MORPHOLOGICAL EFFECTS OF PESTICIDE - CHLORPYRIFOS ON KIDNEY IN ALBINO RATS
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HOW TO CITE THIS ARTICLE:

ABSTRACT: AIM: Pathological lesions have been widely used as biomarkers for health evaluation of organism exposed to pollutants and can be used as warning symptoms for organism health. There are few reports regarding morphological changes in kidney following pesticide Chlorpyrifos exposure which has prompted us to undertake this study. MATERIALS AND METHODOLOGY: The present study was conducted on 45 inbred adult Wistar albino rats of either sex, weighting 145 – 165 gms. These animals were randomly divided into 3 groups A, B, C. Oral Chlorpyrifos was given to the experimental groups B and C in dose of 5 mg/kg body weight and 10 mg/kg body weight respectively. Group A served as control and was left as such. The animals were observed for clinical signs of toxicity and behavioral changes throughout the experimental period. Body weight was recorded at zero day and then at 2 weekly interval for 8 weeks. 3 animals from each group were sacrificed after 1 week, 2nd week, 4th week, 6th week and 8th week of initiation of experiment to see the morphological changes in the kidney architecture. RESULTS: Gross The animals in group A were active, quick to respond and food intake was normal throughout the experimental period where as the animals of groups B & C showed decreased physical activity, dullness, depression, Piloerection, shivering salivation and lacrimation with more severity in Group C. Body Weight Decrease in weight gain was observed in both experimental groups (B & C) as compared to control group A. A statistically significant decrease in weight gain was observed in Group C after 6th and 8th week of Chlorpyrifos treatment. Macroscopic Changes Grossly the kidneys of rats in control group showed no significant changes. The kidneys of rats in Group B showed no change in colour and consistency after 1st, 2nd and 4th weeks of treatment with Chlorpyrifos. However after 6th week there was congestion, thickening and adherence of capsule at some places. With increasing dose and duration of treatment with Chlorpyrifos i.e after 8th week the kidneys were small in size, shrunken and had irregular contour. In Group C upto 1st week of treatment with Chlorpyrifos the kidneys were normal in gross appearance but after 2nd, 4th and 6th weeks the kidneys were grossly congested and friable to touch and the capsule was thickened. After 8 weeks of exposure the kidneys were small in size, shrunken and had irregular contour with thickened outer capsule. Naked eye observations of coronal section of kidney Group A showed clear demarcation of outer cortex and inner medulla. Group B showed no visible change after 1st and 2nd weeks of treatment. After 4th and 6th week there was visible haemorrhage at corticomedullary junction ranging from focal to completely obscuring of corticomedullary junction. After 8th week there was no demarcation between cortex and medulla and whole of medulla was congested. Group C (10 mg/kg b wt) upto 1st week there was no significant visible change in coronal section of kidneys. After 2nd and 4th weeks there was visible haemorrhage at corticomedullary junction. After 6 weeks of treatment whole of the medulla was congested and after 8 weeks there was no demarcation between cortex and medulla, whole of the coronal section of the kidneys showed dark reddish brown colour due to haemorrhage.
CONCLUSION: The present study showed that significant morphological changes were caused in the kidneys of rats administered with Chlorpyrifos. These changes were markedly different from the control rats. Hence this study brought into light the renal toxicity induced by Chlorpyrifos which was found to be significant at high dose level.

KEY WORDS: Pesticide, kidney, capsule, corticomedullary junction

INTRODUCTION: Environmental Pollution, when considered in its broadest context, is a by-product of human activities and its significance is in what ways it affects directly or indirectly the living population. One of the ways of environmental pollution is by chemical pesticides.

