Cytogenotoxic Effects Of A Pyrethroid Insecticide (Fenvalerate) In Channa Punctatus In Vivo

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Abstract- *Pesticides have been known to cause chromosomal aberration and cellular damage in different organism. In the present study, we analysed the induced SCE frequencies, chromosomal aberrations, cellular proliferation rate in mitotically dividing cells of kidney and micronuclei in peripheral erythrocytes of channa punctatus following in vivo of Channa punctatus exposure to three different concentrations of a synthetic pyrethroid insecticide (Fenvalerate). Our result revealed that the insecticide induced significantly high incidences of micronuclei in peripheral erythrocyte, chromosomal aberrations as well as sister chromatid exchanges, inhibited mitotic index and caused considerable delay in the generation time of kidney cells in treated organisms. The effects were found to be dose dependent as well as to the period of exposure to the chemical. The experimental data showed Fenvalerate as cytogenotoxic to fish per se. These results have implications in the use of pesticides in the agricultural field.*

Keywords-
pyrethroid,Genetoxic,Micronucleus,SisterchromatidExchanges ,Chromosomal aberration, Fenvalerate etc.

I. INTRODUCTION

Enormous use of pesticides in modern agriculture to protect crops from pest menance is a matter of great concern to-day, as most pesticides are usually connected with the serious problem of pollution and health hazards (Sternberg 1979, Klopman et al. 1985). A number of reports have shown the mutagenic, carcinogenic and/or clastogenic potentiality of a number of pesticidesis is on record (Bartsch et al. 1980, Waters et al. 1982; Batiste Alentorn et al 1986; Pati and Bhunya, 1989; Atale et al 1992; Porichha et al 1998). However, in view of the importance of pesticides in pest control ,neither their use in agriculture can totally be banned nor can it be minimized until a suitable and safer alternative becomes available. As a compromise, it has been suggested by various workers that the mutagenic and /or carcinogenic potentiality of each and every pesticides should be evaluated and those pesticides that have a neglegibleeffect on the genetic material should be excluded,(ATSDR2003,ATSDR 2007) people involved with the application and formulation of pesticides should be made, aware of the results of such investigations (Epstein and Legator 1971, Waters et al, 1982, Sharma 1984, Kaur 1983, Sinha 1989, Tisch et al, 2005, Paz-y-mino etal, 2002).

In recent years, the harmful effects of many pesticides, such as orgnochlorines, organophosphates and carbamates, have led to the use of pyrethroids as an alternatives. Pyrethroids are analogs of naturally occurring pyrethrins and are widely used in agriculture in many countries (Leahey 1985). Generally, it is known that these pesticides possess high activity against a broad spectrum of insect pests (adult and larvae) (papodopoulou-Markidou, 1983, zebra 1988, Vijvberg & Van Der Bercken, 1990, Abertini etal. 2000).For these reason, pyrethroid insecticides are extensively used in forestry and household products.

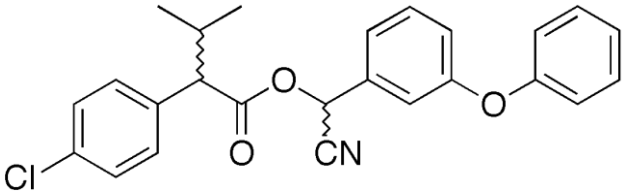
Synthetic pyrethroids are a diverse class of >1000 powerful broad specturm insecticides by virtue of their moderate persistence, low volatility and poor aqueous mobility in soil (Erstfeld, 1999). With the steadily increasing use of pyrethroid insecticides, there is an urgent need to identify their possible effects on living organisms. The genetoxic potential of some pyrethroid insecticides has been shown in previous studies (Carbonel et al, 1989, Puig et al 1989, Surralels et al 1990, Agrawal et al 1994, Institoris et al 1999). The carcinogenic potential of pyrethroids has been discussed in a review by (Litchfield, 1985,kocamen and topaketas,2009)

This paper records the frequency of chromosome aberrations, sister chromatid exchanges, mitotically dividing cell and proliferative kinetics in kidney cells as well as micronuclei in the peripheral erythrocytes following continuous exposure of the fish Channa punctatus) at sub-lethal concentrations of Fenvalerate ,the second most widely used pesticides in agriculture and store-houses (Rumker 1975). We choose this model as this has been considered to be an efficient in vivo cytogenetic test to evaluate the genotoxicity and/or cytotoxicity of chemical mutagens and/or carcinogens (Stich and Action 1976, Kligerman 1982, Mohanty and Prasad 1982, Hooftman and de Raat 1982, Manna et al. 1985, Das and Nanda 1986, Manna and Biswas 1984, Patra 1993, Buxipatra 1994, Porcihha et al. 1998, Bonassi et al 2007, Chauhan et al 1999, Giri et al 2002)

II. MATERIALS AND METHODS

Test animal: Specimens of Channa punctatus measuring about 10-12 cm collected from the local ponds and maintained in laboratory aquaria were used for seven days before treatment.

