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Histological Changes in the Lung and Liver Tissues in Mice Exposed to Pyrethroid Inhalation

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Abstract

Cypermethrin, a type II pyrethroid, is one of the most widely used insecticides in Pakistan. It is considered to be a safe pesticide; however, the possible health hazards of this pyrethroid have been ignored. Cypermethrin may become an air pollutant and adversely affect the health of non-target organisms, leading to acute or chronic disorders. The present work aims to investigate the effects of cypermethrin on lung and liver tissues due to inhalation exposure. The study is performed on 16 mature Swiss albino mice, including controls. The animals are divided into 4 groups (4 mice each) and exposed to 0.5 % dilution of cypermethrin in an inhalation chamber (40×35×25 cm^3) for different time periods, whereas control animals are not exposed to any insecticide. The histopathological changes in lungs and liver tissues reveal that cypermethrin exposure induces time dependent changes in the liver and in the lungs. It damages the normal organization of liver tissues, causing liver injury due to necrosis, significant decrease in number of cells, and widening of sinusoids and fibrosis. Inhalation exposure of cypermethrin results in significant hyperplasia, clumping of cells and necrosis in the lungs. It also induces pulmonary edema, alveolitis, and pulmonary fibrosis by the deposition of collagen. Taking these findings together, it may be concluded that cypermethrin and other pyrethroids cause hazardous effects in non-target organisms through inhalation exposure. Serious efforts and awareness are required to monitor and reduce the insecticide induced health hazards in third world countries.

Keywords: Pyrethroid, cancer, histology, liver, lung

Introduction

Cypermethrin and other pyrethroids have broad-spectrum use in agriculture, domestic and veterinary applications due to their high bio-efficacy, enhanced stability, and considerably low mammalian toxicity [1,2]. These insecticides kill the insects that eat or come in contact with it by quickly affecting the insect's central nervous system [3] by free radical-mediated tissue damage [2] Cypermethrin is classified by the US EPA as a weak category C-oncogene, a possible human carcinogen, and while there is evidence of it causing carcinogenesis in animals, there is no evidence of it causing carcinogenicity in humans [4]. It possesses complete carcinogenic and co-carcinogenic potential, and mice develop benign tumors in their skin upon exposure to it [5]. It is genotoxic in mouse spleen and bone marrow cells, from the inducing of chromosomal aberration and sister chromatid exchange [6,7]. It induces systemic genotoxicity in mammals by causing DNA damage in vital organs like the brain, liver, and kidneys, apart from that in the hematopoietic system [2]. It not only possesses mutagenic activity to

induce dominant lethal mutations in male germ cells of mice [5] but also induces chromosomal aberrations and single stranded breaks in DNA in cultured human lymphocytes. Moreover, it affects the cell cycle causing a decrease in the proliferative rate index [8]. Cypermethrin and other pyrethroids possess the ability to induce coetaneous carcinogenesis in Swiss albino mice, resulting in the development of benign skin tumors [5]. The lungs are an important entry point for airborne contaminants exposed to the toxicants present in the breathing zone which can be absorbed in the nasopharyngeal, trachea-bronchial or pulmonary exchange surfaces of the lung. However, the lung alveoli and the terminal bronchioles are some of the most sensitive surfaces in the body for absorption and the resultant toxicity during respiratory exposure. Toxicants may pass into the systemic circulation from the lungs and affect the other body parts, especially the liver, because the hepatocytes are flooded with the blood for cleaning purpose [9].

Most of the studies on pyrethroids, especially cypermethrin, have been made through oral administration, instead of inhalation exposure which is the major route of exposure to these insecticides. Therefore, the aim of the present work was to better characterize the adverse effects of pyrethroids to public health and to warn the authorities to control human exposure of such chemical agents.

