

Assessment of chlorpyrifos toxicity on certain organs in rat, *Rattus norvegicus*

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Abstract: Chlorpyrifos, an organophosphate insecticide of phosphorothioate group was orally administered to male rats at the doses 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 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). A dose dependent inhibition of acetylcholinesterase (AChE) activity in RBC (22-60%) and brain (7-52%) was observed. Microscopic examination of different tissues of male rats showed minor histopathological changes in brain, liver, testis, epididymis and adrenal. The activity of testicular enzymes SDH, G-6-PDH and testicular content of sialic acid and cholesterol were found increased in animals of high dose (9 mg kg⁻¹d⁻¹). There was decrease in RBC counts and levels of hemoglobin (Hb) and hematocrit (HCT) with increase in WBC counts. While, total protein was decreased significantly at all the dose levels in testes and epididymis, glucose level showed a significant decrease at high dose. A dose dependent increase was observed in the level of serum triglycerides. There was no change in sperm motility and sperm morphology at any dose level except a decrease in sperm counts (114.1 x 10⁶ g⁻¹ in high dose for group against 158.9 x 10⁶ g⁻¹ controls). It is suggested that chlorpyrifos at 9mg/kg/d dose for 90 days has caused toxicological changes along with mild testicular and spermatotoxic effects in male rats.

Key words: Chlorpyrifos, Organophosphate pesticides, Toxicological studies, Histopathology, Sperm analysis
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Introduction

Environmental pollution from pesticides is an important issue that attracts wide spread public concern. Among them, some organophosphate and organochlorine pesticides are routinely used in agriculture (Forget, 1991). Organophosphates (OP's) are the esters of pentavalent phosphorous acid, exhibiting wide range of toxicity in mammals (Sultatos, 1994). 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). Chlorpyrifos causes deleterious effects through acetylcholinesterase inhibition at synapse of central and peripheral nervous system (Gordon *et al.*, 1997) and produces nausea, vomiting, salivation, diarrhoea, tremor and convulsion like symptoms (Kamrin, 1997). The acute oral LD₅₀ of chlorpyrifos is 118-245 mg kg⁻¹ for rats, 1000 mg kg⁻¹ for rabbit and 504 mg kg⁻¹ for guinea pigs (USEPA, 2000). Sub chronic toxicological studies of chlorpyrifos have been reported in rat and dog showing reduced weight gain and slight histopathological changes in adrenal gland with significant brain and plasma AChE inhibition (Zayed, 2002). It is evident that chlorpyrifos causes changes in some hematological and biochemical parameters in experimental animals (Kazmi *et al.*, 2003; Jacobson *et al.*, 2004).

Exposures of organophosphates have caused varying degree of testicular dysfunctions in man (Whorton *et al.*, 1977) and

also in experimental animals (Dikshith *et al.*, 1980; Joshi *et al.*, 2003). Recently, it has been shown that chlorpyrifos modulates *in vivo* phosphorylation of different nuclear protamines of rat sperm chromatin, thus may contribute to adverse reproductive effects (Kadavil *et al.*, 2001). Therefore, the aim of the study is to evaluate general toxicity of chlorpyrifos on certain organs in rats, *rattus norvegicus*.

Materials and Methods

Animals husbandry and treatment: Male albino rats (*Rattus norvegicus*, Wistar strain) of Indian Institute of Toxicology Research animal house, were maintained at temperature (22±3°C) and humidity (30-70%) in controlled animal house with 12:12 hr light: dark cycle. Animals were given commercial synthetic pellet diet and water *ad libitum*. Rats were acclimatized for one week prior to the experiment.

Eighty male rats were divided into four groups having twenty animals in each. The initial body weight of animals ranged between 150-175g. The animals of experimental groups were orally administered with doses (3, 6 and 9 mg kg⁻¹d⁻¹) of chlorpyrifos for 90 days. However, the animals of control group were orally given corn oil (0.4 ml rat⁻¹). The signs 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

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for the estimation of hematological and biochemical parameters respectively.

Organ body weight ratio: The liver, kidney, brain, testes, epididymis, seminal vesicle, and adrenal were removed, weighed individually, and calculated for organ weight ratio (organ weight divided by body weight and quotient multiplied with 100).

