Chlorpyrifos Induced Toxicity in Female Wistar Rats

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ABSTRACT

In the present study 12 healthy female rats of wistar strain were divided into three groups of four rats in each. Two groups were dosed with different doses i.e. 0.1mg/kg (T1) and 2.5 mg/kg (T2) of chlorpyrifos (CPF) by oral intubation for 8 weeks. The control group was given vegetable oil. An increase in the chromosomal aberrations was observed which indicates a mutagenic behavior of (chlorpyrifos) CPF. The increase in the chromosomal aberrations was dose dependent. Microsomal degranulation test is a rapid, reliable and inexpensive method for the detection of the carcinogenicity. The liver microsomes of the treated and control rats were used to test the carcinogenic potential of CPF. A decrease in RNA/Protein ratio is observed in the microsomes due to the loss of RNA with the ribosomes. A dose dependent increase in the per cent degranulation was observed in the treated groups in present study.

Keywords: Chlorpyrifos; mutagenic; carcinogenic; liver; microsome

1. INTRODUCTION

Chlorpyrifos (CPF) is a broad spectrum organophosphate (OP) pesticide. Trade names of CPF include Dursban®, Lorsban®, Empire 20®, Equity® and Whitmire PT270®. Technical CPF is a white crystalline solid with a melting point of 41.5–42.5ºC. Chlorpyrifos is stable in neutral and acidic aqueous solutions; however, stability decreases with increasing pH. Chlorpyrifos is practically insoluble in water, but is soluble in most organic solvents such as acetone, xylene and methylene chloride (Anonymous 2008). Both acute and chronic exposure to CPF caused significantly marked DNA damage in rat tissues, namely liver, brain, kidney, and spleen, when measured 24 hour after treatment; the damage was partially repaired at 48 and 72 hours after treatment (Ojha *et al* 2011). Chlorpyrifos caused increased ratio of DNA migration, as assessed by the comet assay, in human lymphocytes at 10 μM (Sandal and Yilmaz 2011). A significant dose-related increase in mean comet tail length, indicating DNA damage, in mice leucocytes was observed by Rahman *et al* (2002). In our previous studies also we observed an increased proliferation in reproductive organs of female wistar rats (Nishi and Hundal, 2013). Therefore, the present study was designed to assess the mutagenic and carcinogenic effect of CPF in female wistar rats.

2. MATERIAL AND METHODS

Commercial grade chlorpyrifos (Eldrin, 20EC) was purchased from Crystal Phosphate Limited, Nathupur, Sonepat, Haryana, India. Different dilutions for the doses of the insecticide to be administered were made with vegetable oil. The rats were divided into three groups of six animals each. Two groups were given Chlorpyrifos at a dose level of 0.1mg/kg (T1) and 2.5 mg/kg (T2) for eight weeks on daily basis by oral intubation. Same amount of vegetable oil i.e. 1.25ml/kg was given to the control group (C) orally through intubation. The selection of doses was based on our previous study (Nishi and Hundal, 2013). The animals were sacrificed on the completion of the experiment and the liver were collected, weighed and processed for analysis. Bone marrow metaphase cytogenic assay was done by the method of Sharma and Sharma (1994). Microsomes are prepared by method of Kamath and Narayan (1972) and modified by Gupta and Dani (1979). Proteins were estimated by the method of Lowry *et al* (1951) and RNA was estimated by the method of Munro and Fleck (1966).

3. RESULTS AND DISCUSSION

No statistically significant change in the final body weight, feed intake and liver weight was observed in treated and control rats. An increase in the chromosomal aberrations was observed which indicates a mutagenic behavior of CPF. The increase in the chromosomal aberrations was dose dependent (Table 1, Figure 1 (A-D)). Thus, CPF was found to be mutagenic in the present study.

The mechanism of DNA strand breaks due to CPF exposure is poorly understood and little is known about CPF or its metabolites that are responsible for production of DNA strand breaks. The phosphorus moiety in organophosphates appears to be a good substrate for nucleophilic attack (Wild 1975). This may cause phosphorylation of the DNA, which is an instance of DNA damage. In one study by Shadnia *et al* (2005) an increased blood leucocytic and erythrocytic DNA damage in 21 pesticide formulating workers exposed chronically to organophosphates was observed. DNA damage has been observed in the cultured human lymphocytes exposed to CPF (Sandal and Yilmaz 2010). An increased binucleated lymphocyte formation and DNA damage after CPF treatment in both male and female rats was observed (Sandhu *et al* 2013).

