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Studies on The Genotoxicity of An Organophosphorous Pesticide Baytex-1000

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KEY WORDS Chromosome aberrations; sister chromatid exchanges; mitotic index; genotoxic; pesticide.

ABSTRACT Baytex-1000 is an organophorous pesticide. It is widely used both as a larvicide and insecticide in the National Malaria Eradication Programme in India. Malaria control workers are occupationally exposed to Baytex. Genotoxicity of this pesticide was studied by assaying Mitotic Index (MI), Chromosome Aberrations (CA), Sister Chromatid Exchanges (SCE) and Satellite Associations (SA), in short term lymphocyte cultures. Peripheral blood samples were obtained from 60 malaria control workers and equal number of matched controls. MI increased upto exposure period of 5 years and declined thereafter. CA in controls comprised acentric fragment, chromatid gaps and breaks only, whereas in exposed samples, dicentrics, rings, chromosomal gaps, breaks and translocations; chromatid gaps, breaks and isochromatid exchanges were recorded. The frequencies of CA and SCE were significantly increased by smoking and alcohol consumption ($P<0.5$) and depicted positive correlation with period of exposure. D-G-type SA outnumbered 2D-2G types. The mean frequencies of various parameters in controls and workers exposed to Baytex respectively were: MI (4.29 & 6.43), CA (0.933 & 4.200), SCE (4.32 & 7.28) and SA (5.11 & 16.5). The differences were statistically significant ($P < 0.05$). Time and dose-yield effects of Baytex on micronuclei induction in mouse test system were also significant (p-0.05). All the cytogenetic end points investigated conclusively show that Baytex-1000 is a highly genotoxic pesticide. The mechanism of its action has been described.

INTRODUCTION

Green revolution has ushered in a phenomenal growth in the use of a large variety of chemical fertilizers and pesticides in agricultural operations, particularly in Northern India. These xenobiotics are gaining immense importance due to their unquestionable ability to control weeds, insects and other pests. Along with the pest man, the non-target organism, is also environmentally exposed to them. As such, in spite of immense advantages of pesticides, evidences from the available literature clearly indicate that these otherwise readily proven beneficial chemicals are not only hazardous to human health, but also pose a genetic risk to the men working with them.

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Organophosphates are considered 'safe' due to their fast elimination from the environment. As such, these are most widely used. However, now even these compounds seem to pose genetic hazards (Chen et al. 1981). Many such compounds have been tested for mutagenicity by a variety of *in vitro* and *in vivo* assays. Chlorpyriphos, marketed as Dursban, depicted positive results for DNA damage in prokaryotic systems (Waters et al. 1980), significant increase in frequency of sister chromatid exchanges (SCE) in human lymphoid cell cultures (Sobti et al. 1982), and significantly increased values of chromosome aberrations (CA) in rats (de Hondt et al. 1983). Kurinny (1975) also showed positive results for CA in bone marrow cells and Tezuka et al. (1980) showed increased SCE in mammalian cells exposed to organophosphorous pesticides.

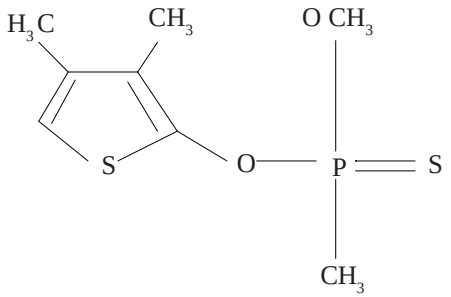
Increased frequency of micronuclei in the erythrocytes of both bone marrow and peripheral blood were reported in chick after chronic exposure to monocrotophos (Jena and Bhunya 1992). Statistically significant CA, micronuclei and sperm abnormalities were reported in an *in vivo* study in mouse system treated with Hinosan (Jayashree et al. 1992).

Baytex-1000, an organophosphorous insecticide, is extensively used in India, to destroy mosquito larvae, adult mosquitoes, flies and other such insects. In view of the other organophosphorous compounds being genotoxic it was thought proper to look into the genetic damage, if any, caused by this compound. The present study was carried out on 120 individuals, 60 occupationally exposed to Baytex-1000 and an equal number of matched controls taking mitotic index (MI), chromosomal aberrations (CA), sister chromatid exchanges(SCE) and satellite associations (SA) in human lymphocytes and micronuclei in mouse test system as the cytogenetic end points.