Biochemical studies: Freshly removed testes and epididymis were homogenized in chilled 0.25 M sucrose solution (10% w/v) for the estimations of biochemical parameters. Testes were homogenized in 0.2 M triethanol amine buffer solution for glucose-6-phosphate dehydrogenase and all homogenates (700 g) were centrifuged at room temperature for 15 minutes to remove cell debris. Serum was used for the of total protein, cholesterol, glucose, triglycerides and phospholipids using fully automated biochemical analyzer CHEMWELL, USA.

The supernatant of testes and epididymis were analyzed for total and free for estimation of, cholesterol and glucose by using fully automated biochemical analyzer. However, the activity of various enzymes lactate dehydrogenase (LDH), sorbitol dehydrogenase (SDH), Glucose-6-phosphate dehydrogenase (G-6-PDH) and Alkaline phosphatase (orthophosphoric monoester phosphohydrolase, ALP) was estimated by Bergmayer et al (1965) and Wooten (1982) methods respectively. The sialic acid content was estimated according to the method of Aminoff (1961). Sialic acid and lactate dehydrogenase (LDH) was measured by the method of Aminoff (1961) and Bergmayer et al. (1965).

Acetylcholinesterase assay: Brain was washed free from extraneous material using chilled saline solution and homogenized in 0.1 M phosphate buffer (10% w/v, pH 7.4). RBC was separated from blood by centrifuging at 2,500 rpm for 5 minutes. The supernatant of brain and RBC were used for the assay of acetylcholinesterase (AChE) by using fully automated biochemical analyzer by the method of King, 1984.

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.

Sperm analysis: The left cauda epididymis was used for sperm motility and right cauda epididymis was used for sperm counts and morphology. Left cauda epididymis was first weighed, placed in a petri dish. An appropriate dilution (1:20) was made with physiological saline (0.9% NaCl). Cauda epididymis was nicked in few sites with a scalpel blade and kept at 37°C to release the spermatozoa from the tubules. The sperm suspension was examined within 5 minute after their isolation from epididymis. The counting of both motile and immotile sperms was done under a phase contrast microscope (Nikon Eclips E600) at 40x magnification. The calculated results were finally expressed as percent motility (Freund and Carol, 1993).

Right cauda epididymis was weighed, diluted in 1:20 with physiological saline (0.9% NaCl) solution in a petri dish and minced with a scalpel blade in the mid-to-distal region. Suspension was kept at 37°C for 5 minutes for the dispersion of sperm into medium. Sperm suspension was pipetted very gently 20 times and placed in a hemocytometer and total number of the sperm head counted (Freund and Carol, 1993) under a Nikon microscope (Nikon Eclips E600) at 40x magnification. Each sample was counted thrice and mean value was taken for calculation.

10 ml of sperm suspension was taken and added with 1ml of 10% neutral buffer saline and mixed gently. Then 2ml of sperm suspension was mixed with 2 drop of 1% eosin Y, followed by 40-60 minutes incubation at room temperature. Sample was placed on a slide, air dried and mounted for permanent slide preparation (Seed et al., 1996). The slides were evaluated by using phase contrast microscope (Nikon Eclips E600) at 100x magnification.

Histopathological studies: Liver, kidney, brain, adrenal, testes and epididymis of rats exposed to different doses (3, 6 and 9 mg kg⁻¹ d⁻¹) of chlorpyrifos and controls were fixed in 10% formal saline solution. After tissue processing paraffin sections of each tissue were cut at 5 µm thickness and stained with haematoxylin and eosin for microscopic examination.

Statistical analysis: Statistical significance between control and experimental values were calculated by Fisher's Student 't' test (1950). Statistical comparison for body weight gain was made using one way ANOVA (Seigel, 1956).

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 1. 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 1. 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 2. The vital organs like liver, brain, kidney, spleen, epididymis and seminal vesicle of rats exposed to different doses of chlorpyrifos were comparable to control. However, testes and adrenal showed a significant decrease and increase in their weights respectively at high dose (9 mg kg⁻¹ d⁻¹) level.