Test Chemical : Fenvalerat is a stomach and contact synthetic pyrethroid insecticide. Chemically it is cyano-[3-(phenoxy)phenyl]methyl] 2-(4-chlorophenyl)-3-methylbutanoate with a structural formula as:



Structure of Fenvalerate

The technical grade of Fenvalerate manufactured by M/s Raills India Ltd. Mumbai, under the brand name Sumicidin is highly effective against a wide-range of cotton pests and, therefore, 90% of the total production of Fenvalerate is used in the areas of plant protection and cultivation of cotton, fruits, vegetables, etc. However, owing to its high insect knockdown activity but a relatively low toxicity, it is also destined for increased used against poultry, dairy and certain household pests (Pluijmen et al, 1984, Mumtaz and Menzer, 1986).

Doses and route of exposure: From among the specimens acclimatized for at least a fortnight in the laboratory aquaria, only strong and active fishes were released into different aquaria containing 250,125 and 50 microgram of fenvalerate which correspond to MC, MC/2 and MC/s doses respectively. MC represent the maximum tolerable concentration of the test compound at which no death of animal beyond 5% was observed during the period of treatment and was determined from preliminary experiments on groups of 20 specimens in aquaria containing 100 litre of water. The test lasted for 25 days with change of water, chemical and food every alternate day. The lowest concentration leading to 50 % death after the treatment was considered as LC_{50} and half of this corresponds of MC. MC/2 and MC/5 represent 1/2 and 1/5 of MC. The treated specimens received in intramuscular injection of 0.02% colchicine solution at the rate of 1ml per 100 mg body weight 2 h prior to their sacrifice on completion of 5,10,15,20 and 25 days of exposure to the test chemical. Control received injection of an equal amount to distilled water Specimens used for analyses of SCEs and proliferation kinetics received, in addition to colchicines i,m. injection of an aqueous solution of bromodeoxy uridine (BrdU) at the rate of 50 mg per 100 g body weight at least 24h prior to their sacrifice.

Micronucleus Test: The smear of peripheral blood drawn from the caudal vein with a heparinized syringe, was prepared and well-dried slides were stained in 10% Giemsa solution (Stock solution diluted with Sorensen's buffer at pH 6.8) for 30 min following the method of Schmid (1976). Four thousand cells per animals (1000 cells per slide) were scored for micro-nuclei and nuclear anomalies.

Chromosome aberration(CA) test: Mitotic metaphase chromosome spread from kidney cells were obtained following colchicines-citrate-flame drying Giemsa technique of (Patro and Prasad (1981). Chromosome aberrations of various kinds were scored from 100 metaphase cells.

Sister-chromatid exchange (SCEs) and proliferative kinetics: Air-dired preparations of mitotic spreads from kidney of BrdU treated specimens were stained for induction of differential staining of sister-chromatids (Mohanty and Prasad 1982) and metaphase cells were classified as M1, M2 and M3 according to staining pattern. At each point of time 600 cells were examined, Proliferation Rate Index (PRI) was determined from the count of M1, M2 and M3 cells by using the formula of Lamberti et al. (1983) where PRI=(M1+2M2+3M3)/100. SCEs were scored from M2 cells only.

Mitotic indices: Mitotically dividing cells were scored from slides prepared and stained for metaphase spreads from kidney of both treated and control specimens. Cells at interphase were omitted from the analysis to avoid any possible error in the result.

Statistical analyses: For comparison of mean ,Student's t-test was applied. Two-way analysis of multiple variance (ANOVA) was done for dose and time response study.

