CYTOGENETIC EFFECTS OF PHOXIM (A - NEW ORGANOPHOSPHOROUS INSECTICIDE) ON BONE MARROW CELLS OF RATS

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ABSTRACT

The present study was designed to investigate the possible mutagenic influence if any of phoxim (1/5 and 1/10 LD50) in 32 immature male rats. The obtained data revealed that the mutagenic effect of phoxim showed dose response relationship.The main types of structural aberrations in treated rats were breaks, gaps, fragments and delation.No numerical aberrations were noticed during the examination of the bone marrow metaphase cells of treated rats with 1/5 or 1/10 LD50 of phoxim.Moreover, phoxim induced a significant increase in the percentage of micronucleated polychromatic and normochromatic cells of treated rats.

INTRODUCTION

Insecticides as well as pesticides constitute a large number of chemicals which are nowadays occupy unique position among many substances which are used daily either for man, animals or pests for killing or injury some forms of life.Most of substances are not highly selective but are generally toxic to many target species including man and other animals **(Casarett et al., 1996)**.

Some of these chemicals are stable persist for several years in the soil as toxic compounds and so constitute a great source of environmental pollution **(Myra et al, 1981)**, causing deleterious effects as neuralgic effects, malignant tumors, abortion, teratogenic and mutagenic effects **(Nafstad et al., 1983)**. Moreover, male reproductive disorders were also recorded as oligo spermia, abnormal spermatozoa and genetic damage of germ cells **(Bellina and Marion, 1993)**.

Organophosphorous compounds are most widely used in veterinary and agriculture practice as for compating external parasites. These substances get access to reach inside the animal through dermal tissue, oral and respiratory routes.Adverse effects include clinical manifesta-

tions, biochemical changes, developmental disorders, pathological and mutagenic changes depend upon the dose used and physiological condition of the animal (**Jerry et al., 1997**).

Phoxim is a new organophosphorous insecticide which is widely used by direct application on animal skin surface for eradication of various ectoparasites in farm animals and some plants (**Muller, 1993 and Castella et al.,1994**) . Accidently ingested phoxim persists in animal tissue for 7 - 12 days after application according to the dose used (**Cargill and Dobson, 1977**).

The present work was conducted to investigate the mutagenic effects of phoxim if any on exposed rats.

MATERIALS AND METHODS

Test chemicals :

Phoxim (Baythion) @ is available as a 50 % emulsifiable concentrate, 5 and 10 % granules and concentrate for ultra low volume spray.Bayer, Leverkusein, Germany.

Animals :

A sum number of 32 immature male Wister rat of average body weight 70 - 80 gm were used in this experiment.They were kept in metal cages during the whole experimental period under a good hygienic condition. They were maintained on balanced diet and freely access to water.

Experimental design :

Rats were divided into 4 groups (each of 8 rats) and treated orally as the following

The 1st group was served as control and given distilled water (negative control).

The 2nd group was given ethyl methane sulfonate (a potential mutagenic substance) at the rate of 250 mg/kg B.Wt. (positive control).

The 3rd group was given phoxim at 1/10 LD50 (162 mg/kg B.Wt) as a single dose .

The 4th group was given phoxim at 1/5 LD50 (324 mg/kg B.Wt) as a single dose.

After the end of treatment, each group was divided into 2 subgroups (4 for each) for studying chromosomal aberration and micronucleated erythrocytic evaluation.

Chromosomal aberrations :

Rats of the 1st subgroup were intraperitoneally injected with colchicine (4 mg/kg B.Wt.) 2 hours prior their scarifying.Rats were scarified 30 hours post phoxim administration and used for cytogenetic analysis of rat bone marrow cells according to **Brusick (1980)** with certain modifications recommended by **Alder et al. (1991)**.