MATERIAL AND METHODS

Baytex-1000 is an emulsifiable concentrate containing 82.5% w/w Fenthion = O, O-

dimmethyl 0-(3-methyl-4-methylthiopheny1) phosphorothioate, 1% phenol, balance emulsifiers marketed by Bayers India Ltd. Empirical formula of Fenthion is $C_8 H_{13} O_3 PS_2$ and its structural formula is:



Although directions for its application call for avoiding inhalation and skin contact while diluting because of spillage or splashes, not to mix with bare hands, and wear full protective clothing, yet none of the workers took these precautions. The history of the workers with respect to drug intake, habit of smoking and consuming alcohol was recorded using a proforma specially prepared for the purpose. The epidemiological survey did not reveal any clinical symptoms. The workers, under National Malaria Eradication Programme, worked for five days a week throughout the year.

Peripheral blood samples were obtained from 60 malaria field workers occupationally exposed to Baytex and another 60 control individuals matched with respect to age, sex, smoking and alcohol consumption. Short term lymphocyte cultures using the whole blood were set up following the method described earlier by Yadav and Kaushik (1996). For CA and SA 100 metaphases per individual were analyzed, for SCE 25 second division metaphases were recorded, while 5000 cells per individual were screened to determine MI.

Approximately 8 weeks-old Swiss albino mice bred in the animal house of National Dairy Research Institute, Karnal were used as test animals for micronuclei analysis to study the *in vivo* effect of Baytex as dose - and time - yield effect. Positive and negative controls were set up. For positive controls 3 mg/Kgbw Mitomycin - C was injected, whereas in the negative control 1.5 ml of distilled water was injected. The controls were sacrificed after 24 hours. To study the dose - yield effect, three different doses 5 mg/Kgbw, 3mg/kgbw and 1.5mg/Kgbw of the pesticide were administered to the experimental mice. For investigating the time - yield effect, the highest dose (5 mg/kgbw) was administered. Bone marrow smears were prepared after 24h, 30h and 48h.

The slides were first cleared in pure ethano1 and then stained with 5% May - Grunwald stain followed by 50% May - Grunwald and 14% Giemsa stains, two changes were given in Sorensons Phosphate buffer, washed in distilled water, air - dried and stored for 24 hours.

The number of micronucleated cells in 1000 polychromatic erythrocytes (PE), in 1000 normochromatic erythrocytes (NE) and number of PEs in 1000 total erythrocytes (PE+NE) were counted.

Statistical analysis of the data was carried out using the student's 't' test (Colton 1980) and Chi square test.

RESULTS

The mitotic index (MI) in exposed individuals and their controls, according to the duration of exposure, is given in table 1. The mean MI was 4.29 in controls, whereas it was 6.43, significantly higher ($p<0.05$), in the exposed workers. It was maximum (8.35) in workers exposed upto a period of five years. Thereafter, the MI showed a gradual decline.

Table 1: Mitotic Index (MI) in workers exposed to Baytex-1000

Duration of exposure (in years)	*No. of individuals*	*No. of cells screened* n_1	*No. of metaphases*	*MI ± SD* $n_2/n_1 \times 100$ n_2
Control	60	301772	12968	4.29 ± 0.94
Exposed	60	301005	19367	*6.43 ± 1.83
0 - 5	5	25012	2090	8.35 ± 0.49
6 - 10	16	82673	6701	8.10 ± 0.27
11 - 15	23	115647	7292	6.30 ± 0.64
16 - 20	13	62822	2780	4.42 ± 0.44
21 - above	3	14825	466	3.14 ± 0.12

* $p < 0.05$
SD = Standard Deviation

Table 2: Types of chromosomal aberrations in exposed and control individuals

Group	*Control*	*Exposed*	*'t'*
No. of individuals	60	60	
No. of metaphases	6000	6000	
Chromosome Type Aberrations			
Dicentric	0	18 (0.300)	
Ring	0	4 (0.670)	
Acentric fragment	3 (0.050)	38 (0.633)	
Chromosome gap	0	7 (0.117)	
Chromosome break	0	2 (0.033)	
Translocation	0	4 (0.067)	
Total	3 (0.050)	73 (1.217)	p<0.01
Chromatid Type Aberrations			
Gap	96 (1.600)	163 (2.717)	
Breaks	53 (0.883)	172 (2.887)	
Isochromatid exchanges	0	7	(0.116)
Total (without gaps)	53 (0.833)	179 (2.983)	p<0.01
Total chromosomal aberrations (without gaps)	56 (0.933)	252 (4.200)	p<0.01

Values in brackets indicate percentage.