Biochemical changes: The results of serum biochemical parameters of male rats are shown in Table 3. There was no

Table - 1: Body weight and mortality pattern of male rats orally administered to chlorpyrifos for 90 days

Chlorpyrifos (mg kg ⁻¹ d ⁻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	171.7 ± 8.36	190.5 ± 3.25	221.7 ± 5.32	244.1 ± 7.23	42	0	0	0(0)*
3	162.0 ± 3.04	196.5 ± 7.20	211.5 ± 4.26	238.1 ± 3.60	47	0	1	1(2)
6	163.7 ± 6.90	189.0 ± 3.11	219.5 ± 2.14	229.7 ± 6.67	40	0	0	2(2)
9	171.0 ± 2.41	199.5 ± 5.58	243.8 ± 3.80	231.3 ± 3.85	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 the level of p<0.05

Table - 2: Relative organ weight (g)* of male rats orally administered to chlorpyrifos for 90 days

Organs	Chlorpyrifos (mg kg ⁻¹ d ⁻¹)			
	Control	3	6	9
Liver	3.02 ± 0.316	3.28 ± 0.093	3.26 ± 0.028	3.15 ± 0.086
Brain	0.684 ± 0.017	0.639 ± 0.011 [#]	0.657 ± 0.012	0.694 ± 0.039
Kidney	0.624 ± 0.063	0.715 ± 0.017	0.646 ± 0.012	0.714 ± 0.047
Spleen	0.303 ± 0.015	0.300 ± 0.008	0.366 ± 0.025	0.391 ± 0.022
Adrenal	0.012 ± 0.0005	0.016 ± 0.010	0.0124 ± 0.001	0.022 ± 0.001 [#]
Testes	1.06 ± 0.046	0.958 ± 0.086	0.914 ± 0.053	0.908 ± 0.052 [#]
Epididymis	0.403 ± 0.024	0.358 ± 0.033	0.343 ± 0.021	0.377 ± 0.011
Seminal vesicle	0.339 ± 0.033	0.344 ± 0.020	0.341 ± 0.004	0.320 ± 0.013

Value represents mean ± SE of 10 rats, # = Significant by Student 't' test at the level of p<0.05, * = Relative organ weight= $\frac{\text{Organ weight} \times 100}{\text{Body weight}}$

Table - 3: Serum biochemical profile of male rats orally administered to chlorpyrifos for 90 days

Parameters	Chlorpyrifos (mg kg ⁻¹ d ⁻¹)			
	Control	3	6	9
Total protein (g dl ⁻¹)	6.90 ± 0.036	6.58 ± 0.233	6.35 ± 0.030	5.81 ± 0.300
Cholesterol (mg dl ⁻¹)	40.16 ± 6.51	37.62 ± 3.97	40.50 ± 2.60	40.30 ± 3.59
Glucose (mg dl ⁻¹)	127.0 ± 7.81	114.2 ± 10.20	100.40 ± 13.37	62.29 ± 4.76 [#]
Triglycerides (mg dl ⁻¹)	13.98 ± 2.11	19.60 ± 3.43 [#]	26.48 ± 4.03 [#]	33.1 ± 3.73 [#]
Phospholipids (mg dl ⁻¹)	36.24 ± 8.74	41.40 ± 9.79	46.44 ± 11.84	41.94 ± 6.34

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

Table - 4: Biochemical profile of testes and epididymis in male rats orally administered to chlorpyrifos for 90 days