The term pesticide covers a wide range of compounds including insecticides, fungicide, Herbicide, rodenticide, plant growth regulators and others (Cope et al., 2004)(1)

Food and agricultural organization (FAO) has defined the term pesticide as: “Any substance or mixture of substances intended for preventing, destroying or controlling any pest including vectors of humans or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with production, processing storage, transport or marketing of food, agricultural commodities, wood and wood products or animal feed stuffs or substances which may be administered to animals for control of insects, arachnids or other pests in or their bodies.” (Food and Agricultural organization of United Nations, 2002)(2)

Pesticides have played vital role in controlling agricultural, industrial, home and public health pest worldwide (Bjorling-Poulsen et al., 2008)(3). However, their use poses animal and human health concerns because of their toxicity, widespread use and release into the environment. According to the World Health Organization, 3 million cases of pesticide poisoning occur every year, resulting in more than 250,000 deaths (Yang and Deng, 2007)(4). Despite this alarming figure, there is currently no global system to track and stem poisoning or diseases associated with pesticide use. The high rate of poisoning may be attributed to a number of reasons, including farmers’ poor knowledge about pesticides and pesticide use, less protection against exposures, little formal education of agricultural workers, minimal understanding of the health risks and, most importantly, inadequate safety warnings on the packages by the manufacturers Gbaruko et al., 2009(5)

If the credits of pesticides include enhanced economic potential in terms of increased production of food, fiber and amelioration of vector borne diseases, then their debits have resulted in serious health implications to man and his environment.

In spite of undesirable and unwanted effects of pesticides on man, there is sequential rise in production and consumption of pesticides in India during last three decades. Many chemical formulations were synthesized, introduced and widely used in pest control programs. These synthetic chemical pesticides can be structurally classified into the following groups:

a. Organochlorine pesticides.

b. Organophosphate pesticides.

c. Carbamates.

d. Synthetic pyrethroids.

(Abdel and Saleh, 1999)(6)

The organophosphate insecticides have superseded the organochlorines owing to their rapid biodegradability and shorter persistence in the environment. The organophosphorus pesticides are among the most widely use insecticides globally and they are readily available commercially for
domestic and industrial purposes. They account for 50% of all insecticides applied worldwide. But as a consequence of their widespread use in agriculture and public health, these insecticides ultimately reach the environment and affect the life there in. Organophosphorus compounds exist in liquid and solid forms and are – Phosphorothioates, Phorodithioates and Phosphates (Tripathi and Srivastava, 2010) (7)

Chlorpyrifos [0, 0-diethyl-o (3, 5, 6-trichloro-2-pyridil) phosphor-thioate] is a member of organophosphate class of pesticides that elicits broad spectrum insecticidal activity against a number of important arthropod pests (Racke, 1993) (8). Chlorpyrifos kills insects upon contact by affecting normal function of nervous system.

Chlorpyrifos is a non-systemic insecticide designed to be effective by direct contact, ingestion and inhalation. Poisoning occurs as a result of agricultural use, accidental exposure, suicide and rarely homicide (Yurumez et al, 2007) (9).

Chlorpyrifos is firstly activated to its active metabolite Chlorpyrifos – oxon by oxidative desulfuration which in turn is responsible for mammalian toxicity through inhibition of cholinesterase (Timchalk et al. ,2002; Betancourt and Carr;2004;Tongbai and Damrongphol, 2011)(10,11,12). Once Cholinesterase has been inactivated, acetylcholine accumulates throughout the nervous system (Latuszynska et al., 1999) (13) Toxicity of pesticide cause adverse effect on many organs like Kidney, Liver, Brain and Blood cell (Bebe and Panemanogalore, 2003) (14). Chlorpyrifos is readily absorbed from gastrointestinal tract. In single dose oral study conducted on Human volunteers Chlorpyrifos was 70% absorbed from gastrointestinal tract (Nolan et al., 1984) (15) and in rats absorption of Chlorpyrifos through Gastrointestinal tract after single dose gavage study ranged from 84-90%. After entering the body of organism Chlorpyrifos is eliminated primarily through kidneys in urine. In rats, following oral intake of Chlorpyrifos, about 90% is removed in urine and 10% is excreted in faeces. Barr et. al. in 2005 (16) reported detectable 3,5,6 trichloropyridil (TCP) a metabolite of Chlorpyrifos in urine of 90% of the approximately 2000 samples collected from US residents (aged 2-59 yrs). The elimination half life for this metabolite (TCP) in humans following oral or dermal exposure was approximately 27 hrs (Nolan et al., 1984) (15).

The mortality rate of organophosphorus poisoning is high and the fatal issue is often related to delay in diagnosis or improper management. Acute treatment includes rapid administration of Atropine, which blocks the muscarinic effects and that of Pralidoxime which reactivates acetylcholine inhibited by organophosphates (Yurumez et al., 2007) (9).

