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CYTOGENETIC EFFECTS OF MALATHION ON BUFFALO BLOOD CULTURES

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THE problem of body pests and external parasites in buffalo and other livestock is acute in India and several types of organophosphorus and organochlorine pesticides are used. Direct applications of these compounds on animals and their dwellings, lead to their entry into the body. Malathion and other related compounds replace in part the chlorinated hydrocarbons in use, as these are degraded relatively quickly. They do, however, have acute toxic effects that may be fatal.

Pesticides, besides their toxic effects, have also been assumed to cause mutagenic and carcinogenic effects. Petropolis and Kamra¹ observed a dose-dependent increase in mitotic and DNA labelling

indices in peripheral lymphocytes exposed to organophosphorus pesticides and chromosomal breaks were found even at $10 \mu g/ml$ dose. Several types of structural and numerical chromosomal anomalies were observed in peripheral blood cultures treated with malathion and other related organophosphates²⁻⁴. The cytogenetic effects of malathion on peripheral blood cultures of Murrah buffalo have been described in this paper.

Commercial grade malathion 50% (O, Odimethyl phosphodithioate of diethyl mercaptic succinate) was obtained from the Pesticide India Ltd and dissolved in Hank's basal salt solution to get concentrations of 125, 250, 375 and 500 μ g/ml. Culture medium TC 199 (Bios Bombay, India) was reconstituted and supplemented with 1-Glutamine, Phytohemagglutinin (PHA Sigma, USA), sodium benzyl penicillin, streptomycin and sterile cattle serum. The reconstituted medium was filtered through millipore filters (0.2 μ) into sterile culture vials (5 ml in each). Forty culture vials were divided into five groups and 0.5 ml whole blood was added to each culture. They were incubated at 37°C for 72 h and 0.1 ml of malathion solution was added at the above mentioned concentrations to each vial of treatment groups I, II, III and IV respectively, 24 h before harvesting the cultures. Group V cultures (negative control) were not treated with pesticide.

The chromosomal preparations were made using colchicine (250 μ g/ml), hypotonic (0.075 M KCl), acetic:methanol (1:3) fixation and air drying protocol⁵. The slides were stained in 2% Giemsa (phosphate buffer, pH 6.8) for 30 min and mounted in DPX.

Under the influence of alkylation of chemicals including the pesticides, both normally dividing (bone marrow) and neoplastic (cell cycle induced tissues) tissues, suffer a reduction in mitotic activity of cells probably due to delay in the synthetic

Table 1 Mitotic index of peripheral blood lymphocytes treated with malathion

Dose level (μg/ml)	Cells scored	Blast cells (%)	Cells at metaphase (%)	Mitotic index
12.5	2250	16.98	0.98	17.96
25.0	2392	16 47	0.88	17.35
37.5	2169	16.50	0.80	17.30
50 0	2039	15/60	0.70	16.30*
Control	2192	17.29	1.00	18.09

^{*}Significant (P < 0.05).

Dose level (μg/ml)	C 11 milele	Types of chromosomal abnormalities						
	Cells with anomalies (%)	Gaps	breaks	Acentric fragments	Dicentric chromosomes	Exchanges	Pulveri- zation	
12.5	4.50		·	i	5	2	1	
25.0	7.50*	3	2	2	5	1	2	
37.5	19.50*	8	4	5	12	4	6	
50.0	22.00*	10	5	7	15	3	4	
Control	4.00	2	2	1	2	1	_	

Table 2 Frequencies of chromosomal aberrations in malathion treated blood lymphocytes

activity of cell cycle⁶. The mitotic activity of peripheral blood lymphocytes decreased with increase in the dose of malathion. However, only $50 \mu g/ml$ dose was affected significantly (table 1). Ahmed et al⁷ reported that malathion and other organophosphorus pesticides induced unscheduled DNA synthesis. The mitotic activity of lymphocytes suffered mild reduction due to malathion as compared to other organophosphates reported earlier⁵.

The incidence of chromosome aberrations in the lymphocytes increased with corresponding increase in the dose of malathion. They were significant in groups II, III and IV cultures. Various types of structural abnormalities were seen. Gaps, acentric fragments and dicentric chromosomes were more frequent (table 2). This indicated that malathion caused breakage in the DNA backbone of buffalo chromosomes at the centromeric region and caused gaps and acentric fragments. Translocation of broken chromosomal fragments with centromere increased the frequencies of dicentric chromosomes. It is interesting to recall in this context that several types of structural and numerical chromosomal aberrations have been reported to be induced in human and hamster blood lymphocytes by

malathion and other related organophosphates. The authors thank the Director, National Dairy Research Institute, Karnal for facilities.

15 May 1986; Revised 3 July 1987

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