Parameters	Chlorpyrifos (mg kg ⁻¹ d ⁻¹)			
	Control	3	6	9
Testes				
Total protein †	0.740 ± 0.064	0.520 ± 0.067 [#]	0.545 ± 0.011 [#]	0.490 ± 0.033 [#]
Cholesterol ‡	9.76 ± 1.06	10.46 ± 0.714	11.68 ± 1.25	13.14 ± 0.554 [#]
Glucose ‡	4.40 ± 1.35	4.03 ± 0.698	2.94 ± 0.632	2.83 ± 0.500
Sialic acid*	57.18 ± 1.49	77.82 ± 5.71 [#]	91.19 ± 8.06 [#]	88.45 ± 6.10 [#]
Alkaline §Phosphatase (ALP)	24.40 ± 4.25	24.79 ± 2.84	25.61 ± 2.96	26.12 ± 0.988
Lactate® dehydrogenase (LDH)	0.937 ± 0.446	0.922 ± 0.030	0.870 ± 0.025	0.818 ± 0.040
Sorbitol ø dehydrogenase (SDH)	2.58 ± 0.490	1.71 ± 0.114	1.14 ± 0.189 [#]	0.738 ± 0.174 [#]
Glucose-6-phosphatasedehydrogenase □	159.9 ± 24.79	193.4 ± 28.28	229.4 ± 31.92	291.7 ± 53.1 [#]
Epididymis				
Total protein †	0.676 ± 0.050	0.536 ± 0.041 [#]	0.530 ± 0.012 [#]	0.498 ± 0.033 [#]
Sialic acid*	60.74 ± 1.07	77.88 ± 5.45 [#]	92.96 ± 7.44 [#]	91.69 ± 5.55 [#]
Lactate® dehydrogenase (LDH)	0.896 ± 0.038	0.846 ± 0.011	0.842 ± 0.029	0.765 ± 0.054

Value represents mean ± SE of 5 rats, # = Significant by Student 't' test at the level of p<0.05, † g dl⁻¹, ‡ mg dl⁻¹, * n mole acetylneuraminic acid formed min⁻¹g⁻¹ tissue, § n mole para-nitro phenol liberated min⁻¹mg⁻¹ protein, ® n mole pyruvate liberated g⁻¹ tissue min⁻¹, ø U L⁻¹, □ n mole NADPH formed min⁻¹

Table - 5: Hematological profile of male rats orally administered to chlorpyrifos for 90 days

Parameters	Chlorpyrifos (mg kg ⁻¹ d ⁻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 ± 2.41	9.26 ± 1.95	9.39 ± 1.32	15.10 ± 1.34 [#]
Hb (g dl ⁻¹)	13.34 ± 0.294	12.52 ± 0.417	11.64 ± 0.392 [#]	11.02 ± 0.469 [#]
HCT (%)	41.44 ± 1.35	40.76 ± 2.41	36.24 ± 1.61 [#]	32.82 ± 2.72 [#]
Lymphocytes (%)	75.0 ± 5.76	70.5 ± 6.11	71.66 ± 3.26	75.20 ± 1.15
Monocytes (%)	2.50 ± 0.425	2.40 ± 0.099	2.24 ± 0.193	2.64 ± 0.355
Granulocytes (%)	22.5 ± 1.09	26.98 ± 5.69	26.06 ± 3.12	22.16 ± 5.42

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

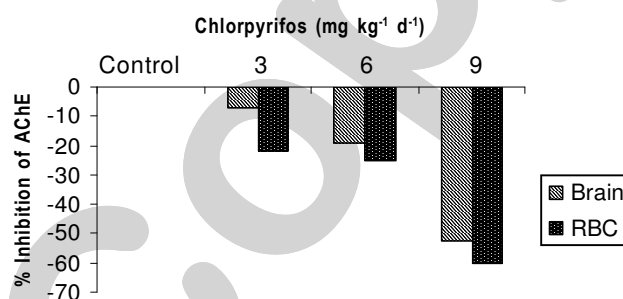
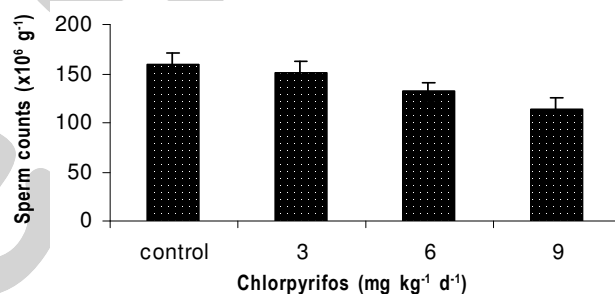
Table - 6: Frequency of abnormal sperm induced in male rats after oral administration of chlorpyrifos for 90 days