The toxicity of organophosphate (CPF) in mammalian animals has received much attention in the recent years. Kidney, the major detoxification organ for many xenobiotics is frequently susceptible to nephrotoxic effects. Nephrotoxicity is one of the toxic manifestations of Chlorpyrifos after its long term as well as acute exposure.

The structure of the kidney of human beings and that of rats is very similar. So in the present study Albino rat were taken as experimental animal to study the effect of Chlorpyrifos in kidney. Also the unilobar kidney of the rat resembles each lobe of multilobar kidney of human beings. And unit of gross structure of kidney is 'lobe'. Moreover general microscopic structure of Nephron and its disposition within tubules in similar in both humans and rats (Ham and Cormach, 1979) (17).

As a consequence of renal heterogeneity, mechanism of chemically induced injury cannot be explored easily, but can be evaluated by studying morphological changes in different parts.
constituting renal tissue. Pathological lesions have been widely used as biomarkers for health evaluation of organism exposed to pollutants and can be used as warning symptoms for organism health.

There are few reports regarding morphological changes in kidney following pesticide Chlorpyrifos exposure which has prompted us to undertake this study.

Keeping in view the above facts, present study is designed to evaluate the effect of orally administered Chlorpyrifos at different doses for 2 months on kidney architecture of Albino Rat.

MATERIALS AND METHODOLOGY: In the present study, Albino rats served as experimental animals:

COLLECTION OF ANIMALS: Healthy Wistar Albino rats, forty five in number of either sex weighing between 145 – 165 mg were taken for the study. The rats were procured from the Central Animal House of Government Medical College, Jammu. The investigation was conducted upon getting clearance from Institutional Animal Ethics Committee (IAEC).

Grouping of Animals
After two weeks of acclimatization, the rats were randomly divided into following groups. Identification number was given to rats of each group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of Animals</th>
<th>Identification Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A :</td>
<td>Control – 15 Animals</td>
<td>A1 – A15</td>
</tr>
<tr>
<td>Group B :</td>
<td>Experimental(15 Animals)</td>
<td>B1 – B15</td>
</tr>
<tr>
<td></td>
<td>Group I</td>
<td></td>
</tr>
<tr>
<td>Group C :</td>
<td>Experimental(15 Animals)</td>
<td>C1 – C15</td>
</tr>
<tr>
<td></td>
<td>Group II</td>
<td></td>
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</tbody>
</table>

The animals were group housed (12 hours light / dark cycle) in labeled cages in a room where temperature was maintained at 25° ± 2°C. The cages were made of solid plastic sides and base and stainless steel grid top. Rice husk was used as bedding material. The animals were fed with standard laboratory feed and water ad-libitum throughout the experimental period. The animals were observed for abnormal physical or behavioral change throughout the experimental period. The study was done from December 2011 to February 2012.

PESTICIDE DETAILS: Chemical used for the study was Chlorpyrifos, an Organophosphorus pesticide. It was purchased from the market with Molecular formula C9H11Cl3 – NO3 PS

EXPERIMENT PROTOCOL: Experimental animals were given oral Chlorpyrifos using a cannula
Group A rats served as control and were left as such.

Drug regime in Group A and Group B were as follows:

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Date of Adm. of 1st Dose</th>
<th>Route of Adm.</th>
<th>Duration</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1 – B3</td>
<td>7/12/2011</td>
<td>Oral</td>
<td>1 Week</td>
<td>5mg/kg</td>
</tr>
</tbody>
</table>
Chlorpyrifos was diluted in distilled water to obtain desired concentration. Fresh dosing solution was made every time.

Parameters Studied:

Clinical signs and behavioral activities: All animals were observed daily for physical or behavioral change throughout the experimental period.