Various types of chromosomal aberrations (CA) observed in the samples were listed in table 2. Frequencies of all types of aberrations were significantly higher in the exposed sample as compared to the controls. The frequency of total CA for 100 metaphases was 4.20 in exposed workers and 0.933 in the controls. the difference being significant (P<0.01). The frequency of CA was the least in non smoker-non alcoholics and maximum in smoker-alcoholics both among exposed workers and their controls (Table 3). Whereas among controls alcoholics had less CA than smokers, it was the other way round in the exposed sample. However, differences between exposed workers and controls were always significant ($p < 0.05$).

Frequency of sister chromatid exchanges (SCE) in Baytex exposed workers and controls is presented in table 4. The mean of SCE per cell was 4.22 in controls and 7.28 in exposed workers. The difference was significant ($p < 0.05$). The values were higher in exposed sample as compared to controls for alcoholics, smokers, alcoholic - smokers and non-alcoholic non-smokers. Smoker-alcoholics among workers showed highest values of SCE followed by smokers, alcoholics and non-smoker - non-alcoholics. The differences in SCE frequencies among different sub groups were always significant ($p < 0.05$).

Both the CA and SCE showed a positive corelation with the duration of exposure (Table 5).

As compared to 5.11 associations per cell in the controls, the value of SA among the exposed workers was 16.15 in the exposed sample (Table 6). The increase was significant ($p < 0.05$). D-G type of associations were the maximum (407), whereas 2D-2G associations were the lowest.

Table 7 shows the result of the micronucleus test. Negative controls treated with saline showed an average of 0.08 ± 0.10 micronucleated NE (MNE), 0.14 ± 0.13 micronucleated PE (MPE) and 50.4 ± 4.17 polychromatic erythrocytes in total erythrocytes (PE/PE +NE). Mice treated with Mitomycin-C were all positive. Significant increases in the incidence of both MNEs and MPEs were noted ($p < 0.05$). Incidence of MNEs

Table 3: Frequency of chromosomal aberrations in exposed and control individuals

	Exposed		*Control*		
	n	*Mean ± SEM*	*n*	*Mean ± SEM*	*'t'*
Alcoholics	8	4.50 ± 0.24	10	0.77 ± 0.81	p < 0.05
Smokers	14	3.64 ± 0.16	19	1.00 ± 0.79	p < 0.05
Smoker-alcoholics	22	5.68 ± 0.24	21	1.14 ± 0.62	p < 0.05
Non-smoker-non-alcoholics	16	2.25 ± 0.33	10	0.50 ± 1.34	p < 0.05

n = Number of individuals
SEM = Standard Error of Mean

Table 4: Frequency of sister chromatid exchanges in exposed and control individuals

	Exposed		*Control*		
	n	*Mean ± SEM*	*n*	*Mean ± SEM*	*'t'*
Total SCEs	60	7.28 ± 0.08	60	4.22 ± 0.19	p < 0.05
Alcoholics	8	7.11 ± 0.04	10	3.69 ± 0.08	p < 0.05
Smokers	14	7.25 ± 0.01	19	4.20 ± 0.10	p < 0.05
Smoker-alcoholics	22	7.79 ± 0.04	21	4.85 ± 0.16	p < 0.05
Non-smoker-non-alcoholics	16	6.52 ± 0.03	10	3.46 ± 0.17	p < 0.05

Table 5: Frequency of CAs and SCEs with duration of exposure

Duration of exposure (in years)	*No. of individuals*	*CAs (Mean ± SEM)*	*SCEs (Mean ± SEM)*
0 - 5	5	3.00 ± 0.36	6.78 ± 0.08
6 - 10	16	3.93 ± 0.29	6.90 ± 0.06
11- 15	23	4.21 ± 0.52	7.38 ± 0.06
16 - 20	13	4.23 ± 0.36	7.54 ± 0.08
21 - 25	3	6.00 ± 0.13	8.21 ± 0.05

and MPEs showed a correlation with increase in dose from 1.5 mg/Kg bw to 5 mg/Kg bw. With the duration of time, a peak at 3.3 ± 0.40 was achieved in the mice sacrificed after 30 hours. The value was, however, least in mice dissected after 48 h (1.98 ± 0.14). Differences in the values are significant ($p < 0.05$).

DISCUSSION

In the present investigations the mean mitotic index was found to be significantly higher in the exposed workers than the control individuals. The maximum increase was recorded up to exposure of five years after which a decline was observed and the MI showed very significant decrease. It fell almost to the level of controls after 15 years and below that after 20 years exposure. Rupa et al. (1989) also reported a significant increase in MI in the workers exposed to a mixture of pesticides, including organophosphorous compounds, up to 10 years of exposure and thereafter it decreased significantly. Sobti et al. (1982) reported inhibition of the MI when human lymphoid cells were treated with organophosphorous pesticides at higher concentration.