No. of rats	Control (0.4 ml rat ⁻¹)	Chlorpyrifos (9 mg kg ⁻¹ d ⁻¹)
	5	5
Acrosome abnormality		
Bent upward	10	7
Bent downward	14	8
Absent	11	15
Head abnormality		
Double head	-	-
Banana head	18	10
Amorphous head	10	2
Tail abnormality		
Double tail	-	-
Folded tail	9	11
Loop tail	14	8
Total no. of sperm counted	1198	1199
Total no. of sperm (normal)	1112	1124
Total no. of sperm (Abnormal)	86	75
% Abnormality	7.17	6.25

change in the total protein, cholesterol and phospholipids of male rats exposed to chlorpyrifos for 90 days. However, a significant decrease was noted in the level of glucose at high dose (9 mg kg⁻¹d⁻¹) of chlorpyrifos. A significant dose dependent increase in serum triglyceride level was observed.

The results of testicular biochemical parameters of male rats are shown in Table 4. Male rats exposed to chlorpyrifos up to 9 mg kg⁻¹d⁻¹ did not show any change in the testicular enzyme activities of LDH, ALP and glucose level. An increase in the activity of G-6-PDH and cholesterol was observed in the testes of rats exposed to high dose (9 mg kg⁻¹d⁻¹) of chlorpyrifos. Sialic acid content was significantly increased at all doses but the activity of SDH was increased only at 6 and 9 mg kg⁻¹d⁻¹ doses of chlorpyrifos. In contrast, decreased level of protein content was observed in the testes of rats exposed to different doses of chlorpyrifos.

Epididymal protein content was significantly decreased in a dose dependent manner and sialic acid was increased in rats

**Fig. 1:** Percent inhibition of acetylcholinesterase in Brain and RBC of male rats orally administered to chlorpyrifos for 90 days**Fig. 2:** Histogram showing epididymal sperm counts (x10⁶ g⁻¹) of rats orally administered to chlorpyrifos for 90 days

exposed to different doses of chlorpyrifos. However, there was no change in the activity of LDH at any doses of chlorpyrifos (Table 4).

Acetylcholinesterase assay: The inhibition of AChE in brain and RBC of male rats exposed to different doses (3, 6 and 9 mg kg⁻¹d⁻¹) of chlorpyrifos and control are shown in figure 1. Inhibition of AChE was dose dependent in both brain and RBC. Percent inhibition ranges from 7-52 in brain and 22-60 in RBC.

Hematological changes: The results of hematological parameters in male rats exposed to different doses (3, 6 and 9 mg kg⁻¹d⁻¹) of chlorpyrifos are shown in Table 5. The Hb and HCT content were significantly decreased in animals exposed to chlorpyrifos at 6 and 9 mg kg⁻¹d⁻¹ doses. However, RBC showed significant decrease at all doses. There was no change in WBC counts at 3 and 6 mg kg⁻¹d⁻¹ but showed a significant increase at 9 mg kg⁻¹d⁻¹ dose level.

Sperm studies: The results of epididymal sperm counts and morphology are shown in figures 2 and table 6 respectively. There

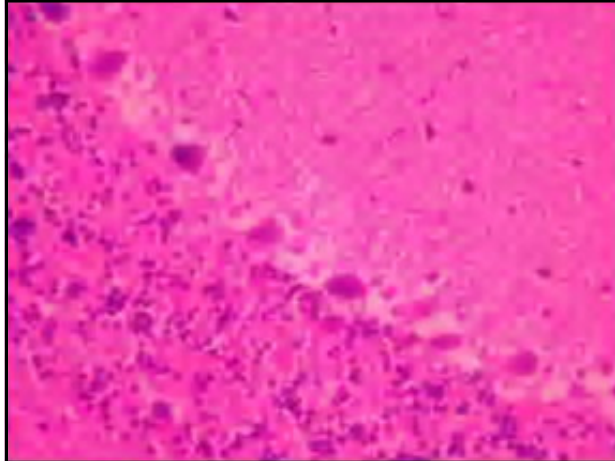


Fig. 3: Section of cerebellum of male rats exposed to chlorpyrifos ($9 \text{ mg kg}^{-1}\text{d}^{-1}$) for 90 days showing necrosed purkinje cells with loss of dendrites and loss of granules in granular layer H&E x 151