Metaphases with well spread chromosomes were used for scoring chromosomal aberrations (structural and numerical or both).Some of these metaphases were photographed.

b- Micronucleus method :

Rats of 2nd subgroup were scarified 30 hours post phoxim administration and were employed for evaluation of the frequency of micronucleated (polychromatid and normochromatid) erythrocytes in femoral bone marrow according to **Schmid (1976)** with some modifications as recommended by **Alder et al (1991).**

Statistical analysis :

The significance of the results obtained from treated rats and control was tested according to **Berly and Lindgren (1990).**

RESULTS AND DISCUSSION

Chromosomal aberrations :

Results of the potential mutagenic influence of a single acute oral dose of phoxim (1/10 and 1/5 LD50) in bone marrow of rats were shown in tables (1 and 2) and illustrated in Fig. (1 and 2).The obtained data revealed that the mutagenic effect of phoxim was dose-related, as the 1/5 LD50 produced marked and highly significant effect than 1/10 LD50. Moreover, the results showed that, cells with one aberration were more commonly prevalent as mutagenic evidence than cells with more than one aberration. No numerical aberrations were obtained during the examination of the bone marrow metaphase cells of the phoxim.

The main types of structural aberrations induced by phoxim in treated rats were breaks, gaps, fragment and delation. On the other hand, there were no aberrant cells with centromeric attenuation or ring chromosomes of treated rats.

There was a total increase in aberrant cells with breaks (2.5% and 5.5%), gaps (12.5% and 22%), delation (1% and 1%) and fragments (1.5% and 1%) in rats given phoxim at 1/10 and 1/5 LD50, respectively. Formation of chromosomal and chromatid gaps suggested that phoxim interact with DNA independent of the cell cycle stage. **Gebhart (1977) and Anderon and Richardson (1981)** considered gaps to be sensitive indicator of chemically induced chromosomal damage as other types of aberration. On the other hand, **Schmid (1976)** considered the scoring of gaps in highly subjective only, therefore unsuitable indicator for mutagenic potential. Meanwhile, **El-Nahas et al (1997)** recorded chromosomal aberration and cellular proliferation in maternal fatal mice cell after treatment with organophosphorous phenthoate. Moreover,

Fahmy and Abdalla (1998) stated that, profenofos induced an increase in the percentage of chromosomal aberrations in bone marrow cells of male rats.

Micronucleated polycromatic and normochromatic cells :

As shown in tables (3 & 4) and Fig. (3), there was a significant increase in the percentage of total cells with micronucleated polychromatic (1.9% and 2.2%) in rats orally received phoxim at 1/10 and 1/5 LD50, respectively. The micronucleated normochromatic cells were 0.55% in control rats received saline and increased to 1.4% (at 1/10 LD50) and 1.75% (at 1/5 LD50) in rats treated by phoxim.Our data paralleled with the results obtained by **Adhikari and Grover (1988)** who observed an increase in chromosomal abnormalities after treatment rats with dimecron. Furthermore, **Agrawal et al (1994)** found that, deltamethrin induces increase of micronucleated erythrocytes in bone marrow cells of rats. In addition, **Jayashree et al (1994)** reported that, the organophosphorous hinosan revealed a significant chromosomal aberrations and micronucleus abnormalities in mice.

Table 1 : Effect of phoxim on the induction of chromosomal aberrations in rat bone marrow cells.