Materials and methods

Animals

Sixteen Swiss albino mice (about 20 g body weight and 3 - 4 months age) were taken from the Animal House of Zoology Department of Punjab University, Lahore, to perform the experiment. Animals were kept under standard conditions with a 12 h light/dark cycle and access to freshwater and food at room temperature of 23 - 27 °C. Four mice served as the control (group 1) and the others were divided into 3 groups, 4 in each group, and exposed to cypermethrin through inhalation. The experiment was performed as a whole-body exposure. For this purpose, mice were kept in an inhalation chamber (40×35×25 cm) (**Figure 1**) and were exposed to 0.5 % dilution of cypermethrin (1 L water + 5ml cypermethrin) for 24 h (group 2), 72 h (group 3) and until the development of skin tumors (group 4). The animals in group 4 were the progeny of mice exposed to pyrethroid for 10 days. The animals in this group developed tumors after the second lactation round. A tap system was arranged in the chamber to ensure the drop delivery of cypermethrin into the chamber.

Histology procedure

Animals were sacrificed at time points of 0, 24 and 72 h and until the development of skin tumors. For histopathological study, the tissue samples of lungs and liver were removed, fixed in Bouin's fixative, dehydrated, embedded in paraffin wax, sectioned at 5 μm, and subsequently stained with Hematoxylin and Eosin. Slides were examined and photographed using a light microscope (Kyowa, Germany) attached with a digital camera (DCM35, Japan).

Results

Mice treated with cypermethrin did not show clinical signs of acute intoxication, including salivation, hyperactivity, incoordination, seizures and tremors. They did not show any symptoms of respiratory changes, such as nasal irritation and dyspnea. However, their activity was reduced with the passage of time and the mice became sluggish and lazy. No deaths occurred during their exposure to cypermethrin. The excised liver and lung tissues of experimental group 2 and 3 appeared as of the control, whereas tissues from group 4 were slightly dark in color and comparatively stiff.

Histological analysis

Changes in the histology of the lungs and liver of the mice fumigated with cypermethrin were observed under the microscope after hematoxylin and eosin staining. Significant changes were observed in the tissue integrity as well as in the pathology of the tissues.

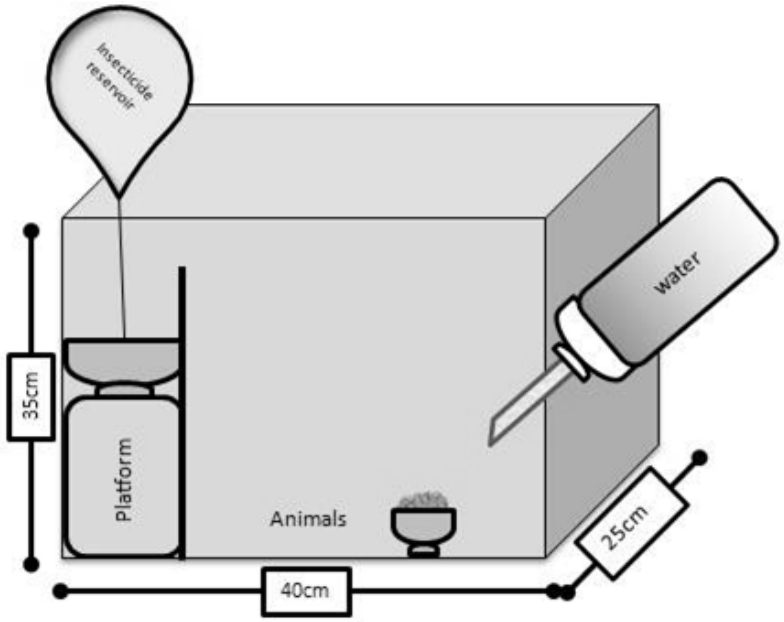

Figure 1 Inhalation chamber used to house the mice. The chamber was provided with food and water *ad libitum*. The source of insecticide fumes was demarked and was in contact with an insecticide reservoir out of the chamber. The animals were monitored on a daily basis through a transparent observation panel.

