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Mutagenicity of Monocrotophos in Mice

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The chemical induction of mutations has alarmed the general population since early ninteen forties. The concern over potential mutagenicity of organophosphates increased when trimethyl phosphate was found to be mutagenic in mice (Epstein et al. 1970). Chromosome damage in humans due to organophosphorous insecticides through accidental and occupational exposure has been reported by Yoder et al. (1973) and Trin Van bo (1974). Subsequently marked increase in morphologically abnormal sperms had been demonstrated following the exposure of mice to mutagenic agents like dichlorvos and trimethyl phosphate (Wyrobeck and Bruce 1978) and dimethoate (Rani and Reddy 1985). Monocrotophos a commonly used agricultural pesticide is reported (Janardhan et al. 1983) to have teratogenic potential in rats. Recent studies in our laboratory revealed the mutagenic potential of monocrotophos that induced micronuclei in mice (Vijaya Kumar & Janardhan 1987). Since sperm abnormalities are a measure of genetic damage to spermatogenic cells (Bruce et al 1974) sperm abnormality assay was employed to further confirm or otherwise the mutagenic potential of monocrotophos in mice.

MATERIALS AND METHODS

Monocrotophos (3-hydroxyl-N-methyl-cis-crotonamide dimethyl phosphate) technical grade (98%) has been supplied by M/s. National Organic Chemical Industries Ltd., Bombay.

Swiss Albino mice weighing between 23 - 25g procured from M/s. Biological E.Ltd., Hyderabad were housed at 25°C with access to pelleted feed supplied by M/s. Hindustan Lever Ltd. and tap water ad libitum. The light period was from 6.00 AM to 6.00 PM.

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Monocrotophos was administered at the dose rate of 0 (control), 0.9, 1.8 and 3.6mg/kg body weight (i.e. 0, 1/20, 1/10 and 1/5 of LD 50). The chemical miscible in water was administered orally by intragastric intubation. The concentration was adjusted to have maximum ingestion volume of 0.2ml. The mice in control groups received appropriate quantities of distilled water.

Forty male mice were randomised into four groups of ten mice each. The mice were administered with 1/5th of the corresponding dose daily for five days (Wyrobek and Bruce 1978) the lowest dose being maximum tolerated dose. All the animals were sacrificed by cervical dislocation at 35th day following the first dose. The reproductive tract was exposed, testis was disected out and fixed in Bouins fluid. The cauda epididymis was disected out and placed in a petri dish containing phosphate buffered physiological saline. It was minced with scissors and pipetted up and down and then transferred into a test tube in which the volume was made upto 2ml and the debris was permitted to settle over a few minutes. Each cell suspension was then mixed with 0.1% aqueous Eosin Y (10:1). Thirty minutes later smears were made, allowed to dry in air and mounted under a cover slip. For each suspension 1000 sperms were examined at 400 X with blue green filter. Abnormal sperms that were readily recognisable were counted.

Differences between experimental and control values were analysed for statistical significance by applying "Student's 't' test" (Snedecor and Cochran 1967).

One of the testes from each mouse was fixed in Bouin's fluid. Paraffin-wax sections were stained with conventional haemotoxylin and eosin and examined under microscope for histopathological changes.

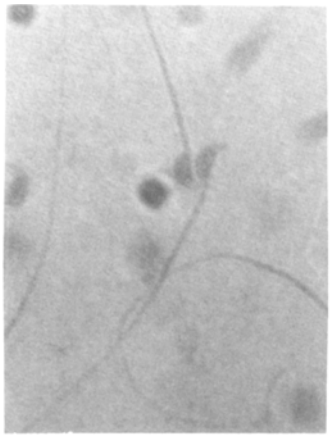
RESULTS AND DISCUSSION

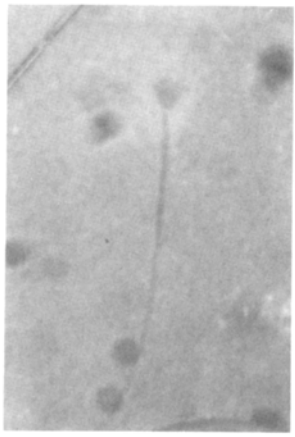
Sperm morphology test is a relatively new in vivo assay for the effects of chemicals on the germ cells of male mice. It is seen from Table-1 that the percentage of abnormal sperms increased from 2.09 in control group to 2.12, 3.20 and 5.36 in groups that received monocrotophos. Significant increase ($P<0.01$) in abnormal sperms occurred at the dose range of 1.8 and 3.6 mg/kg body weight. The abornal types of sperms (Figure-1) were scored. Abnormalities

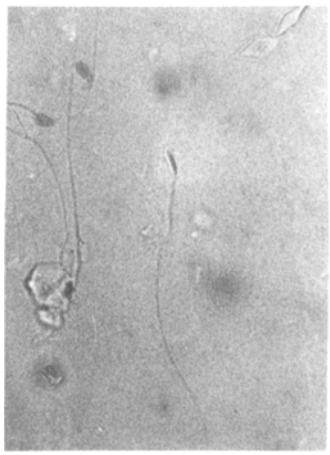
Table-1: Sperm abnormalities in mice treated with monocrotophos

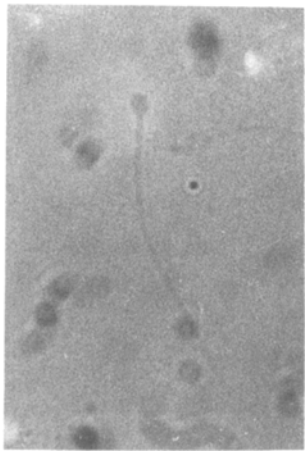
Group (dose mg/kg)	No.of mice	Total No. of sperms scored	Normal sperms				Abnormal sperms			
			No.	±	S.E.	%	No.	±	S.E.	%
0	10	10,000	9,791	±	1.04	97.91	209	±	1.03	2.09
0.9	10	10,000	9,788	±	2.22	97.88	212	±	2.22	2.12
1.8	9	9,000	8,680	±	4.66	96.44	320	±	4.67	3.56*
3.6	10	10,000	9,464	±	4.47	94.64	536	±	4.47	5.36*

* $P < 0.01$









Banana Shaped Head Lack the Usual Hook

Fig.1. Various types of sperms recorded in mice after administration of monocrotophos.

in sperms morphology are a consequence of chromosomal aberrations (Bruce et al. 1974). The increase in the abnormality may be due to the damage to the genes responsible for spermatogenesis. Since organophosphorus insecticides are chemical alkylating agents (Wild 1975), alkylation of DNA bases either directly or indirectly via protein alkylation is probably involved in the DNA disintegration (Mohan 1973, Green et al. 1974). Thus it is probable that monocrotophos could cause alterations in testicular DNA resulting in disruption of the process of differentiation of sptermatozoa.

Microscopic examination of sections from testes from control and treated groups showed well developed seminiferous tubules with all stages of spermatogenesis indicating that the drug in the doses studied did not bring about any histological changes. The absence of any histological changes in testicular tissue might probably be due to insufficient period of exposure to the chemical.

From the present study it is concluded that monocrotophos could bring about induction of sperm abnormalities in mouse testis by interfering either with the integrity of the DNA itself (Lawley et al. 1974 Wennerberg and Lafroth 1974) or with the expression of this genetic material (Wyrobek and Bruce 1975). However, mechanics of this aspect need further investigation. The results of this investigation in conjunction with those of micronucleus test (Vijaya Kumar and Janardhan, 1987) may help in guarding the genetic hazard to human and animal population through judicious and careful use of monocrotophos in agriculture and animal husbandry.

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