Treatment	Rat Ident. No	Total No. of exam. cells	No. of normal cells	No. of aberrant cells			Structural Aberrations								Numerical Aberrations
				Cells with one Ab	Cells with 71 Ab	total Ab. cells	Chromatide type				Chromatide type				
							G	B	D	F	G	B	R	CA	
Negative control	1	50	49	1	-	1	1	-	-	-	-	-	-	-	-
	2	50	49	1	-	1	1	-	-	-	-	-	-	-	-
	3	50	50	-	-	-	-	-	-	-	-	-	-	-	-
	4	50	47	3	-	3	3	-	-	-	-	-	-	-	-
	Total	200	195	5	-	5	5	-	-	-	-	-	-	-	-
Positive control EMS	1	50	28	14	8	22	13	1	3	2	10	2	-	1	1
	2	50	25	18	7	25	20	3	4	-	10	2	1	3	-
	3	50	31	9	10	19	11	3	1	3	8	-	2	1	2
	4	50	32	10	8	18	25	2	3	3	10	3	-	1	-
	Total	200	116	51	33	84	69	9	11	8	38	7	3	6	3
Phoxim 1/10 ld_{50}	1	50	42	8	-	8	4	1	1	1	1	1	-	-	-
	2	50	45	5	-	5	4	-	1	-	-	-	-	-	-
	3	50	41	6	3	9	7	4	-	2	3	-	-	1	-
	4	50	47	2	1	3	5	-	-	-	1	-	-	-	-
	Total	200	175	21	4	25	20	5	2	3	5	-	-	1	-
phoxim 1/5 LD_{50}	1	50	39	8	3	11	10	3	-	-	2	-	1	1	-
	2	50	41	9	-	9	4	1	1	-	1	1	-	-	-
	3	50	38	8	4	12	8	4	1	1	4	-	1	2	-
	4	50	40	8	2	10	15	2	-	1	1	-	-	-	-
	Total	200	158	33	9	42	37	10	2	2	8	1	2	3	-

Table 2 : Effect of phoxim on the induction of chromosomal aberrations in rat bone marrow cells.

Treatment	Total No. of exam. cells	No. of normal cells	No. of aberrant celles		Structural Aberrations								Numerical Aberrations
					Chromatide type				Chromatide type				
			No.	%	G	B	D	F	G	B	R	CA	
Control	200	195	5	2.5	5 2.5%	-	-	-	-	-	-	-	-
1/10 LD_{50}	200	160	25	12.5	20 10%	5 2.5%	2 1%	3 1.5%	5 2.5%	-	-	1 0.5%	-
1/5 LD_{50}	200	130	42	21.0	37 18%	10 5%	2 1%	2 1%	8 4%	1 0.5%	2 1%	3 1.5%	-

G = Gaps B = Breaks D = Deletions
F = Fragments R = Ring CA = Centromeric attenuation

Table 3 : Effect of phoxim on the frequency of micronucleated polychromatic and normochromatic cells in rat bone marow.

Treatment	Rat Ident. No	Total Exam. cells	Total M.N cells	Micronucleated PCE						Structural Aberrations						
				Big			Small			Total Exam. Cells	Total M. N cells	Big			Small		
				1	2	>2	1	2	>2			1	2	>2	1	2	>2
Negative control	1	500	4	1	-	-	3	-	-	500	1	1	-	-	-	-	-
	2	500	1	-	-	-	1	-	-	500	3	1	-	-	2	-	-
	3	500	4	2	-	-	2	-	-	500	5	2	-	-	3	-	-
	4	500	2	-	-	-	2	-	-	500	2	1	-	-	1	-	-
	Total	2000	11	3	-	-	8	-	-	2000	11	5	-	-	6	-	-
Positive control EMS	1	500	56	6	3	1	32	11	2	500	55	25	-	-	26	4	-
	2	500	63	5	1	3	48	5	1	500	43	21	4	-	15	2	1
	3	500	52	5	3	2	39	3	-	500	51	19	-	-	28	3	1
	4	500	74	8	4	-	52	9	1	500	62	29	1	-	30	2	-
	Total	2000	245	24	11	6	172	28	4	2000	211	94	5	-	99	11	2
Phoxim 1/10 ld_{50}	1	500	10	4	-	-	-	-	-	500	7	-	-	-	5	1	-
	2	500	6	1	1	-	6	-	-	500	5	1	1	-	3	1	-
	3	500	10	3	-	-	4	1	-	500	9	1	-	-	8	-	-
	4	500	12	3	1	-	6	1	-	500	7	2	-	-	5	-	-
	Total	2000	38	11	2	-	16	2	-	2000	28	4	1	-	21	2	-
phoxim 1/5 LD_{50}	1	500	8	-	-	-	8	-	-	500	7	-	1	-	7	-	-
	2	500	12	2	-	-	9	1	-	500	6	-	-	-	6	-	-
	3	500	14	2	-	-	10	2	-	500	12	1	-	-	9	2	-
	4	500	10	2	-	-	7	1	-	500	10	-	-	-	9	1	-
	Total	2000	44	6	-	-	34	4	-	2000	35	1	1	-	31	3	-