III. RESULTS

Chromosome Aberration Test

Chromosome slides prepared from the kidney cells of Channa punctatus treated with Fenvalerate was found to be a relatively a good inducer of chromosome aberrations in the kidney cells of C. punctatus as compared to other organophosphorous pesticides so far tested in our laboratory as well as elsewhere. As can be judged from the data of chromosome aberrations summarized in table 01, the frequency increased from 0.084 ± 0.031 in control to respectively 0.388 ± 0.094, 0.521± 0.062 and 0.696± 0.066 in specimens exposed for

5 days to MC/5, MC/2 and maximum tolerable concentraion(MC) of fenvalerate, which terms of percent increase in aberrations are quite higher, than caused by most organophosphorous pesticides described earlier. Furthermore, the frequency increased with the increase in the concentration or the period of exposure upto 20 days. The frequency however, declined in specimens exposed to any of the three concentrations beyond 20 days, although the same was still very high as compared to controls.

Table-01

Frequency of chromosome aberrations (per 100 chromosomes) in kidney cells of Channa punctatus following their in vivo exposure to three different concentrations of fenvalerate for 5-25 days and in control.

Periods of Exposure (in days)	Concentrations		
	MC/5	MC/2	MC
0	0.084 ± 0.031	0.084±0.031	0.084±0.031
5	0.388±0.094	0.521±0.062	0.696±0.066
10	0.497±0.056	0.627±0.081	0.842±0.074
15	0.637±0.088	0.754±0.073	0.956±0.086
20	0.749±0.094	0.844±0.107	1.083±0.106
25	0.676±0.093	0.762±0.076	0.979±0.083

The data when subjected to Student's 't' test made if evident that the increase in the frequency of chromosome aberrations was significant in all the treated groups of specimens as compared to the control and that the level of significance increased with the increase in the concentration of the pesticide or in the period of exposure. Two way analysis of multiple variance (ANOVA test) testified that the pesticide induced Chromosomal Aberrations in dose as well as period dependent manner.Infact, the calculated values of 'F' for concentration ($F=616.06;d.f14,2,p<0.001$) and period of exposure ($F=270.95$, d.f. $14.4p<0.001$) were much higher than their respective tabulated values.

Micronucleus Test:

The frequency of micronuclei and nuclear anomalies of various kinds in the peripheral erythrocytes in Channa punctatus exposed to different concentrations of fenvalerate for varying periods of time and in control is summarized in table 02. Evidently, all the treated group of specimens had higher frequency of erythrocytes with MN and nuclear anomalies as compared to controls. In fact, the frequency increased from 0.055±0.007 in the controls to 0.134 ± 0.015, 0.192±0.021 and 0.294±0.024 in specimens exposed for 5 days to concentrations of MC/5, MC/2 and MC respectively. Furthermore, the frequency increased progressively to a maximum of 0.452±0.048, 0.562±0.054 and 0.713±0.69 respectively in specimen exposed for 20 days. The specimens exposed for 25 days, in the other hand had slightly a lower frequency than those exposed for 20 days to any of the three concentrations, as was the case with most organ phosphorous pesticides described earlier. Statistical analyses of the data, however, revealed that the increase in the frequency in all specimens was statistically significant as compared to the controls and that the level of significance increased with increase in the concentration or period of exposure. Two-way analyses multiple variance also revealed that the compound induced MN and nuclear anomalies in concentration as well as period of exposure dependent manner as was the case with all pesticides studies herein. In fact, the calculated values of 'F' for concentration ($F=20.62;d.f.14.2,p<0.001$) and period of exposure ($F=15.74.df$ $14,4,p<0.001$) were significantly higher than their respective tabulated values.

TABLE-02

Frequency of peripheral erythrocyte with micronuclei and nuclear anomalies of various kidney in Channa punctatus following their in vivo exposure to three different concentrations of fenvalerate for 5-25 days and in control.

Periods of Exposure (in days)	Concentrations		
	MC/5	MC/2	MC
0	0.055 ± 0.007	0.055 ± 0.007	0.055 ± 0.007
5	0.134±0.015	0.192±0.021	0.294±0.024
10	0.214±0.018	0.312±0.028	0.445±0.036
15	0.345±0.035	0.435±0.046	0.582±0.049
20	0.452±0.048	0.562±0.054	0.713±0.069
25	0.386±0.039	0.492±0.042	0.623±0.056

Sister-chromatide Exchanges:

Chromosome slides prepared by flame drying method from the kidney cells of Channa punctatus treated with Fenvalerate-BrdU-colchicine-giemsa,it was found that Fenvalerate as the more potent inducer of SCEs in Kidney Cell of C.punctatus at maximum tolerable concentration in (MC) as compared to the five organophosphorous insecticides analysed in this study. At MC/5 and MC/2 concentrations, however, it induced SCEs in relatively lower frequency than chloryriphos and malathion. A reference to the average data on SCEs is kidney cells per 100 metaphase chromosomes enumerated in table 03 would make it evident that the frequency increased from 0.197±0.011 in control to 0.346±0.032, 0.460±0.044 and 0.660±0.074 in specimens exposed for 5 days to MC/5, MC/2 and MC respectively which in terms of percent increase was much higher than induced by die other organophosphorous

compounds studied herein. However, like all other pesticides under study, save for dimethoate and chlorpyriphos, the progressive increase in the frequency with the increase in the period of exposure was observed only in specimens exposed upto 20 days. This notwithstanding, statistical analyses of the data by way of Student's 't' test testified that elevated rate of sister-chromatid exchanges in specimens exposed to each concentration and/or each period of exposure was significant, as compared to the controls. Also, the level of significance increase with the increase in the concentration and /or the period of exposure. Two-way analyses of the multiple variance, too reinforced that induction of sister-chromatid exchanges by fenvalerate was dependent significantly both on the concentration as well as the period of exposure. The calculated values of 'F' for concentration (F=2744.74;d.f.14,2,p<0.001) and period of exposure (F-1276.48; d.f. 14,4.p<0.001) were unexpectedly higher than their respective tabulated

Table-03

Incidence of sister-chromatid exchanges (per 100 chromosomes) in kidney cells of Channel punctatus following their in vivo exposure to three different concentrations of fenvalerate for 5-25 days and in control.

Periods of Exposure (in days)	Concentrations		
	MC/5	MC/2	MC
0	0.197 ± 0.011	0.197 ± 0.011	0.197 ± 0.011
5	0.346±0.032	0.460±0.044	0.660±0.074
10	0.469±0.046	0.572±0.051	0.791±0.071
15	0.586±0.053	0.690±0.059	0.991±0.096
20	0.718±0.057	0.810±0.091	1.030±0.106
25	0.632±0.061	0.722±0.079	0.931±0.090

Mitotic index:

Table 04 potrays the frequency of mitotically dividing cells in the kidney of Channa punctatus following their in vivo exposure to three sublethal concentrations (MC/5, MC/2 and MC) of fenvalerate of the period varying from 5-25 days and in controls. A cursory survey of the data would make it evident that all the treated grups of specimens had highiy depressed .

Table-04

Frequency of mitotically dividing cells in kidney cells of Channa punctatus following their in vivo exposure to three different concentrations of fenvalerate for 5-25 days and in control.

Periods of Exposure (in days)	Concentrations		
	MC/5	MC/2	MC
0	1.325 ± 0.172	1.325 ± 0.172	1.325 ± 0.172
5	1.188±0.082	1.104±0.097	1.025±0.091
10	1.088±0.091	1.070±0.098	0.910±0.091
15	0.963±0.088	0.954±0.093	0.816±0.074
20	0.875±0.093	0.825±0.084	0.687±0.052
25	0.946±0.089	0.875±0.086	0.725±0.091

mitotic index as compared to the control,thereby indicating that fenvalrate is also mitostatic to larger cells. In fact, the frequency declined from 1.325 ± 0.172 in control to 1.188±0.082, 1.104±0.097 and 1.025±0.091 in specimens exposed fro 5 days to MC/5, MC/2 and MC respectively and then progressively with the increase in the period of exposure and the concentration of the pesticide. The maximum depression was found in specimens exposed for 20 days. The specimens exposed for 25 days, on the other hand, had slightly higher MI than those exposed for 20 days-thereby indicating a reversal in the deceasing trend, as was the case with dichlorvos methyl parathion and malathion. Satistical analyses of the data employing Student's 't' test clearly indicated that the decrease in the rate of mitotically dividing cells in kidney of control specimens was significant as compared to the controls while application of ANOVA test (two-way analysis of multiple) testified that fenvalerate affected the mitotically dividing kidney cells in both concentration as well as period dependent manner. The calculated values of 'F' for concentration (F=91.71, d.f. 14.2,p<0.001) and period of exposure (F=101.91, d.f. 14,4, p<0.001) were in fact significantly higher than their respective tabulated values.