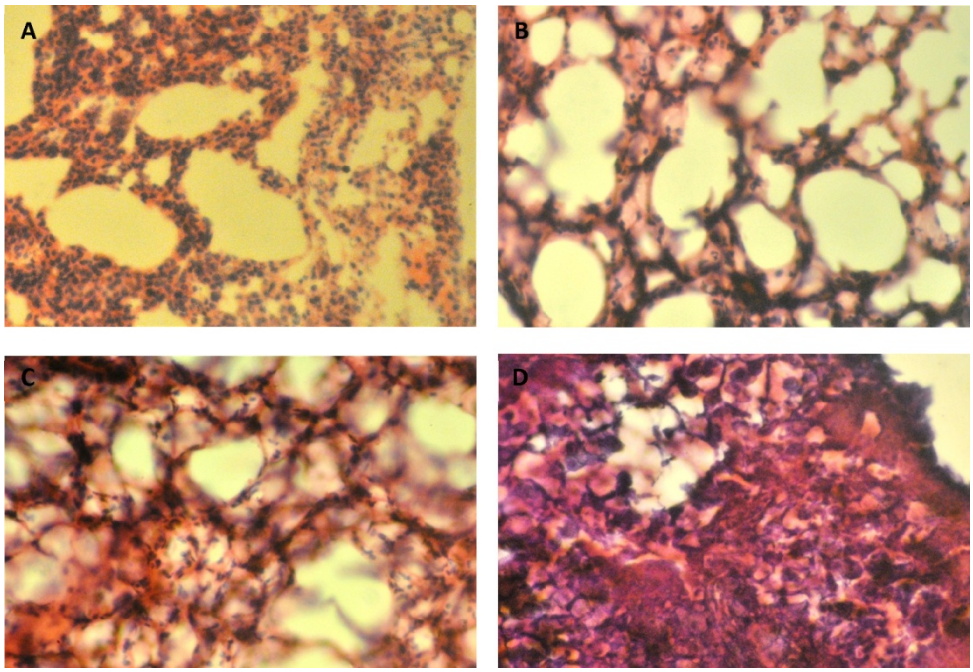

Figure 2 Hematoxylin and Eosin staining of the control (a; 50×) and cypermethrin exposed for 24, 72 h and post lactation stage when tumors appeared (b - d) lung tissues (50×). Control group lung shows

normal architecture of the lung tissue, whereas cypermethrin exposed groups show progressive alterations in the normal histology of lung with the passage of time of exposure.