Chromosome aberrations in control individuals included only acentric fragments, chromatid gaps and breaks. The background frequency (0.933) is in fair comparison with other similar studies (Galloway et al. 1986; Bender et al. 1988; Yadav and Kaushik 1996). However, all the types of CA were observed in the workers exposed to Baytex. Their frequency was also significantly increased as compared to the controls. The present observations are in accordance with Doulout et al. (19850 who reported a significant increase in exchange type aberrations in floriculturists exposed to various pesticides, including organophosphates, the exchange type of aberrations being nearly exclusively dicentrics without fragments. During the present investigation also a high frequency of dicentrics was found. Whereas Doulout et al. (1985) related the presence of dicentrics to the increased presence of MII cells because of 72h culturing, the same cannot be said about the present study in which the CA were scored in lymphocytes cultured for 48 hours. A significant increase in CA was also recorded in various human populations exposed to pesticides *in vitro* (Rupa et al. 1988a,b,c,1989; Yoder et al. 1973; Paldy et al. 1987; Van Bao et al. 1974; Vijayraghavan and Nagarajan 1993; Rita et al. 1987; Nehez et al. 1981; Kourakis et al. 1992) as well as mouse system *in vivo*

Table 6: Frequency of satellite associations in exposed and control individuals

No. of individuals	*Total cells scored*	*Types of satellite association between D and G group acrocentric chromosomes*							*Total*	*Ass. per cell*	*± SEM*
		D-D	*D-G*	*G-G*	*2D-G*	*2GD*	*2D2G*	*3D*			
E	6000	212	407	121	83	80	31	35	969	16.15%*	± 0.44
60		(3.53)	(6.78)	(2.01)	(1.38)	(1.33)	(0.51)	(0.58)			
C	6000	60	156	47	12	14	11	7	307	5.11%	± 0.41
60		(1.00)	(2.60)	(0.78)	(0.02)	(0.23)	(0.18)	(0.11)			

* Significant ($p<0.05$), E = Exposed, C = Control, Ass. = Association

Table 7: Micronucle test with Baytex-1000

Dose mg/kg	*Expression time*	*PE/PE ± NE (Mean ± SD)*	*MNE/NE (Mean ± SD)*	*MPE/PE (Mean ± SD)*
Negative Control (Normal Saline)	24 hrs	50.4 ± 4.17	0.08 ± 0.10	0.14 ± 0.13
Positive Control (MMC 3.0)	24 hrs	34.3 ± 0.09	0.30 ± 0.96	5.72 ± 0.91
1.5 mg/kg	24 hrs	50.16 ± 1.30	0.12 ± 0.08	2.04 ± 0.11
3 mg/kg	24 hrs	49.62 ± 2.09	0.12 ± 0.08	2.08 ± 0.79
5 mg/kg	24 hrs	49.76 ± 4.06	0.16 ± 0.05	3.12 ± 0.31
	30 hrs	48.36 ± 4.02	0.18 ± 0.08	3.34 ± 0.40
	48 hrs	47.78 ± 4.84	0.16 ± 0.05	1.98 ± 0.14

*Significant at $p < 0.05$, MMC = Mitomyocin-C, PE = Polychromatic Erythrocytes, NE = Normochromatic Erythrocytes MNE = Micronucleated Erythrocytes

(Jayashree et al. 1992; Amer and Farah, 1983; Amer and Mikhael 1983; Amer and Sayed 1986; Amer and Aly 1992; Usha Rani et al., 1980).

A significant increase was observed in the frequency of SCE in the exposed workers during the present investigation. A clear correlation was noticed between the period of exposure and increase in CA and SCE. This confirms the earlier reports. Chen et al. (1982) found that Fenthion (Baytex has 82.5% w/w of this compound) and Oxydemeton-methyl induced a significant increase in SCE in Chinese hamster V79 cells. Rupa et al. (1989) also reported significant increase in SCE which was related to the duration of exposure to pesticides. Other organophosphates viz. Malathion, Methyl parathion, Parathion, Dimethoate, Dursban, Phorate, Phosdime and Viozene also increased SCE in human lymphoid cells (Sobti et al. 1982). Monocrotophos induced SCE in Chinese hamster ovary cells, rat tracheal epithelial cells (Wang et al. 1987) and human lymphocytes (Rupa et al. 1988c).Similar results were earlier reported by Crossen et al. (1978), Nicholas et al. (1979) and Dulout et al. (1985).

