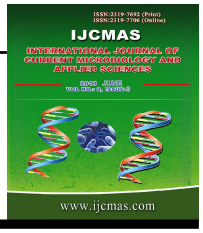
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 2 Number 6 (2013) pp. 30-40**
http://www.ijcmas.com



Original Research Article

Evaluation of Cytogenetic Effects of β-Cyfluthrin in Swiss Albino Mice

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A B S T R A C T

The aim of present investigation was to evaluate β-cyfluthrin induced oxidative stress and genotoxicity in male Swiss albino mice. Two doses of pesticide *viz.* 1/20 of LD_{50} (Low dose) and 1/10 of LD_{50} (High dose) were administered for 7 and 14 days to different groups of mice. Activity of antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD) were estimated in the liver. Genotoxicity of pesticide was assessed in bone marrow cells of mice using chromosomal aberration assay. Cyclophosphamide was used as positive control. β-cyfluthrin reduced the body weight and food consumption in treated animals. CAT and SOD activities were not affected in 7 days group but these were significantly reduced in 14 days group. Mitotic index decreased in a days as well as dose dependent manner. A significant number of chromosomal aberrations like acentric fragements, chromosome breaks, centric rings and polyploidy occurred in 14 days groups. It may therefore be inferred that β-cyfluthrin has the ability to elicit oxidative stress and cause genotoxicity in mice at the tested dose levels.

Keywords

β-cyfluthrin, genotoxicity, chromosomal aberration assay, mitotic index, oxidative stress.

Introduction

Worldwide application of pesticides is increasing continually to the extent that they have become an integral part of the ecosystem. Approximately 5.6 billion pounds of active pesticide ingredients are used annually throughout the world (USEPA, 2001). Post market surveillance has revealed that as many as 25 million agricultural workers worldwide experience unintentional pesticide poisoning each year (Alavanja *et al.,* 2004). It has been reported that pesticides available in the market may cause cancer in humans (Alavanja *et al.,* 2004; 2005; Bonner *et al.,* 2005; Beane-Freeman *et al.,* 2006; Hou *et al.,* 2006; Van *et al.,* 2008). Zahm and Ward (1998) showed that childhood cancers such as leukemia, neuroblastoma, Wilms tumor, soft tissue sarcoma, Ewing's sarcoma, non-Hodgkin's lymphoma and cancers of brain and testes are linked to pesticide exposure. Synthetic pyrethroids are the most widely used pesticides and comprise approximately 25% of worldwide insecticide market (Casida and Quistad, 1998). Human exposure to pyrethroids is well documented (Heudorf and Angerer 2001; Schettgen *et al.,* 2002)

including exposure to pregnant women, infants and toddlers (Whyatt *et al.,* 2002; Berkowitz *et al.,* 2003; Lu *et al.,* 2006; Morgan *et al.,* 2007).

Synthetic pyrethroids are derived from natural insecticide pyrethrin, which is extracted from *Chrysanthemum cinerariaefolium* flowers (Casida, 1980). They are environmentally compatible due to their moderate persistence, low volatility and reduced aqueous mobility in soil. These favorable properties of this class of insecticides have promoted its widespread application in almost all sectors of food protection and pest control programs. β-cyfluthrin is a synthetic fluorinated pyrethroid, widely used in agriculture and in houses to control pests and disease vectors (Surgan *et al.,* 2002). It is an active ingredient of many insecticide formulations such as Attatox, Baygon, Laser and Solfac (Meister, 1995). DCCA (cis-/trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid) and FPBA (4-flouro-3-phenoxybenzoic acid) are the main metabolites of cyfluthrin formed in humans and these get eliminated renally.

As the use of pyrethroids is steadily increasing, there may be an urgent need to identify the adverse effects that may be associated with their use. The carcinogenic potential of pyrethroids has been discussed in a review by Litchfield in 1985. β-cyfluthrin is a newer pyrethroid insecticide used all over the world. It is reported to be neurotoxic (Satpathy *et al.,* 1997), hepatotoxic (Omotuyl *et al.,* 2006) and teratogenic (Soni *et al.,* 2011). β-cyfluthrin toxicity is exhibited by its metabolites which in turn generate free radicals (El-Demerdash 2007). *In vitro* study on human erythrocytes demonstrated that cyfluthrin generates the reactive oxygen species in cells (Sadowaska-Woda *et al.,* 2010). Reports on the genotoxicity of β-cyfluthrin are limited. Therefore, the present work was aimed to evaluate its *in vivo* oxidative stress, and genotoxic effects in the bone marrow cells of male Swiss albino mice.

