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Mutagenic Properties of Pirimiphos-methyl in Male Mice

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The potential genetic effects of several organophosphorus pesticides have been extensively investigated and considerable information is available regarding the potential risk of this group of pesticides for the human genome (Wild, 1975). However, only a few published data are available regarding the mutagenic properties of Pirimiphos-methyl, a phosphorothioate insecticide, which is gaining widespread acceptance as a substitute for Malathion (WHO/FAO 1975). It has been clearly demonstrated to be mutagenically positive in different strains of *Salmonella typhimurium* with or without activation system (Seiler 1973; Hanna and Dyer 1975; Moriya et al. 1983). However, studies are wanting to demonstrate its mutagenic effect in *in vivo* mammalian systems.

Hence the objective of our study was to assess the cytogenetic effect of Pirimiphos-methyl in the bone marrow cells and spermatogonia of male mice, by employing the micronucleus and sperm morphology assays respectively.

MATERIALS AND METHODS.

Technical grade Pirimiphos-methyl (90.5% pure) obtained from The Alkali and Chemical Corporation of India Ltd., Bangalore, India, was suitably diluted with coconut oil (commercial) and used. Male mice (Swiss, inbred CFT strain), 8-10 weeks old were orally administered Pirimiphos-methyl at the dosages of 100, 200 and 400 mg/kg body weight. The animals marked for 'Micronucleus test' received the doses in two equal instalments, separated by an interval of 24 h. Six hours after the second treatment, mice from each group were killed by cervical dislocation and bone marrow preparations were made and stained in May Grunwald-Giemsa stain according to the procedure of Schmid (1975).

The mice marked for 'sperm morphology assay' received the doses in five equal instalments for five consecutive days. The animals were killed at 1,3,5 and 7 weeks following the end of the treatment. The testes were excised and fixed in Bouin's fluid and processed for histological examination. Sperm morphology assay was performed according to the method of Wyrobeck and Bruce (1978).

RESULTS AND DISCUSSION

Data on the incidence of micronuclei in polychromatic and normochromatic erythrocytes are presented in Table 1. The mean incidence of micronuclei in polychromatic and normochromatic erythrocytes of control mice were 0.36 and 0.10% respectively. There were no significant alterations in the incidence of micronuclei in either class of erythrocytes at any of the dosages tested. Further, the ratio of polychromatic and normochromatic erythrocytes (P/N ratio) with or without micronuclei was not affected at any of the dosages except at the highest dosage, where there was a slight reduction in P/N ratio.

Sperms observed at 1,3,5 and 7 weeks were presumably corresponding to the treatment of mature sperms, spermatids, spermatocytes and spermatogonia respectively. The percent of abnormal sperms at the various dosages and intervals are presented in Table 2. The results did not reveal any evidence of

Table 2. Percent of abnormal sperms in mice administered Pirimiphos-methyl

Group	Dosage* (mg/kg b.w.)	% abnormal sperms**			
		Week			
		1	3	5	7
A	Control	1.2±0.7	1.5±0.6	2.1±0.4	2.4±0.3
B	100	1.6±0.8	2.5±0.9	3.1±2.3	3.2±1.2
C	200	2.3±0.5	4.2±0.9	5.2±0.4	4.1±0.7
D	400	2.4±0.9	4.4±1.0	7.3±0.8	5.2±0.6

* Administered in five equal instalments.
** Values are Mean ± SE of 4 animals each.

treatment-related effect on the sperm morphology. There was only a slight increase in the frequency of abnormal sperms at 5 and 7 weeks following administration of the insecticide at the higher two dosages. The main types of abnormalities encountered were the 'nubbed hook', 'hammer headed' 'mishappen' and 'macrocephalic'.

Microscopic examination of the testis revealed atrophy and shrunken seminiferous tubules with reduced germinal epithelium. (Fig. 1). Mild to moderate spermatogenetic arrest was seen in most tubules. These changes were evident at all 3 dosages especially at 5 and 7 weeks after treatment.