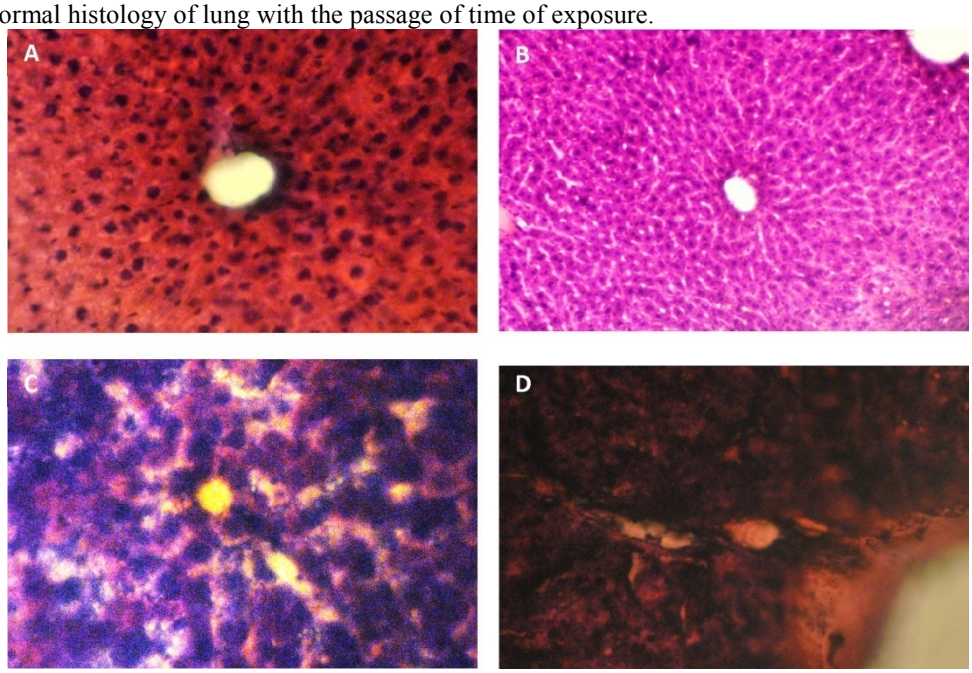

Figure 3 Hematoxylin and Eosin staining of the control (a; 50×) and cypermethrin exposed for 24, 72 h and until tumor development (b - d) liver tissues (50×). Control group exhibits normal hexagonal structure of the liver lobules, having a central vein, hepatocytes, sinusoids and portal triads, while cypermethrin exposed liver tissues show atypical architecture of the liver tissue.

Lungs

Histological examination of the control mice showed that the alveoli were thin walled polyhedral chambers surrounded by single layered squamous epithelium. Between the alveoli there was a thin layer of connective tissue. The alveolar septum can be distinguished into 2 specialized portions, i.e. thin and thick portions. Alveolar septum was composed of type I alveolar cells, also known as type I pneumocytes, and type II alveolar cells, or type II pneumocytes. In the lung tissue of the control samples, type I alveolar cells were thin, squamous and flat, and lined most of the surface of alveolar lining, while type II cells were cuboidal secretary, interspersed among the type I cells but tending to congregate at the septal junction. There were also alveolar macrophages present in the alveolar wall which also moved into the lumen to remove inhaled particulate matter (**Figure 2a, 2b**).

Compared with the control group, mice exposed to cypermethrin showed severe changes in the lung tissues, as the normal architecture of the lung tissue exhibited gradual distortion with the passage of time of exposure. The alveolar walls were destroyed in some places and thickened in other places. There was deposition of collagen in the extracellular matrix, due to pulmonary fibrosis, and subsequent reduction in the alveolar spaces. Cypermethrin induced hyperplasia, due to size and number of the alveolar cells, was significantly augmented. There was a progressively increasing degree of necrosis and pycnosis, and also inflammation of lung tissue, leading to pulmonary edema and alveolitis (**Figure 2c - 2d**).

Liver

The histology of the liver tissue from control mice was normal. Liver was composed of lobules with a roughly hexagonal arrangement of plates of hepatocytes radiating outward from a central vein in the center. The central vein was quite prominent and provided an easy mean of orientation in sections of the

liver. Hepatocytes were arranged into single cell thick hepatic cords separated from each other by sinusoidal capillaries or sinusoids. The hepatocytes were polygonal in shape with spherical central nuclei. At the vertices of the lobule were regularly distributed portal triads composed of a bile duct and branches of the hepatic artery and hepatic portal vein which were connected to the central vein through the sinusoids (**Figure 3a, 3b**).

In the case of the cypermethrin exposed mice, the normal architecture of the liver lobules was gradually distorted as a result of liver injury. The normal polygonal shape of the hepatocytes was distorted, their nuclei were enlarged, and the hepatocytes had undergone increasing degrees of necrosis. The sinusoids were widened & the central vein was damaged. The sinusoids and central vein were filled with collagen due to fibrosis. There was a decrease in the number of hepatocytes and their size was increased. Tissue was also damaged due to the appearance of blood streaks among the hepatocytes. All of these findings were time dependent, being more prominent in the tissues exposed for longer times (**Figure 3c - 3d**).

Discussion

In the present work, the histopathological changes in the lung and liver tissues of cypermethrin exposed mice were studied. The inhalation exposure of cypermethrin to the mice induced significant time dependent changes in the histopathology of the lungs and liver tissue. Inhalation is a major route of exposure to air born pollutants such as pesticides [9,10]. The lungs and liver are the organs which are at the highest risk to environmental pollutants, especially air born chemicals [11].