Materials and Methods

Animals

Adult male Swiss albino mice were procured from the Indian Veterinary Research institute, Bareilly (U. P.). The animals were housed at a temperature of 29°±2°C (relative humidity 33-40%) and 12 hrs. light and dark cycle. They were kept in sanitized polypropylene cages containing saw dust as bedding and given *ad libitum* access to food (Hindustan Lever Limited, Chandigarh, India) and tap water. Healthy and sexually mature animals (6-8 weeks, 30±5 g) were selected for the experiments.

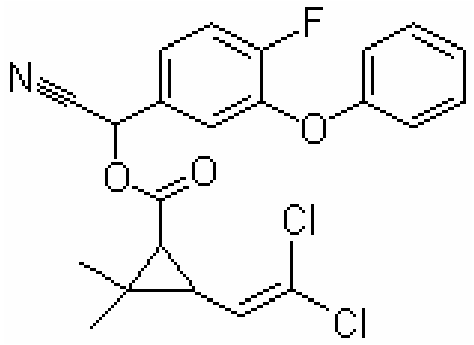
The experiments were performed according to the guidelines for care and use of the animals in scientific research, of the Indian National Science Academy (2000), New Delhi. The study had the consent of the Institutional Ethical Committee (IEC).

Compound Administered

Technical grade β-cyfluthrin was purchased from Bayer (Sigma-Aldrich, CAS No. 68359-37-5). The purity of pesticide was 96.1%, molecular weight 434.3 g/mol and chemical formula is $C_{22}H_{18}Cl_2FNO_3$. Its chemical name is 3-(2,2-dichloro-vinyl)-2,2-dimethyl-cyclopropane-carboxylic acid cyano-(4-fluoro-3-phenoxy-phenyl)-methyl ester (Fig 1). It is an enriched isomeric form of the two biologically active diastereo

isomeric pairs of isomers of cyfluthrin; 3-(2,2-dichloro -vinyl)-2, 2-dimethyl-cyclopropane -carboxylic acid cyano-(4-fluoro-3-phenoxy-phenyl)-methyl ester. It is an optical isomer of cyfluthrin and acute toxicity of former is approximately 2-5 times higher than cyfluthrin (European Commission, 2002). The primary mechanism of its toxicity is interaction with sodium-ion gated channels, depolarization of the membrane and loss of electrical excitability in nervous system. The second mechanism is through inhibition of calcium transport enzymes in cell (Wolansky *et al.,* 2006).

Figure.1 Chemical structure of β-cyfluthrin.



Experimental Protocol

To assess the antioxidant enzyme activity and genotoxicity of β-cyfluthrin, the mice were administered technical grade pesticide dissolved in corn oil (0.1 ml) by oral gavage. The doses of pesticide selected were 1/20 of LD_{50} (Low dose) and 1/10 of LD_{50} (High dose). The oral LD_{50} of this pesticide as reported by EMEA (2003) is 291 mg/kg b. wt. for male mice. Both doses were administered for 7 and 14 days daily to different experimental groups, each consisting of 6 animals. The control animals received only the vehicle *i.e.* corn oil every day. Cyclophosphamide was used as positive control (25 mg/kg b. wt.) and given to animals intraperitoneally. After 24 hrs. of last dose administration, the animals were sacrificed by cervical dislocation, their femurs and liver were excised. Liver was washed with ice cold normal saline and used for enzyme assay for oxidative stress. Chromosomal aberration assay was performed in bone marrow cells.

Body weight and food consumption

The body weight of the experimental and control animals were recorded on every alternate day and food consumption was recorded daily during the period of treatment.

Assessment of antioxidant enzyme activity

10% (w/v) homogenate of liver was prepared as described by Awasthi *et al.,* (2013). Activity of CAT and SOD was estimated by the methods suggested by Aebi (1974), and Marklund and Marklund (1974) respectively.