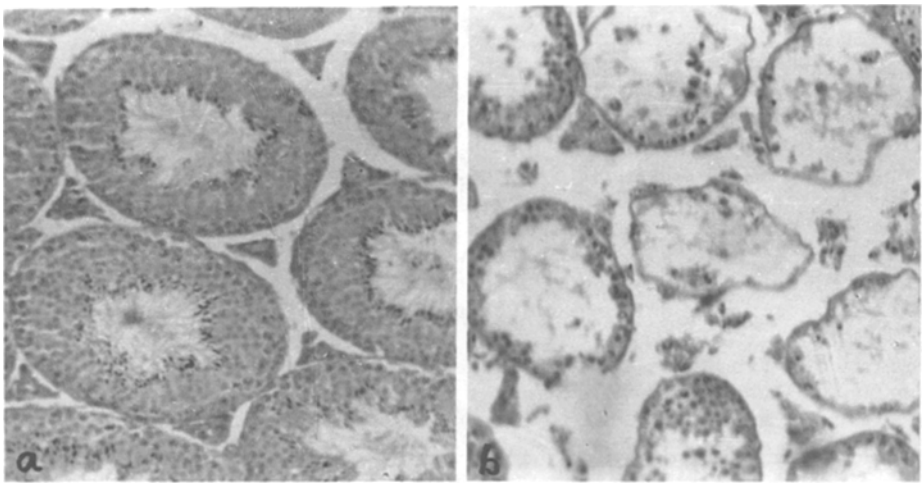

Fig. 1. Histo-architecture of testis of mice *a* control, showing normal histology; *b* Pirimiphos-methyl administered (400 mg/kg b.w.) showing atrophied and shrunken seminiferous tubules and spermatogenetic arrest H&E, x35

The results of the present study do not reveal any significant cytogenetic effect in bone marrow cells and spermatogonia. The results also parallel the lack of genetic effect in germ cells as demonstrated by Mc Gregor (cited in JMPR 1976) in dominant lethal test in mice. However, the observed histological alterations in the testis are indicative of the fact that germline exposure to the insecticide has taken place *in vivo*. Such negative mutagenic activity in dominant lethal test, accompanied by histological alterations in testis (viz. reduction of germinal epithelium) of mice has been reported for dichlorvos (Dean and Thorpe 1972; Krause and Homola 1972). Recently Degraeve et al. (1984) have reported negative result in dominant lethal and micronucleus tests in mice for Bromophos, though it has been shown to impair spermatogenesis in certain laboratory animals (Kinkel et al. cited in Derache 1977). The slight decrease in the P/N ratio observed presently is in accordance with the decreased bone marrow proliferation reported earlier (Rajini 1984) in rats administered repeated oral doses of Pirimiphos-methyl.

From the present study it is evident that Pirimiphos-methyl does not induce any cytogenetic alterations in mice. However, at the tested concentrations, it seems to exert some effect on germinal epithelium and bone marrow proliferation in mice which needs further investigation.

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Table 1. Incidence of micronuclei (MN) in bone marrow-erythrocytes of mice orally administered Pirimiphos-methyl

Group	Dosage* (mg/kg b.w.)	Polychromatic (P) cells with MN** (%)	Normochromatic (N) cells with MN** (%)	Poly-Normochromatic cells with MN** (%)	Polychromatic cells** (%)	P/N ratio**
A	Control	0.36 (63/17492)	0.10 (20/19868)	0.46 (83/37360)	44.5	0.88
B	100	0.33 (56/17120)	0.11 (20/18408)	0.44 (76/35528)	47.1	0.93
C	200	0.38 (53/14064)	0.12 (17/14650)	0.50 (70/28714)	43.4	0.96
D	400	0.26 (20/7727)	0.16 (18/11198)	0.42 (38/18925)	39.0	0.69

* Administered in two equal instalments.
** Values represent the mean in each group.
Values in parentheses denote number of total micronuclei in cells scored.

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