The present study indicated that cypermethrin has a carcinogenic effect in the lungs of exposed mice. Exposure to cypermethrin caused a gradual distortion of the normal structure of alveoli in the lung. The lungs became more compact due to clumping of the cells and condensation of nuclei in a time dependent exposure to the cypermethrin. Cypermethrin induced hyperplasia and necrosis among the alveolar cells. The inflammation in the lungs led to pulmonary edema, alveolitis and pulmonary fibrosis due to the size of alveolar sacs being reduced and alveolar walls becoming thicker. Epidemiologic data show an increase in the number of cancer cases in persons involved in agricultural production using pesticides [12]. Pyrethroids induce benign tumors in the lungs of mice [13]. The findings are in agreement with those of Tian, stating that inhalation of pyrethroids causes alveolitis, pulmonary edema and damage to lung cells [14]. Lung cancer develops through a series of progressive pathological changes occurring in the respiratory epithelium [15]. Pyrethroids are known to be genotoxic and may interact with DNA and damage its structure. They induce chromosomal aberrations and single strand breaks in DNA [8,12]. Pyrethroids may lead to molecular alterations, such as loss of heterozygosity, gene mutations, and aberrant gene promoter methylation, which are potentially promising molecular biomarkers of lung carcinogenesis [15].

It has been suggested that pyrethroids induce oxidative stress [16]. Oxidative stress is caused by an imbalance in the production of reactive oxygen, including free radicals and peroxides which cause damage to the cells and collagen deposition leading to pulmonary fibrosis and edema [1,17-19].

Cypermethrin also damaged the normal architecture of liver lobules. The number of hepatocytes was reduced, with distortion in their polygonal shape, widened sinusoids with necrosis, and fibrosis [20]. The magnitude of these findings was time dependent, being more prominent in the tissues exposed for longer time periods. Pyrethroids have hepatotoxic potential in mammals [21-24].

The carcinogenic property of cypermethrin observed may be attributed to its ability to interact with DNA and damage its structure [12]. Such interactions are critical for the initiation of cells to transform into neoplastic cells. Cypermethrin may also induce the frequency of well-established markers of genotoxicity, such as chromosomal aberrations and micronuclei formation [25].

Conclusions

Taking these findings together, it can be concluded that cypermethrin and other synthetic pyrethroids with large scale use target not only the targets but also non-target organisms, i.e. humans and other animals with a high potential of carcinogenesis and fibrosis. Prolonged exposure of cypermethrin may cause carcinogenic mutations in the genome. Therefore, the use of these insecticides should be monitored and controlled seriously to protect non-target organisms like farmers from the hazardous effects of this mutagenic agent.