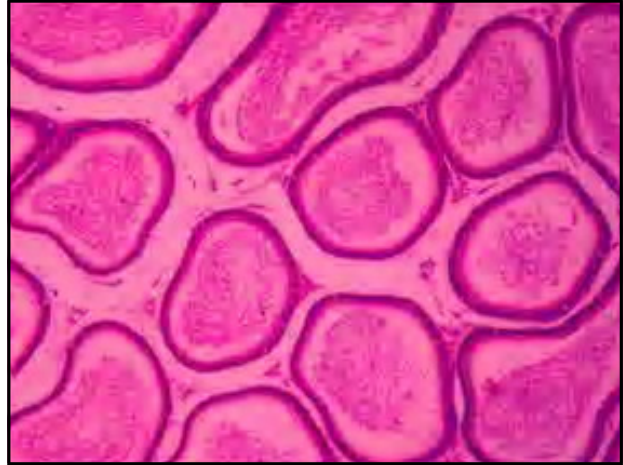


Fig. 6: Section of epididymis of male rats exposed to chlorpyrifos ($9 \text{ mg kg}^{-1}\text{d}^{-1}$) for 90 days showing loss of sperm with increased interstitial space H&E x 118

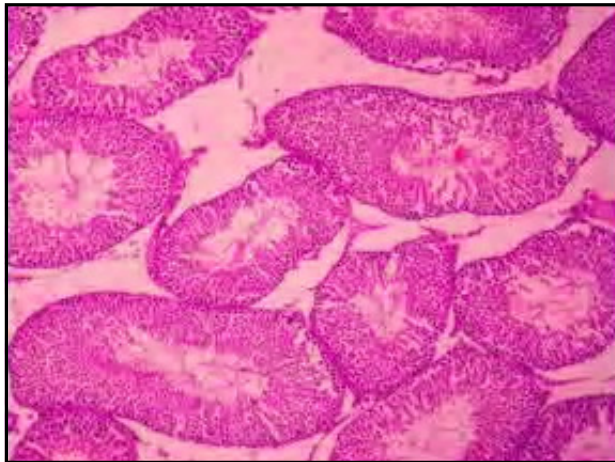


Fig. 4: Section of testes of male rats exposed to chlorpyrifos ($9 \text{ mg kg}^{-1}\text{d}^{-1}$) for 90 days showing degeneration of seminiferous tubules with large interstitial space H&E x 118

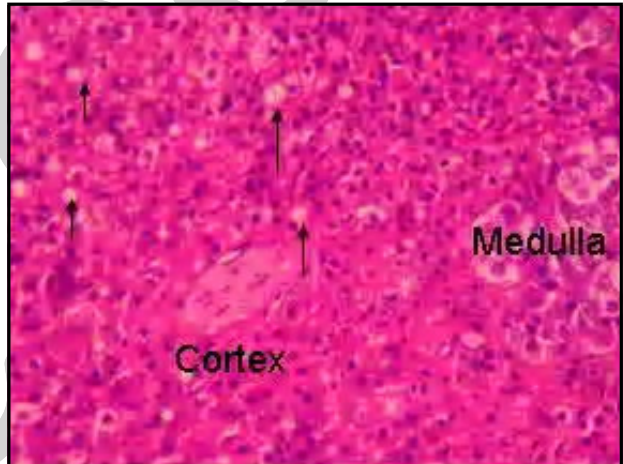


Fig. 7: Section of adrenal of male rats exposed to chlorpyrifos ($9 \text{ mg kg}^{-1}\text{d}^{-1}$) for 90 days showing cortical vacuolization H&E x 151

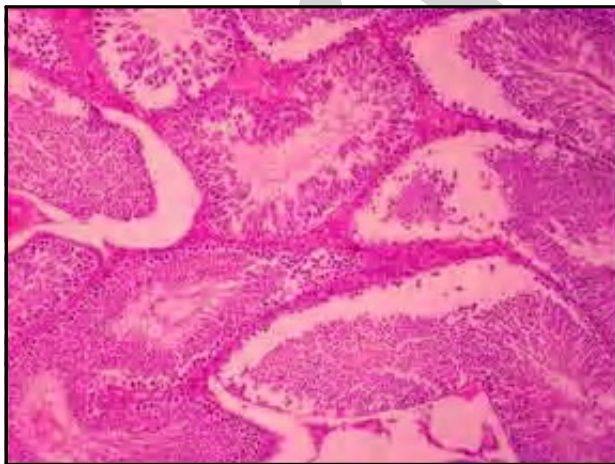


Fig. 5: Section of testes of male rats exposed to chlorpyrifos ($9 \text{ mg kg}^{-1}\text{d}^{-1}$) for 90 days showing sloughing of germinal cells into the lumen and loss sperm H&E x 118