M. N. = Micronucleated P. C. E = Polychromatic erythrocytes N.C.E = Normochromatic erythrocytes

Table 4 : Effect of phoxim on the frequency of micronucleated polychromatic and normochromatic cells in rat bone marrow

Treatment	Total exam. cells	Total M. N.P.C.E.		Micronucleated P. C. E.		Total exam. cells	Total M. N.P.C.E.		Micronucleated P. C. E	
		No.	%	Big	Small		No.	%	Big	Small
Control	2000	11	0.55%	3	8	2000	11	0.55%	5	6
1/10 LD_{50}	2000	38	1.9%	13	25	2000	28	1.4%	5	26
1/5 LD_{50}	2000	44	2.2%	6	38	2000	35	1.75%	1	34

M. N. P. C. E. : Micronucleated polychromatic erythrocytes.
M. N. N. C. E. = Micronucleated normochromatic erythrocytes.

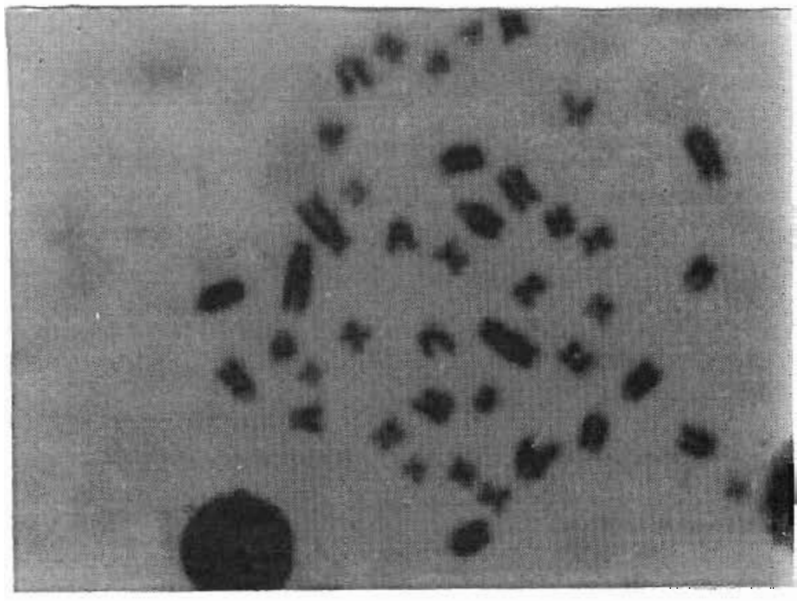

Fig. 1 : Rat metaphase spread showing chromatid gap induced by phoxim.