IV. DISCUSSION

Compared to various organophophorous insecticides, there has been only a limited number of studies relating genotoxic potentiality of pyrethroids in general and fenvalerate in particular. However, almost all studies so far undertaken, save for mutagencity testing in some Salmonella typhimurium strains, namely TA97, TA98. TA100, TA104,TA1535, TA1537 and TA1538. Where fenvalerate did not induce reverse mutation (Pluijmen el at 1984; Herrera and Laborda,19S8) have clearly demonstrated the genotoxic potential of the compound. According to Baliste-Alentorn et al. (1987) fenvalerate induced aneuploidy in Drosophila after adult feeding. Geetha and Rudrama Devi (1992) observed chromosome aberrations and micronuclei in high frequency in the bone marrow cells of mouse in vivo following intraperitoneal and oral administration of fenvelarate. These author also observed a significantly high frequency of sperm head obnormality in mice treated with fenvalerate in vovo. The genotoxic potential of this has also been demonstrated by Jayaraman et ah (1984) in the bone marrow cells of the male squirrel, Funabulus palmarum. The

results obtained in this study clearly revealed dose as well as period dependent increase in chromosome aberrations in kidney cells as well as micronuclei in the peripheral erythrocytes of Channa punctatus following their exposure to three different sub-lethal concentrations (MC,MC/2 and MC/5) of fenvalerate for varying periods of time (5-25 days) and thus reinforce the clastogenic potentiality of fenvalerate as reported by earlier workers. Failure of Pluijmen et al (1984) and Herrera and Laborda (1988) to obtain reverse mutation in various strains of Salmonella typhimurium may, therefore, be attributed to the low dose of fenvalerate and /or technical difference in the setting of the culture. The contradictory results obtained in various microorganisms in so far as the mutagenic and/or carcinogenic potentiality of fenvalerate is concerned, may, also be attributed to the differential response by different types of organisms as found with several chemicals (Kihlman, 1966; Fishbein et al, 1970)

The results obtained in this study also disagree with the views of Pati and Bhunya (1989) insofar as the route of administration is concerned. In this study we reared the specimens of C.punctatus for varying period of time in different concentrations of fenvalerate. This means that the higher incidence of micronuclei and sister-chromatid exchanges and decreased mitotic index in treated groups of fishes must be due to absorption of chemical through dermal tissues. Furthermore, the observed effect was almost similar to those obtained in-'jn-other studies. Although the possibility that little amount of chemical might have entered in the body of the specimens through oral routes cannot be totally ruled out, it seems unlikely that the effect is exclusively, due to oral inhalation of the chemical. In fact, fenvalerate, like other synthetic pyrethroids is known to be rapidly absorbed, distributed to tissues, metabolised and excreted from tested mice (Mumtaz and Manzer, 1986). Thus in significant increase in the incidence of micronuclei and chromosome aberrations in mice treated through intraperitoneal route as observed by Pati and Bhunya (1989) must be due to rapid metabolism and excretion of the chemical in mice. Yet, another possibility is that the difference in the result is due to different responses of the cell types of organism in question.

Our results, also differ from those of the results obtained in earlier studies insofar as the reversal of trend ,the increase in the frequency of SCEs, chromosome aberrations and MN and decrease in the frequency of mitotically dividing cells in the kidney after certain period of exposure (20 days) in concerned. In fact, we observed a higher frequency of SCEs, chromosome aberrations and MN in specimens exposed for 20 days as compared to those exposed for 25 days. Similarly, the mitotic index was higher in specimens exposed for 25 days to any of the three concentrations (MC/5, MC/2 or MC) of fenvalerate as compared to dose exposed for 25 days. The possible reasons for such a reversal in the increasing/decreasing trend in the frequency of CAs, MN and SCEs, MI after certain period of exposure has been discussed in detail in general conclusion part of the thesis, since such a reversal in the trend has also been observed with several other pesticides studied herein.

In the present study, we also observed significantly high frequency of SCEs in the kidney cells of specimens exposed to fenvalerate as compared to the controls vis-a-vis reduction in the rate of mitotically dividing cells in the kidney with concurrent increase in concentration and/or period of exposure, thereby indicating that fenvalerate has genotoxic as well as cytotoxic effect in target organisms. As a result this insecticide when not used properly can cause harm to Aquatic organisms, man and environment and show cytotoxic and Genotoxic effects (Kocamen and Topakatas ,2009). Thus, our result clearly demonstrate that fenvalerate acts on the genetic material of the target organisms exactly in the same manner as several organophosphorous pesticides do (see Chen et al, 1981; Waters et al, 1982; Nicolas and Vandenberghe, 1982; Sobti et al, 1982 and Balaji and Sasikala, 1993, Bonassi et. al. 2005, 2007).

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