Chromosomal aberration assay

To analyze the chromosomal damage in the cells, bone marrow preparations were made (Adler, 1984). Colchicine was injected in the animals intraperitoneally 1.5 hr. prior to sacrifice. Bone marrow cells were aspirated with pre-warmed (37°C) Potassium chloride (0.59%) from the femurs and treated hypotonically for 20 min. at 37°C in incubator. The material was then centrifuged at 1000 rpm for 10 mins, and supernatant was discarded. The cell pellet was fixed in chilled methanol:acetic acid (3:1 v/v). The centrifugation and fixation steps were repeated thrice with an interval of 30 mins.

The slides were prepared by dropping method, dried completely and then stained with 4% giemsa. Chromosomal aberrations were scored under 1000X total magnification with oil immersion under the light microscope. Only well spread metaphases with 40±1 chromosomes were analyzed. Structural chromosomal aberrations (SCAs) like chromatid breaks, chromosome breaks, acentric fragments, centric ring, polyploidy, pulverization and robertsonian translocation were scored. One hundred metaphase cells per animal were analyzed to determine the total chromosomal aberrations. The mitotic index was obtained by counting the mitotic cells/1000 cells per mouse. The frequency of aberrant cells was calculated as follows:

$$\text{Frequency of aberrant cells} = \frac{\text{Total aberrant cells}}{\text{Total No. of cells studied}} \times 100$$

Statistical Analysis

The data for all groups is expressed as Mean ± SEM and subjected to variance analysis by using one way ANOVA followed by Tukey's honestly significant difference (HSD) test for comparison between the treated and control groups and within the treated groups. The $p<0.05$ level was set as significant and $p<0.01$ level was set as highly significant.

Result and Discussion

Body weight and food consumption

Treated animals that received β-cyfluthrin showed behavioral toxicity such as dullness, abnormal gait, burrowing behavior and loss of hair. Similar results were reported by Sankar *et al.*, (2010) in rats treated with cypermethrin. A significant reduction in body weight of experimental animals was observed in this study (Fig. 2). Tiwari *et al.* (2008) and Sankar *et al*. (2010) also reported loss of weight in animals treated with cypermethrin and deltamethrin. This may probably be due to effect of the insecticide on gastrointestinal tract, resulting in decreased appetite and absorption of nutrients from gut (Venkatesanwarlu *et al.*, 1997). It is further supported by decreased food consumption in treated animals in comparison to controls (Figure.3).

Exposure to β-cyfluthrin for 7 days did not cause any change in CAT and SOD activity at both the dose levels. However, 14 days exposure caused a significant attenuation in CAT and SOD activity in comparison to control in a dose dependent manner (Fig. 4, 5).

The probable mechanism of this decline may involve their inhibition during the breakdown of free radicals and the high level of superoxide anion and hydrogen peroxide following pyrethroid treatment (Maiti and Kar, 1997; Kale *et al.*, 1999; Giray *et al.*, 2001). Cyfluthrin has been reported to increase free radical generation and decrease SOD and CAT activity in treated mice (Omotuyl *et al.*, 2006; Eraslan *et al.*, 2007) and in cultured human erythrocytes (Sadowaska-Woda *et al.*, 2010). .

Deltamethrin treatment too caused an induction in the level of lipid peroxidation and malondialdehyde (MDA), and decreased SOD and CAT activity in mice (Yarsan *et al.*, 2002; Rehman *et al.*, 2006). Kale *et al.*, (1999) earlier demonstrated an increase in erythrocytes SOD and CAT activities in rats following fenvalerate and cypermethrin treatment.

Table.1 Mitotic index, frequency of aberrant cells in control and β-cyfluthrin treated mice.