was a significant decrease in the number of sperm in rats exposed to high dose ($9 \text{ mg kg}^{-1}\text{d}^{-1}$) of chlorpyrifos. However, sperm morphology and sperm motility were not affected at any dose levels.

Histopathological studies: Repeated exposure of high dose ($9 \text{ mg kg}^{-1}\text{d}^{-1}$) of chlorpyrifos to male rats for 90 days has produced significant patho-morphological changes in vital organs. Cerebellum of brain has showed loss of granules in granular layer and necrosed purkinje cells with absence of dendrites (Fig. 3) as compared to controls. Hepatocytes of liver showed mild focal necrosis in the animals exposed to chlorpyrifos. Microscopical examination of testes and epididymis showed slight changes in seminiferous tubules. The observed changes in the testes were increased interstitial space along with sloughing of germinal cells (Fig. 4,5) into the lumen. The epididymal tubules showed increased interstitial spaces with loss of sperm (Fig. 6). Cortex region of adrenal gland showed slight vacuolization (Fig. 7). There was no microscopic change in kidney as compared to controls. Histopathology of liver, brain, kidney,

testes, epididymis and adrenal of male rats exposed to lower doses (3 and 6 mg kg⁻¹d⁻¹) of chlorpyrifos did not exhibit any significant pathological changes.

The present study has shown significant signs of toxicity in animals during long-term exposure of chlorpyrifos. These included piloerection, diarrhoea, 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⁻¹d⁻¹) has produced loss in body weight gain. In contrast, no change in the body weight at 15 mg kg⁻¹d⁻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⁻¹d⁻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).

Chlorpyrifos irreversibly inhibits AChE activity in the central nervous system and lowers plasma, erythrocyte and brain cholinesterase (Ogawa *et al.*, 1989; Sakai, 1991). In this study, significant inhibition of AChE in brain and RBC along with cholinergic symptoms in animals were associated with loss of granules from granular layer and necrosed purkinje cells of cerebellum after chlorpyrifos exposure.

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

The increased weight and presence of cortical vacuolization in adrenal of male rats in the present study supports the findings of Yano *et al.* (2000) indicating absence or unclear toxicological significance. This has been supported by the findings of USEPA (2000) and Anonymous (1997). It is important to note that organophosphorous pesticides have been reported to produce testicular toxicity in animals (Contreras and Bustos-Obregon, 1999; Pant and Srivastava, 2003). The decrease in the testicular weight of rats in the present study appears to be due to loss of spermatogenic elements and spermatozoa. Sherin and 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. Sloughing of germinal cells in the present study may be due to the inhibition of microtubules formation. Similar to our findings, Hess *et al.* (1991) have reported a decrease in testicular weight and sloughing of germinal cells with benomyl. Though, the present study has described the reproductive toxicity of chlorpyrifos but there is no cytotoxic description (mechanism of toxicity has not been dealt). Russel *et al.* (1981) and Christensen (1965) have advocated sertoli cells to be responsible for bringing such changes.

The increase in the testicular marker enzymes (Shen and Lee, 1976) such as sialic acid, G-6-PDH and cholesterol was noted leading to the adverse changes in the male reproductive system. Organophosphorous pesticides are known to cause changes in testicular marker enzymes (Hodgen and Sherins, 1973; Joshi *et al.*, 2003). Chlorpyrifos was reported to affect profoundly hypothalamic gonadotrophic releasing hormone gene expression and growth hormones (Gore, 2001). 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 be concluded from the present study that subchronic oral exposure of male rats to chlorpyrifos has caused organ toxicity as well as morphology and number of sperms at 9 mg kg⁻¹ dose level.

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