The synergistic effect of confounding factors, smoking and alcohol consumption, was clearly noticeable, the frequency of CA and SCE being the maximum in smoker-alcoholics and minimum among non smoker - non alcoholics in the exposed workers as well as the controls. Maximum number of dicentrics were present in individuals who were both alcoholics and smokers and only a few in persons who were neither smokers nor alcoholics, but non dicentric was present in alcoholics and smokers in the control group. Alcohol consumption and smoking along with Baytex exposure increase the incidence of dicentrics.

All the translocations were limited to the group smoker - alcoholics with none in the control group. Obe and Herha (1975) earlier reported increase in the exchange type aberrations (translocations, dicentric etc.) in chronic alcohol users, while Kissin and Kaley (1974) reported that alcohol acts as a carcinogenic augmenter, presumably by increasing the diffusion of carcinogenic substances into the tissues. As many as 6 out of 7 exchanges were present in individuals belonging to smoker - alcoholic group, whereas 3 out of 4 rings were found in persons having an exposure of between 11-15 years, and only one ring was observed in an individual with exposure of 5 years. This individual was both a chronic alcoholic and a chain smoker. Earlier Gill (1991) reported that translocations appeared in persons who consumed a lot of alcohol daily, whereas dicentrics were more common in chain smokers. The frequency of SCE and CA in two day culture of peripheral lymphocytes of alcoholics and heavy smokers were significantly higher than their controls (Natrajan and Obe 1980; Obe and Herha 1978; Obe et al. 1980).

Age and smoking were the confounding factors which showed an increase in SCE on exposure of agricultural workers to a mixture of pesticides (Carbonell et al. 1990).

It is clear from the present study that exposure coupled with the habit of taking alcohol and smoking or a combination of the three can have a synergistic effect on enhancing the cytogenetic damage.

It was found that CA and SCE increased with duration of exposure showing a maximum frequency of 6.00 ± 0.13 and 8.21 ± 0.05 SCE in persons who had been employed in this profession for 20 years. Paldy et al. (1987) reported an increase in CA upto an exposure of 15 years, thereafter CA decreased since elderly workers had to work less. In the present study, however, CA increased with duration of exposure. It may be so since all the workers had to work for equal number of hours irrespective of age.

The satellite associations (SA),by their nature are primarily associations between non-homologous chromosomes (Reitalu 1964). Ultimately, the SA lead to translocations and nondisjunction between satellited chromosomes (Hansson 1970). In the exposed group 16.15 SA per cell, about three times that of control (5.11), were recorded, thereby increasing an obvious risk of translocations and non-disjunction.

Baytex proved to the toxic to the bone marrow even in the relatively low doses and induced a high number of micronucleated polychromatic erythrocytes in mice with the maximum tolerated dose,. Maximum number of micronucleated PCEs and NCEs were observed after 30 hours. Significant increase in micronuclei has also been reported in a large variety of organisms or cultures treated with different doses of pesticides (Bhunya and Behera 1984; Grower and Malhi 1985; Gonzalez et al. 1990; Mathew et al. 1990; 1992; Surralles et al. 1990; Bhunya and Jena 1992, 1993).

The ability of organophosphates to react with DNA by transalkylation may reasonably explain their mutagenecity (Epstein and Legator 1971). Wild (1975) focused attention on the electrophilic activity as the fundamental cause of the

toxicity of these compounds and considered DNA alkylation as one of the reasons for the production of chromosomal aberrations. In P-O-C, the common structural element of all organophosphates, phosphorous and carbon are electrophilic sites which offer insight into the understanding of the reactions of organophosphates with nucleophiles. A nucleophil can preferentially attack either phosphorous or carbon atom with subsequent cleavage of P=O or C=O bonds and undergo phosphorylation or alkylation, as the case maybe. Such reactions of nucleophilic substitution constitute the primary chemical lesions, resulting ultimately in cytotoxic or genotoxic effects (Jayashree et al. 1992).

Benke and Murphy (1975) found that liver microsome oxidase in the liver of rat oxidizes Methyl parathion, containing phosphorothioate (P=S), to oxon (P=O) and subsequently to methyl paroxons. Vijayraghavan and Nagaraj (1994) consider these oxons, which are highly toxic compounds, to account for the profound cytotoxic effect of Methyl parathion. Since Baytex contains 82.5% w/w Fenthion, which also has phosphorothioate group, the above discussed mechanisms may also hold true for its reaction with the DNA leading to genotoxicity. The high cytotoxicity of Baytex may be due to Fenthion carrying methyl groups since methyl esters have higher alkylating potential than ethyl esters (Gerret et al. 1986).

All the genetic/cytogenetic end points lead to the conclusion that Baytex - 1000 is a highly genotoxic pesticide which must be handled with utmost care. Further, the workers exposed to this pesticide must abhor smoking and alcohol consumption.

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