Groups	Doses	Mitotic Index	Chromosome Break	Acentric Fragment	Polyploidy	Centric Ring	Total Chromosomal Aberrations	Frequency of aberrant cells
7 days	Control	10.90±1.04	0	3.50±0.64	0	0	3.50±0.64	3.50±0.64
	Low Dose	9.35±0.99	0	5.0±1.08	0	0.25±0.02	5.25±1.19	5.0±0.91
	High Dose	6.75±0.54*	0.50±0.29	5.25±0.63	0.25±0.02	0	5.50±0.87	5.50±0.87
14 days	control	9.97±0.73	0.25±0.02	2.50±0.85	0	0.250±0.02	3.0±0.70	3.0±0.71
	Low Dose	7.15±0.61*	0.50±0.05	5.75±0.63	1.0±0.41	0.50±0.29	7.75±0.95	6.75±0.63*
	High Dose	4.72±0.43**$^{\Omega}$	0.750±0.04	11. 0±0.92	1.50±0.50	1.0±0.58	14.25±1.80	12.25±1.1**$^{\#}$
Cyclophosphmide		2.52±0.48	12.75±1.80	10.75±1.89	1.0±0.41	0.50±0.23	25.0±1.29	22.75±0.85

*P< 0.05, Significant to their respective control, **P<0.01, Highly significant to their respective control Within the both dose level

Ω P<0.05, Significant to low dose level in the same days group

#P<0.01, Highly significant to low dose level in the same days group

Figure.2 Body weight gain or loss in control and β-cyfluthrin treated animals.

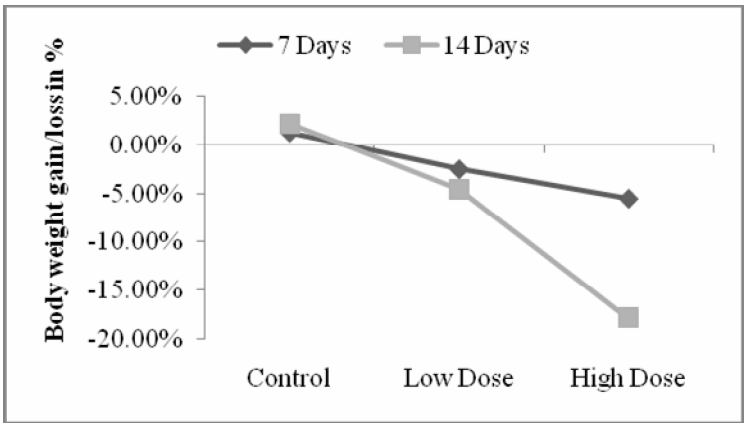


Figure.3 Food consumption in control and β-cyfluthrin treated animals.

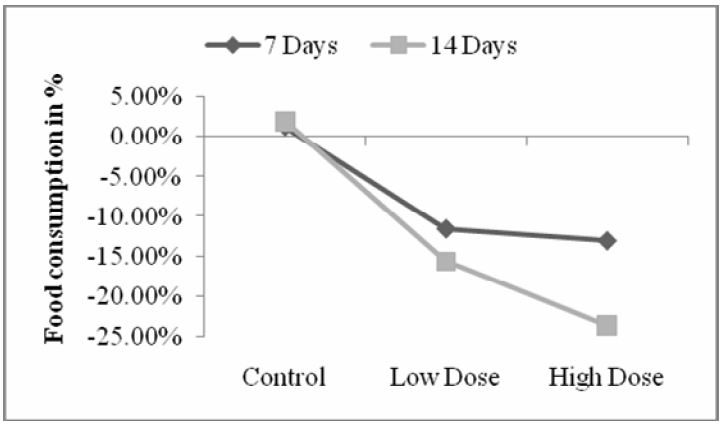


Figure.4 Changes in the CAT activity in mice liver exposed to different doses of β-Cyfluthrin. Values were calculated and expressed as mean ± SEM.
a; significant at $p<0.05$ level b; highly significant at $p<0.01$ level.