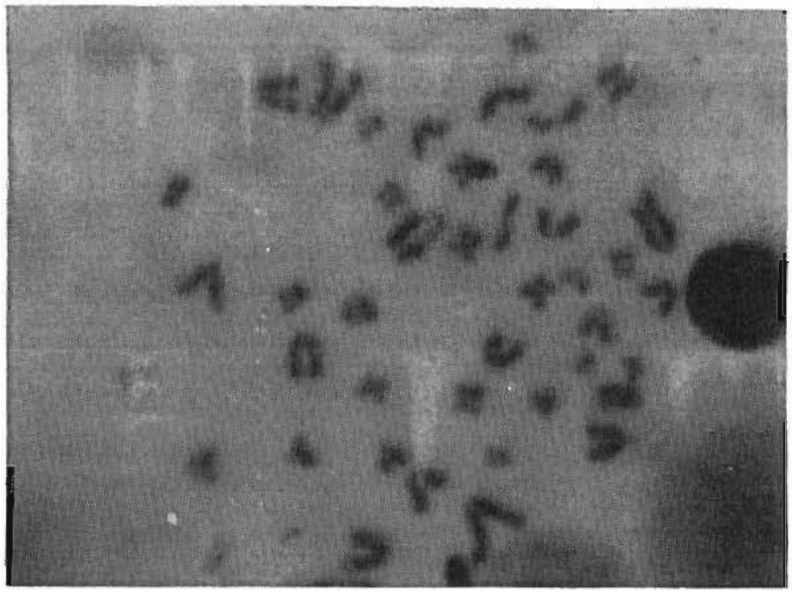

Fig. 2 : Rat metaphase spread delation ang fregment induced by phoxim.

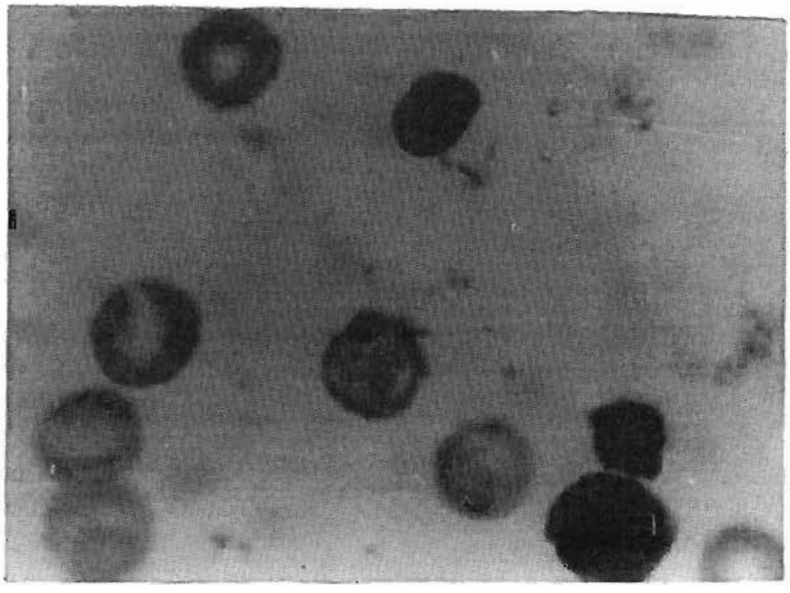

Fig. 3 : Rat anaphase spread showing micronucle in erythrocytes induced by phoxim.

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الملخص العربى

التأثيرات الطفرية للفوكسيم (مبيد حشرى فسفورى عضوى حديث) على خلايا نخاع العظام للفئران

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أجريت هذه التجربة على عدد ٣٢ فأر من الذكور الغير بالغة والتى يتراوح وزنها بين ٧٠-٨٠جم لدراسة التأثير الطفرى لمركب الفوكسيم على خلايا نخاع العظام عند معالجة الفئران بجرعة واحدة (عشر، خمسة الجرعة النصف مميتة) عن طريق الفم.

هذا وقد أظهرت النتائج مايلى :-

١- أن الفوكسيم أحدث تأثيراً طفرياً معنوياً حيث إستدل على ذلك من زيادة نسبة عدد الخلايا المحتوية على شذوذات كروموسومية، وكانت التغيرات الكروماتيدية هى السائدة، ونسبة الخلايا المحتوية على شذوذ كروموسومى واحد أكثر من الخلايا المحتوية على أكثر من شذوذ.

٢- زيادة نسبة خلايا الدم الحمراء المتعددة الصبغة والمحتوية على نويات.