Body weight: Live body weight of animals was recorded using electronic weighing machine. 3 animals from each group were weighed at zero day and after every 2 weeks till 8 weeks. Body weight gain was calculated as compared to the zero day weight of the same group. Body weight gain in Chlorpyrifos treated rats were compared for statistical analysis [analysis of variance (ANOVA) and Bonferroni test was applied for comparing two groups- i.e. Control and Chlorpyrifos treated, by using body weight of control rats of respective week]. Animal were anaesthetized by using Diethyl ether. A piece of cotton soaked in ether was placed in desiccators’ jar and then the animals to be sacrificed were placed in the jar and lid was closed. 3 rats from each of 3 groups were sacrificed after last dose at the end of 1st week (14th of December 2011), 2nd week (21st of December 2011), 4th week (4th of January 2012), 6th week (18th of January 2012) and 8th week (1st of February 2012) from the date of initiation of experiment. Sacrificing of animals was followed by dissection a mid-line incision through the skin extending from xiphisternum to pubic symphysis was given with a knife and was extended laterally at its lower end to achieve maximum exposure of abdominal cavity and the kidneys were exposed by displacing intestines. Later each kidney was removed in total and then washed with distilled water. After dissection and removal of kidney following parameters were studied

Macroscopic Changes: Kidneys were examined first grossly and later on section of kidney was made in coronal plane.

RESULTS AND OBSERVATION:

<table>
<thead>
<tr>
<th>GROUP B (Table 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental group</strong></td>
</tr>
<tr>
<td>B4 – B6</td>
</tr>
<tr>
<td>B7 – B9</td>
</tr>
<tr>
<td>B10 – B12</td>
</tr>
<tr>
<td>B13 – B15</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GROUP C (Table 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experimental group</strong></td>
</tr>
<tr>
<td>C1 – C3</td>
</tr>
<tr>
<td>C4 – C6</td>
</tr>
<tr>
<td>C7 – C9</td>
</tr>
<tr>
<td>C10 – C12</td>
</tr>
<tr>
<td>C13 – C15</td>
</tr>
</tbody>
</table>
I. Clinical signs and Behavioral activities

Group A (Control) – 15 rats

The animals of this group served as control and were left as such. The animals in this group were active, quick to respond and the food intake was normal throughout the experimental period i.e. 8 weeks.

Group B (5 mg/kg body weight Chlorpyrifos) – 15 rats.

This group was given 5 mg/kg body weight Chlorpyrifos orally. The clinical signs observed in this group were characterized by decreased physical activity and dullness as compared to control group in which the animals were seen moving around actively. In group B, the activity decreased with passage of time and the rats grouped together in corner of the cages with dull activity and moved initially on sound and later by being teased by hand. Besides this there was lacrimation, salivation, shivering and Piloerection which was observed as puffing of fur. These signs were observed within half-an hour of giving the oral dose. There was reduced intake of food gradually with passage of time with Chlorpyrifos treatment.

Group C (10 mg/kg body weight Chlorpyrifos) – 15 rats

This group was given 10 mg/kg body wt of Chlorpyrifos orally. The animals of this group also showed decreased physical activity, dullness, depression, Piloerection, shivering, salivation and lacrimation. The animals in this group were slow to respond even on touch and had reduced intake of food because of decreased appetite. In few experimental animals, brownish yellow staining of hair near anal region was seen which was suggestive of diarrhoea.

II. Body Weight Analysis.

The daily oral administration of Chlorpyrifos was continued for 2 months and their live body weights were recorded. 3 animals from each group were weighed at zero day and after every 2 weeks till 8 weeks. Mean body weights were calculated as shown in Table-1. Body weight gain was calculated as compared to the zero day weight of the same group. Body weight gain in Chlorpyrifos treated rats was compared for statistical analysis. [Analysis of variance (ANOVA) and Bonferroni test was applied for comparing two groups – control and Chlorpyrifos treated, by using body weight of control rats of respective week.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Zero day</th>
<th>2 Weeks</th>
<th>4 Weeks</th>
<th>6 Weeks</th>
<th>8 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>152.33</td>
<td>166.0</td>
<td>174.33</td>
<td>185.33</td>
<td>198.66</td>
</tr>
<tr>
<td>B</td>
<td>155.33</td>
<td>168.0</td>
<td>177.33</td>
<td>182.0</td>
<td>190.0</td>
</tr>
<tr>
<td>C</td>
<td>148.33</td>
<td>160.0</td>
<td>166.33</td>
<td>170.0</td>
<td>173.0</td>
</tr>
</tbody>
</table>

(Table-3) Mean Body Weight

Results of body weight gain in Group B
Rats treated with Chlorpyrifos 5 mg/kg have shown a decrease in body weight gain. There was decrease in mean body weight gain and percent weight gain after 6th and 8th weeks of treatment, however it was not statistically significant (p>0.05).