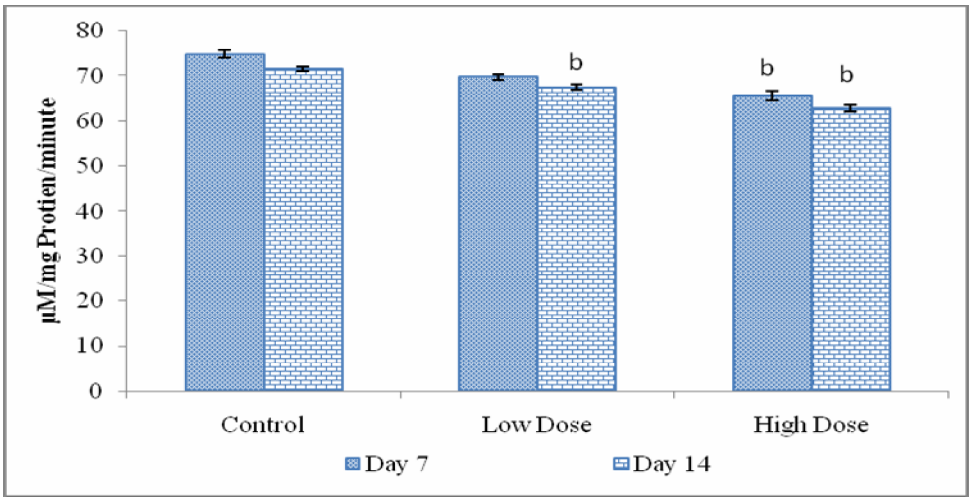
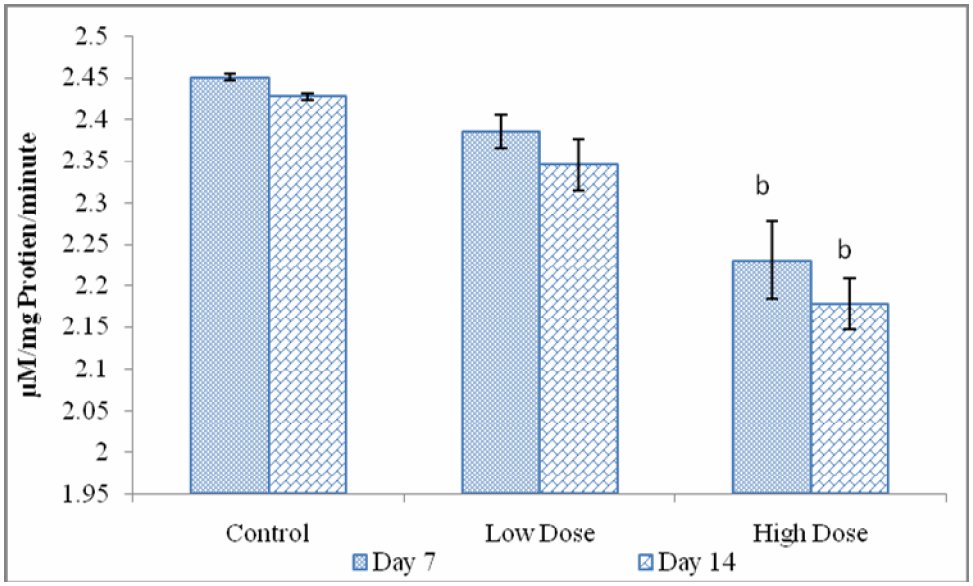


Figure.5 Changes in the SOD activity in mice liver exposed to different doses of β-Cyfluthrin. Values were calculated and expressed as mean ± SEM. a; significant at p<0.05 level b; highly significant at p<0.01 level.



The mitotic index was calculated as percentage of dividing cells. It decreased significantly in the treated groups. The decrease in mitotic index was significant at low dose level and highly significant at high dose level in 14 days group in comparison to control animals, indicating the inhibition of cell proliferation in bone marrow.

This suggests the probable microtubular or spindle disturbing nature of pesticide and illustrates its cytotoxicity. Deltamethrin too has been reported to reduce the mitotic index in rat bone marrow (Agarwal *et al.*,1994). Supermethrin also elicited cytotoxicity *in vivo* and *in vitro* conditions (Dianovsky and Sivikova, 1995).

The cytogenetic analysis of mice bone marrow cells revealed chromosomal aberrations like chromosomal breaks, acentric fragments, polyploidy and centric rings (Table 1). Acentric fragments were the most frequent type of aberration seen. Treatment for 7 days with both doses caused no significant change in frequency of aberrant cells. However, in 14 days group, the frequency of aberrant cells increased significantly and highly significantly at low and high dose levels respectively. Thus, pesticide treatment for 14 days showed toxicity in mouse bone marrow. This is in concomitance with the results of Ila *et al.*, (2008) who reported that β-cyfluthrin treatment increased the frequency of SCAs in rats, and cultured human peripheral blood lymphocytes. Clastogenic effects of synthetic pyrethroids have also been reported in rodent bone marrow earlier by Tyrkiel *et al.* (2001).