References

[1] S Erdogan, EH Zeren, M Emre, O Aydin and D Gumurdulu. Pulmonary effects of deltamethrin inhalation: an experimental study in rats. *Ecotoxicol. Environ. Saf.* 2006; **63**, 318-23.
[2] S Patel, AK Pandey, M Bajpayee, D Parmar and A Dhawan. Cypermethrin-induced DNA damage in organs and tissues of the mouse: evidence from the comet assay. *Mutat. Res.* 2006; **607**, 176-83.
[3] SM Bradberry, SA Cage, AT Proudfoot and JA Vale. Poisoning due to pyrethroids. *Toxicol. Rev.* 2005; **24**, 93-106.
[4] Anonymous. United States Environmental Protection Agency, Office of Pesticide Programs reference dose tracking report. Washington D.C., 1997.
[5] Y Shukla, A Yadav and A Arora. Carcinogenic and cocarcinogenic potential of cypermethrin on mouse skin. *Cancer Lett.* 2002; **182**, 33-41.
[6] SM Amer, AA Ibrahim and KM El-Sherbeny. Induction of chromosomal aberrations and sister chromatid exchange in vivo and in vitro by the insecticide cypermethrin. *J. Appl. Toxicol.* 1993; **13**, 341-5.
[7] S Giri, A Giri, GD Sharma and SB Prasad. Induction of sister chromatid exchanges by cypermethrin and carbosulfan in bone marrow cells of mice *in vivo*. *Mutagenesis* 2003; **18**, 53-8.
[8] M Puig, E Carbonell, N Xamena, A Creus and R Marcos. Analysis of cytogenetic damage induced in cultured human lymphocytes by the pyrethroid insecticides cypermethrin and fenvalerate. *Mutagenesis* 1989; **4**, 72-4.
[9] IS Richards. *The Respiratory System. In*: Principles and Practice of Toxicology in Public Health. 1st ed. Jones & Bartlett Publishers, 2007, p. 239-52.
[10] A Emmendoerffer, M Hecht, T Boeker, M Mueller and U Heinrich. Role of inflammation in chemical-induced lung cancer. *Toxicol. Lett.* 2000; **112-113**, 185-91.
[11] D Dixon, RA Herbert, GE Kissling, AE Brix, RA Miller and RR Maronpot. Summary of chemically induced pulmonary lesions in the National Toxicology Program (NTP) toxicology and carcinogenesis studies. *Toxicol. Pathol.* 2008; **36**, 428-39.
[12] N Kornuta, E Bagley and N Nedopitanskaya. Genotoxic effects of pesticides. *J. Environ. Pathol. Toxicol. Oncol.* 1996; **15**, 75-78.
[13] J Ishmael and MH Lithfield. Chronic toxicity and carcinogenic evaluation of permethrin in rats and mice. *Fundam. Appl. Toxicol.* 1988; **11**, 308-22.
[14] HL Tian. Biochemical .and toxicological studies of fenvalerate on the lung. *Zhonghua Yu Fang Yi Xue Za Zhi.* 1993; **27**, 290-3.
[15] P Vineis and K Husgafvel-Pursiainen. Air pollution and cancer: biomarker studies in human populations. *Carcinogenesis* 2005; **26**, 1846-55.
[16] M Kale, N Rathore, S John and D Bhatnagar. Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocytes: a possible involvement of reactive oxygen species. *Toxicol. Lett.* 1999; **105**, 197-205.
[17] MD Evans and MS Cooke. Factors contributing to the outcome of oxidative damage to nucleic acids. *BioEssays* 2004; **26**, 533-42.

[18] FQ Schafer, GR Buettner. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic. Biol. Med.* 2001; **30**, 1191-212.
[19] I Rahman and W MacNee. Oxidative stress and regulation of glutathione in lung inflammation. *Eur. Respir. J.* 2000; **16**, 534-54.
[20] PB Singh and V Singh. Cypermethrin induced histological changes in gonadotrophic cells, liver, gonads, plasma levels of estradiol-17beta and 11-ketotestosterone, and sperm motility in Heteropneustes fossilis (Bloch). *Chemosphere* 2008; **72**, 422-31.
[21] U Mani, AK Prasad, V Sureshkumar, P Kumar, K Lal, BK Maji and KK Dutta. Hepatotoxic alterations induced by subchronic exposure of rats to formulated fenvalerate (20% EC) by nose only inhalation. *Biomed. Environ. Sci.* 2004; **17**, 309-14.
[22] Y Okuno, S Ito, T Seki, T Hiromori, M Murakami, T Kadota and J Miyamoto. Fenvalerate-induced granulomatous changes in rats and mice. *J. Toxicol. Sci.* 1986; **11**, 53-66.
[23] N Tuzmen, N Candan, E Kaya and N Demiryas. Biochemical effects of chlorpyrifos and deltamethrin on altered antioxidative defense mechanisms and lipid peroxidation in rat liver. *Cell Biochem. Funct.* 2008; **26**, 119-24.
[24] SA Sakr. Pyrethroid inhalation-induced hepatotoxicity in albino rats. *Oxford Research Forum J.* 1999; **1**, 27-31.
[25] J Surralles, N Xamena, A Creus, J Catalan, H Norppa and R Marcos. Induction of micronuclei by five pyrethroid insecticides in whole-blood and isolated human lymphocyte cultures. *Mutat. Res.* 1995; **341**, 169-84.