Results of Body Weight gain in Group C
Rats treated with Chlorpyrifos (10 mg/kg/day) have shown a decrease in mean body weight gain and percent weight gain. A significant decrease in body weight gain recorded after 6th (P<0.03) and 8 weeks (P<0.004) Chlorpyrifos treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Zero day</th>
<th>2 Week</th>
<th>4 Weeks</th>
<th>6 Weeks</th>
<th>8 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A(control)</strong></td>
<td>152.33±1.85</td>
<td>166±1.52</td>
<td>174.33±0.88</td>
<td>185.3±2.02</td>
<td>198.6±1.76</td>
</tr>
<tr>
<td>Percentage gain/significance</td>
<td>-/-</td>
<td>8.9/-</td>
<td>14.4/-</td>
<td>21.6/-</td>
<td>30.4/-</td>
</tr>
<tr>
<td><strong>Group B(5 mg/kg b wt)</strong></td>
<td>155.33±2.40</td>
<td>168.0±2.30</td>
<td>177.33±4.37</td>
<td>182.0±4.61</td>
<td>190.0±5.29</td>
</tr>
<tr>
<td>Percentage gain/significance</td>
<td>-/-</td>
<td>8.1/NS</td>
<td>14.1/NS</td>
<td>17.1/NS</td>
<td>22.3/NS</td>
</tr>
<tr>
<td><strong>Group C(10mg/kg b wt)</strong></td>
<td>148.33±2.20</td>
<td>160.0±2.64</td>
<td>166.33±1.45</td>
<td>170.0±1.145</td>
<td>173.0±1.15</td>
</tr>
<tr>
<td>Percentage Gain/significance</td>
<td>-/-</td>
<td>7.8/NS</td>
<td>12.1/NS</td>
<td>14.6/p&lt;0.03</td>
<td>16.6/p&lt;0.004</td>
</tr>
</tbody>
</table>

Table 4. Body weight (gm) and percent gain of rats orally administered Chlorpyrifos for 8 weeks.

NS, NOT SIGNIFICANT.
Each value represents mean ± s. E. Of three rats; percent gain was calculated comparing with the zero day value of respective group; anova –p< 0.05 was considered as significant; bonferroni test was used for comparing significance between two groups- control and chlorpyrifos (by using body weight of control rats of respective week).

Group A (Control)
Macroscopic Observations: No gross changes observed

Group B (5 mg /kg body weight Chlorpyrifos)
Macroscopic Observations: Grossly, the kidney in group B showed no change in shape, colour and consistency after 1st, 2nd and 4th weeks of treatment with Chlorpyrifos. However after 6th week of treatment with Chlorpyrifos, the kidney showed congestion and there was thickness of capsule and adhesions at places. 8 weeks after treatment with Chlorpyrifos, the kidney was small in size, shrunken and had irregular contour and the outer capsule was thickened.

Coronal section of kidney when viewed with naked eye in Group B, showed clearly visible cortex and medulla after 1st week and 2nd week of treatment with Chlorpyrifos and respectively.

After 4 weeks, cortex and medullary zones were clearly demarcated but there was focal haemorrhage at corticomedullary junction.
Following 6 weeks of treatment with Chlorpyrifos, there was observed congestion at corticomedullary junction and even the base of the medulla was affected by the congestion.

After 8 weeks of treatment whole of the medulla was seen to be congested due to which corticomedullary junction was obscured. Cortex was congested especially along the upper poles.

Group C (10 mg/kg body weight)
Macroscopic Observations: Grossly the kidney in group C was normal in shape, colour and texture after 1st week of treatment with Chlorpyrifos however after 2nd, 4th and 6th weeks of Chlorpyrifos exposure the kidneys showed no change in shape, but there was congestion and friability. The outer capsule was thickened and adherent to the Kidney at some places. 8 weeks after Chlorpyrifos treatment the kidneys of the exposed rats were small in size, shrunken and had irregular contour with thickened outer capsule.