Fenvalerate was reported to increase the frequency of sister chromatid exchange (Giri *et al.*, 2002). Lambda-cyhalothrin significantly increased micronucleus frequency and decreased the number of polychromatic erythrocytes in the bone marrow cells of Wistar rats (Celik *et al.*, 2005).The possible clastogenicity of β-cyfluthrin may therefore be attributed to

free radical formation in exposed animals. It is further supported by the decreased activity of antioxidant enzymes as evident from the results of antioxidant enzyme assay (Fig. 4 and 5). In conjunction with this, pyrethroids are also lipophilic in nature and may easily penetrate into the cells (Nausti *et al.,* 2003), thereby inducing oxidative stress and further damage to the genetic material.

It may thus be concluded that β-cyfluthrin induced oxidative stress and DNA damage in mouse at the tested dose levels. The results of this investigation may help in creating awareness about the possible genetic hazards to human population and the environment. This in turn may lead to careful usage of these pesticides in agricultural fields and at human dwellings.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Acknowledgements

The financial support by Council of Scientific and Industrial Research (CSIR), New Delhi, India and the facilities provided by the Centre for Advanced Studies, Department of Zoology, University of Rajasthan are thankfully acknowledged.

References

Adler, I. D., Cytogenetic tests in mammals. In: Mutagenicity testing, a practical approach (Venitt S and Parry J M, Eds.) IRL Press, Oxford, England, U K. 275, 1984.

Aebi, H., 1974. Catalase. In: Bergmeyer, H.U. (Ed.), Methods of Enzymatic Analysis.2nd English ed. Verlag Chemic, Weinheim, pp. 673–684.

Agarwal, D. K., Chauhan, L. K. S., Gupta, S. K. and Sundararaman, V. 1994.Cytogenetic effects of deltamethrin on rat bone marrow.Mutat Res.311: 133-138.

Alavanja, M. C. R., Hoppin, J. A. and Kamel, F. 2005. Health effects of chronic pesticide exposure: Cancer and Neurotoxicity.Annu Rev Public Health.25: 155-197.

Alavanja, M. C. R., Dosemeci, M., Samanic, C., Lubin, J., Lynch, C. F., Knott, C., Barker, J., Hoppin, J. A., Sandler, D. P., Coble, J., Thomas, K. and Blair, A. 2004. Pesticides and lung cancer risk in the Agricultural Health Study cohort.Am J Epidemiol. 160: 876-885.

Awasthi, K.K., John P.J., Awasthi A. and Awasthi K. 2013. Multi walled carbon nano tubes induced hepatotoxicity in Swiss albino mice.Micron. 44: 359-364.

Beane-Freeman, L. E., Bonner, M. R., Blair, A., Hoppin, J. A., Sandler, D. P., Lubin, J., Dosemeci, M., Lynch, C. F., Knott, C. and Alavanja, M. 2006. Cancer incidence among male pesticide applicators in the Agricultural Health Study cohort exposed to diazinon.Am Epidemiol.162: 1070-1079.

Berkowitz, G. S., Obel, J., Deych, E., Lepinski, R., Godbold, J. and Liu, Z. 2003. Exposure to indoor pesticides during pregnancy in a multi ethnic, urban cohort.Environ Health Perspect.111:79-84.

Bonner, M. R., Lee, W. J., Sandler, D. P., Hoppin, J. A., Dosemeci M., and Alavanja, M. C. 2005.Occupational exposure to carbofuran and the incidence of cancer in the Agricultural Health Study. Environ Health

Perspect.11: 285-289.

Casida, J. E. 1980. Pyrethrum flowers and pyrethroid insecticides.Environ Health Perspect. 34: 189-202.

Casida, J. E. and Quistad, G. B. 1998. Golden age of insecticide research: past, present and future. Ann Rev Entomol. 43: 1-16.

