood factors in rats isupporting the earlier reports of Siddique *et al.* (1991) and, Morowati (1998). A significant change in hematological parameters seems to be related with dysfunction of haemopoitic system.

The decrease in the testicular weight of rats in the present study appears to be due to loss of spermatogenic elements and spermatozoa. Sherin & Hawards (1978) and Takihara *et al.* (1987) have also reported decrease in testicular weight and advocated that may be due to loss of spermatogenic elements.

Increased level of cholesterol in testes in the present study also supports the decreased androgen concentration (Bedwal *et al.*, 1994) which further leads reduced sperm density in testes (Sinha *et al.*, 1995).

It may, therefore, be concluded from the present study that subchronic oral exposure of male rats to chlorpyrifos has caused organ toxicity as well as morphology and haematology at 9 mg $kg^{-1}$ dose level.

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