Coronal section of kidney when viewed with naked eye was normal after 1st week.

After 2nd week of treatment with Chlorpyrifos cut section showed a focal band of haemorrhage at corticomedullary junction. Rest of the cortex and medulla were normal in appearance.

After 4 weeks, the cut section of kidney showed dark red-brown granular cortex and pale looking medulla with apex fitting into the minor calyx of the pelvis. But a thick band of haemorrhage was seen to obscure corticomedullary junction.

After 6 weeks of treatment with Chlorpyrifos, red-brown coloured outer cortex was seen and visible haemorrhage was seen in whole of the medulla.

8 weeks after treatment with Chlorpyrifos the cut section of the kidney showed no demarcation between cortex and medulla and whole of it was congested.

These features were suggestive of acute renal failure leading to chronic renal failure.

**DISCUSSION:** Pesticides have been one of the most effective weapons discovered by man to protect agricultural products from the attack of Pests. Among these Chlorpyrifos is an extensively used organophosphate pesticide. Due to its wide-spread use it poses potential harm to non target organisms including humans. Chlorpyrifos exposure leads to extensive structural damage to the kidney. The unusual susceptibility of mammalian kidney to the toxic effects of noxious chemical can be attributed in part to the unique anatomic and physiologic features of this organ. Although the kidneys constitute only 0.5% of total body mass they receive about 20-25% of the resting cardiac output. Consequently any drug or chemical in the systemic circulation will be delivered to these organs in relatively high amounts. The process involved in forming concentrated urine also serves to concentrate potential toxicants into tubular cells. Therefore, a non-toxic concentration of chemical in the plasma may reach toxic concentration in the kidney. Progressive concentration of toxicants along the nephron may result in intraluminal precipitation of relatively insoluble compounds, causing acute renal failure secondary to tubular obstruction. Finally, renal transport, accumulation and metabolism of xenobiotics contribute significantly to the susceptibility of the kidney to toxic injury.

Several studies have supported the fact that major route of excretion of Chlorpyrifos is through kidneys in urine (Nolan R. J et al., 1984, Griffin et al., 1999) (15, 19)
In the present study, some clinical signs characterized by decreased physical activity, dullness, depression, piloerection, lacrimation, salivation, shivering, diarrhea and loss of appetite were observed with doses of 5mg/kg b wt and 10 mg/kg b wt. Similar results were observed by Lotti et al (1986), Akhtar N et al. (2009), Kammon et al. (2010), Ambali et al. (2011a), Ambali et al. (2011b), Issa AM et al (2011), Bhadaniya et al. (2012), Galakatu et al. (2012), Heikal et al. (2012)(20-28). These clinical signs were attributed to cholinergic crisis due to accumulation of acetylcholine (neurotransmitter).

Body Weight Gain: In the present study, a decrease in weight gain was observed after 6th and 8th week, of treatment with Chlorpyrifos in doses of 5 mg/kg b wt and 10 mg/kg b wt for 8 weeks. This decrease in weight gain was statistically significant after 6th and 8th weeks in Group C (10 mg/kg b wt). This observation is in conformity with observations of Malik et al. (2004) Akhtar et al. (2009) Tripathi and Srivastava (2010) Ambali et al. (2011a) Mansour and Mossa (2011) Heikal et al. (2012) Bhandaniya et al. (2012) Galakatu et al. (2012), Mossa & Abbassy (2012)(29,21,7,23,30,28,26,27,31)

Macroscopic Changes
(a) Grossly, the kidneys in Chlorpyrifos treated rats in the present work were seen to be congested with increasing dose and duration of treatment. Also there was thickening and adherence of capsule at some places after 6th week 8 weeks after treatment with Chlorpyrifos the kidneys were small in size, shrunken and with irregular contour similar facts have been reported by Kumar et al. (2012), Kammon et al. (2010), Bhandaniya et al. (2012) (32, 22, 26)

(b) Coronal section of kidney, when observed with naked eye revealed clearly distinct 2 zones – outer red-brown cortex and inner lighter medulla after 1st and 2nd weeks of treatment with Chlorpyrifos (5 mg/kg body weight) in Group-B and after 1st week of treatment with Chlorpyrifos in Group-C. As the duration of treatment advanced variable degrees of corticomedullary haemorrhage was observed ranging from focal to complete obscuration of corticomedullary junction. In Group-C, after 8 weeks of treatment with Chlorpyrifos there was no demarcation between cortex and medulla and whole of the section was congested (giving dark red-brown appearance). Similar facts have been given by Kumar et al. (2012) (32). The present study showed variable intensities of changes, depending upon dose and duration of treatment and these changes were significantly different from those of control rats.