Celik, A., Mazmanci, B., Camlica, Y., Comelekoglu, U. and Askina, A. 2005.Evaluation of cytogenetic effects of lambda-cyhalothrin on Wistar rat bone marrow by gavage administration.EcotoxicolEnviron Saf.61: 128-133.

Dianovsky, J. and Sivikova, K. 1995.In vivo and in vitro cytogenetic effect of supermethrin.Biomed Environ Sci.8: 359-366.

El-Demerdash, F. M. 2007. Lambda-cyhalothrin-induced changes inoxidativestess biomarkers in rabbit erythrocytes and alleviation effects of some antioxidants, Toxicol in Vitro. 21: 392-397.

EMEA, European agency for the evaluation of medicinal products,. Veterinary medicines and information technology:EMEA/MRL/746/100-final, July 2000. Committee for veterinary medicinal products, cyfluthrin, summary report (2).http://www.eudra.org/emea.html.

Eraslan, G., Saygi, S.and Essiz, D. 2007.Evaluation of aspect of some oxidative stress parameters using vitamin E, proanthocynidin and N-acetylcysteine against exposure to cyfluthrin in mice.Pesticide Biochem and Physio.88: 43-49.

European Commission, Health and consumer protection directorate-general (β-cyfluthrin), 2002, Appendix 1.

Giray, B., Gurbay, A. and Hincal, F. 2001. Cypermethrin-induced oxidative stress in rat brain and liver is prevented by vitamin E or allopurinol. Toxicol.Lett. 118(3): 139-146.

Giri, S., Sharma, G. D., Giri, A. and Prasad, S. B. 2002.Fenvalerate-induced chromosome aberrations and sister chromatid exchanges in the bone marrow cells of mice in vivo, Mutat Res. 520: 125-132.

Heudorf, U. and Angerer, J. 2001. Metabolites of pyrethroid insecticides in urine specimens: current exposure in an urban population in Germany. Environ. Health Perspect. 109:213-217.

Hou, L., Lee, W. J., Rusiecki, J. A., Hoppin, J. A., Blair, M. R., Lubin, I., Samanic, C., Sandler, D. P., Blair, A. and Alavanja, M. 2006. Cancer incidence, pendimethatin exposure among pesticide applicators: A report from the Agricultural Health Study.Epidemiol. 17, 3: 302-307.

Ila, H. B., Topaktasa, M., Rencuzogullari, E., Kayraldisb, A., Donbakb, L. and Daglioglu, Y. K. 2008.Genotoxic potential of cyfluthrin.Mutat Res. 656: 49-54.

Kale, M., Rathore, N., John, S. and Bhatnagar, D. 1999. Lipid peroxidative damage on pyrethroid exposure and alterations in antioxidant status in rat erythrocyte: a possible involvement of reactive oxygen species. ToxicolLett. 105(3): 197-205.

Litchfield, M. H. 1985. Toxicity to mammals, In: Leahay, J. P. (Ed.), Thepyrethroid insecticides, Taylor and Francis, London, pp. 99, 1985.

Lu, C., Barr, D., Pearson, M., Bartell, S., and Bravo, R. 2006. A longitudinal approach of assessing urban and suburban children's exposure to pyrethroid pesticides. Environ Health Perspect 114: 1419-1423.

Maiti, P. K. and Kar, A. 1997.Dual Role

of Testosterone in Fenvalerate-treated Mice with Respect to Thyroid Function and Lipid Peroxidation. JApplToxicol. 17(2): 127-131.

Marklund, S. and Marklund, G. 1974. Involvement ofthe superoxide anion radical in theautoxidation of pyrogallol and a convenientassay for superoxide dismutase.Eur JBiochem. 47: 469-547.

Meister, R. T. Farm chemicals handbook 95. Willoughby, OH: Meister Publishing Company, 1995.

Morgan, M. K., Sheldon, L. S., Croghan, C., Jones, P. A., Chuang, J. C. and Wilson, N. K. 2007. An observational study of 127 preschool children at their homes and daycare centers in Ohio: environmental pathways to cis- and trans-permethrin exposure. Environ Res. 104:266-274.