DNA is considered to be an important target for reactive oxygen species (ROS). Oxidative stress has been implicated in many diseases, including cardiovascular disease, macular degeneration, pancreatitis, and cancer (Halliwell and Gutteridge 1999). Pesticides can induce oxidative stress via a multi-step pathway, resulting in an imbalance between pro-oxidant and antioxidant defense mechanisms in different tissues, including alterations in antioxidant enzymes (Banerjee *et al* 2001). Studies in experimental animals and tissue culture studies show that pesticides, especially organophosphate pesticides, induce oxidative stress (Nasuti *et al* 2007).

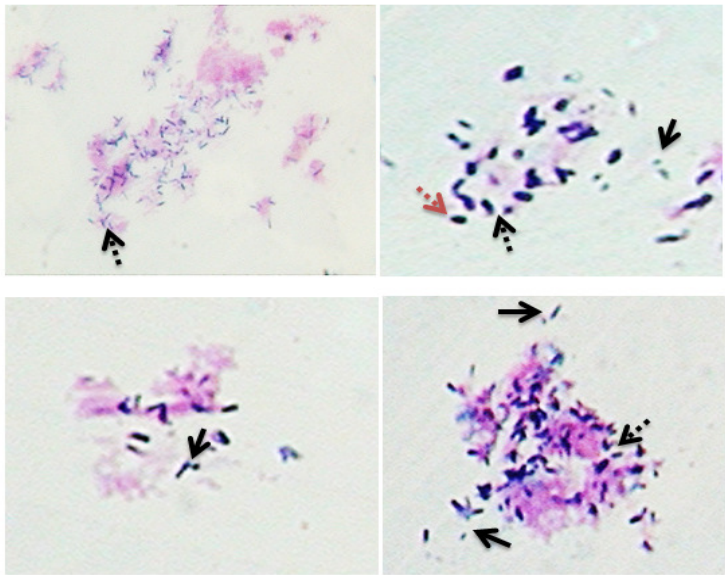

Figure 1. Different types of chromosomal aberrations in the treated rats. Solid arrow showing fragmentation, black dotted arrow showing chromatid break, red solid arrow showing ring formation, red dotted arrow showing chromatid gap. Fig. A-D (1000X, Giemsa)

A decrease in the RNA/protein ratio in the test samples as compared to control is taken as an index of degranulation. Degranulation more than 5 per cent was taken as a positive result. A dose dependent increase in the per cent degranulation was observed in the treated groups in present study (Table 10).

Table 9 Mean values of chromosomal aberrations in the treated and control rats

Treatment	**Control**	**T1**	**T2**
Chromatid break	0.500±0.223	1.333±0.494	1.500±0.500
chromatid gaps	0.1667±0.1	1.00±0.632	0.667±0.333
Rings	0.00±0.00	0.333±0.21	0.666±0.333
Fragmentation	0.667±0.333	2.833±0.4104**	3.00±0.3651**

All the values are Mean ± SE values of 4 animals in each groups.
***Values are significant at P<0.01*

Table 10 Microsomal Degranulation

Treatment	T1	T2	C	Control
RNA / Protein	0.011 ± 0.002	0.0273 ± 0.004	0.0043 ± 0.0001	0.088
per cent Degranulation	13.346 ± 2.475*	29.132 ± 2.832**	4.852 ± 0.1539	___

All the values are Mean ± SE values of 4 animals in each groups.
***Values are significant at P<0.01*

Researchers have demonstrated that electrophiles of a carcinogen can disrupt ribosome-membrane interaction in rough microsomes by their attacks on nucleophilic components of the reticular membrane-ribosome complex involved in protein synthesis for export from the cytosol (Dani and Kaur 2001). Lack of exported proteins can adversely affect signal transduction across plasma membrane, possibly leading to carcinogenesis. Chlorpyrifos has been found to show carcinogenic activities at lower doses in breast cancer cell lines (Ventura *et al* 2012).

4. CONCLUSIONS

In the present study CPF was observed to be mutagenic and carcinogenic. Such activities of CPF even at low doses may be responsible for the increased proliferation in the ovarian surface epithelium and mammary glands. Thus, the indiscriminate use of CPF in domestic as well as agricultural fronts may be detrimental to the animal as well as human health.

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