SUMMARY AND CONCLUSION: The present study is based upon the observations made on 45 albino Wistar rats weighing 145-165 gms to determine the effect of Chlorpyrifos on histomorphology of kidneys of these animals. The animals were group housed (12 hr light/dark cycle) with ad-libitum access to food and water. They were divided into 3 groups A, B and C with 15 animals each. Group A served as control group, Group B were daily administered Chlorpyrifos at a dose of 5 mg/kg b wt and animals in Group C received daily an oral dose of 10 mg/kg b wt Chlorpyrifos. All the animals were observed for ½ an hour after giving the dose and also throughout the experimental period. 3 animals from each group were weighed at 2 weekly intervals for 8 weeks and weight gain was evaluated in each group. Rats were sacrificed on 1st, 2nd, 4th, 6th and 8th week after initiation of the experiment. Kidneys were removed after dissection, examined grossly and also after making coronal section.
Gross Observations
Clinical signs and Behavioral activities –
Group A (Control)
The animals in this group were active, quick to respond and food intake was normal throughout the experimental period.

Group B & C
The animals of these groups showed decreased physical activity, dullness, depression, piloerection, shivering, salivation and lacrimation. Though severity of these signs were more in Group C. Both groups showed decreased food intake with passage of time. Few of the animals in Group C also had diarrhoea.

Body Weight: Decrease in weight gain was observed in both experimental groups (Group – B & C) as compared to control group A. A statistically significant decrease in weight gain was observed in Group C after 6th and 8th week of Chlorpyrifos treatment.

Macroscopic Changes
Grossly the kidneys of rats in control group showed no significant changes.

The kidneys of rats in Group B showed no change in colour and consistency after 1st, 2nd, and 4th weeks of treatment with Chlorpyrifos. However after 6th week there was congestion, thickening and adherence of capsule at some places. With increasing dose and duration of treatment with Chlorpyrifos i.e. after 8th week the kidneys were small in size, shrunken and had irregular contour. In Group C up to 1st week of treatment with Chlorpyrifos the kidneys of exposed rats were normal in gross appearance but after 2nd, 4th and 6th weeks the kidneys were grossly congested and friable to touch and the capsule was thickened. After 8 weeks of exposure the kidneys were small in size, shrunken and had irregular contour with thickened outer capsule.

Naked eye observations of coronal section of kidney
Group A (control) showed clear demarcation of outer cortex and inner medulla.
Group B (5 mg/kg b wt Chlorpyrifos) showed no visible change after 1st and 2nd weeks of treatment. After 4th and 6th week there was visible haemorrhage at corticomedullary junction ranging from focal to completely obscuring of corticomedullary junction. After 8th week there was no demarcation between cortex and medulla and whole of medulla was congested.
Group C (10 mg/kg b wt) up to 1st week there was no significant visible change in coronal section of kidneys. After 2nd and 4th weeks there was visible haemorrhage at corticomedullary junction which was obscuring it. After 6 weeks of treatment whole of the medulla was congested and after 8 weeks there was no demarcation between cortex and medulla, whole of the coronal section of the kidneys showed dark reddish brown colour due to haemorrhage.

These changes were markedly different from the control rats. All these changes leading to acute renal failure progressing to chronic renal failure with increasing duration. Hence this study brought into light the renal toxicity induced by Chlorpyrifos which was found to be significant at high dose level.
REFERENCES:
FIG. 1: GROSS STRUCTURE OF THE KIDNEY SEEN IN CONTROL GROUP

FIG. 2: CORONAL SECTION GROUP A (CONTROL) SHOWING OUTER CORTEX (C) AND INNER MEDULLA (M).

FIG. 3: GROSSLY CONGESTED KIDNEY WITH THICKENED CAPSULE (C) IN GROUP C.
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