Nausti, C., Cantalamessa, F., Falconi, G. and Gabbianelli, R. 2003. Different effects of type I and type II pyrethroids on erythrocyte plasma membrane properties and enzymatic activity in rats. Toxicology.191: 233-244.

Omotuyl, I. O., Oluyemi, K. A., Omofoma, C. O., Josiah, S. J., Adesanya, O. A. and Saalu, L. C. 2006, Cyfluthrin- induced hepatotoxicity in rats. African J of Biotech. 5, 20: 1909-1912.

Rehman, H., Ali, M., Atif, F., Kaur, M., Bhatia, K. and Raisuddin, S. 2006.Themodulatory effect of deltamethrin on antioxidants in mice.ClinChemActa. 369:61-65.

Sadowaska-Woda, I., Wojcik, N., Agata, K. and Bieszczad-bedrejczuk, E. 2010.Effect of selected antioxidants in β-cyfluthrin-induced oxidative stress in human erythrocytes in vitro.Toxicol in Vitro. 24: 879-884.

Sankar, P., Telang, A. G. and Manimaran, A. 2010. Protective effect of curcumin on cypermethrin–induced oxidative stress in Wistar rats.ExpToxicolPathol. (in press).

Satpathy S. K., Tyagi, P. K., Das, B. S., Srivastava, P. and Yadav, R. S. 1997. Evaluation of possible toxic effects of cyfluthrin during short-term, relevant community exposue. Environ ContamToxico, B.59:681-687.

Schettgen,T., Heudorf, U., Drexler, H. and Angerer, J. 2002. Pyrethroid exposure of the general population-is this due to diet. ToxicolLett. 134: 141-145.

Soni, I., Syed, F., Bhatnagar, P. and Mathur. R. 2011. Perinatal toxicity of cyfluthrin in mice: developmental and behavioural effects. Hum ExperToxicol. 30, 8: 1096-1105.

Surgan, M. H., Congdon, T., Primi, C. Lamster, S. and Louis-Jacques, J. 2002. Pest control in public housing, schools and parks: Urban Children at risk, 5-116, Department of law Environmental protection bureau, New York state Government publications.

Tiwari, V. K., Suresh, B. and Pilo, B. 2008. Evaluation of maternal toxicity in rats treated with deltamethrin 1%+triazophos 35% EC. Toxicol Int. 15, 2: 127-131.

Tyrkiel, E. B., Wiadrowska, J. K. and Ludwicki, J. K. 2001.Comparative study of the effect of synthetic pyrethroids on the induction on genetic changes in mice somatic and sex cells depending on exposure route.RoczPanstwZaklHig.52, 2: 97-109.

USEPA, Pesticide industry sales and usage: 1996 and 1997 market estimate, 2001 available at www.epa.gov /oppbead1/peatsales/97pestsales/table1 htm.2001.

Van, B., Visvanathan, K. Beane, F. L., Coble, J. Hoppin, J. A. and Alavanja, M. C. 2008. S-ethyl-N,N-dipropylthiocarbamate exposure and

cancer incidence among male pesticide applicators in the Agricultural Health Study: a prospective cohort. Environ HealthPerspect.116: 1541-1546.

Venkatesanwarlu, P., Sharma B. J. R., Kalakumar, B., Reddy, K. S. and Ravikumar, P. 1997. Comparative evaluation of toxicity of carbaryl, cypermethrine and malathion of testies in mice. Indian J Toxicol. 4: 33-37.

Whyatt, R. M., Caman, D. E., Kinney, P. L., Reyes, A., Ramirez, J. and Dietrich, J. 2002. Residential pesticide use during pregnancy among a cohort of urban minority women. Environ Health Perspect. 110: 507-514.

Wolansky, M. J., Gennings, C. and Crofton, K. M. 2006. Relative potencies for acute effects of pyrethroids on motor functions in rats.Society of Toxicol. 89:271-277.

Yarsan, E., Bilgili A., Kanbur M. and Celik, S. 2002. Effects of deltamethrin on lipid peroxidation in mice. Vet Hum Toxicol 44(2): 73-75.

Zahm, S. H. and Ward, M. H. 1998.Pesticides and Childhood Cancer. Environ Health Perspect. 106, 3: 893-908.