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In Vitro Mutagenicity of The Fungicide Ziram

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ABSTRACT The fungicide ziram (zinc dimethly dithiocarbamate) was evaluated for its *in vitro* mutagenicity on human leucocytes. We employed six doses of ziram (0.001, 0.01, 0.1, 1.0, 5.0 and 10.0 µg per ml) to study its effect on the mitotic index, chromosomal aberrations, satellite associations and micronuclei. Chromosomal aberrations, satelite associations and micronuclei were found to be high at dose 0.1 µg; 1.0 µg and above resulted in 100% cell death.

INTRODUCTION

The use of pesticides has become inevitable in modern agriculture and has led to measurable pollution of our environment. The pesticides (insecticides, fungicides and herbicides) are toxic to animals, which may cause alterations of genetic material and therefore represent risk of cancer (IARC, 1974-76). They could also be a source of hazard to humans, being present in small amounts in food stuffs (Berg, 1975) and particularaly to farmers and pesticide manufacturers who handle them directly. The dithiocarbamate fungicide ziram (zinc dimethyl dithiocarbamate) is used widely on seeds and vegetables, and as an industrial fungicide in paper, plastic rubber and textile industries to increase the rate of vulcanization. The annual consumption of ziram in India is 450 million tons (Pesticide Information, 1987).

Ziram is available as a white crystalline powder. The fungicide has been already reported to be a potent mutagen *in vitro* by Pilinskaya (1971), Elizarova and Nuzhdina (1971) and Kurinniy and Pilinskaya (1976). We thought of studying the mutagenicity of the fungicide applying four different parameters. The objective of this work is to find out whether it causes any occupational hazards, and residue risks.

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MATERIALS AND METHODS

Ziram (containing 97.6% of the active principle CS2) was dissolved in 5% DMSO to obtain 10.0, 5.0, 1.0, 0.1, 0.01, and 0.001 µg/ml; the chemical was prepared fresh prior to use.

Peripheral blood cultures were set contaning 0.5 ml of heparinised blood sample, with 4 ml of Eagles minimum essential medium (M), 1 ml of serum, 0.2 ml of phytohemagglutinin and 0.1 ml of ziram following the method of Moorhead et al. (1960) for chromosomal preparation. The cultures were incubated at 37°C and harvested at the end of 48 h.

Six doses of the fungicide were tested, and for each dose, three repeat experiments were set. For each treatment a control was also set with 5% DMSO. All the cultures were done in sterile conditions under UV hood.

To each culture vial 0.1 ml of 0.01% colchicine was added and after 45 minutes they were centrifuged for 5 minutes at 800 rpm. Cells were suspended in 6 ml of 0.75 M KCl and incubated at 37°C for 5 minutes and centrifuged. Cells were fixed in (3:1) methanol acetic acid. Chromosome preparations were made by the flame drying method and stained with 4% Giemsa.

The following parameters were recorded.

1. *Mitotic Index (MI)* : more than 5,000 cells were scored for each repetition.

$$MI = \frac{\text{No. of dividing cells}}{\text{Total no. of cells scored}} \times 100$$

2. *Chromosomal Aberrations* : 100 well spread metaphases were scored for each repeat experiment.

3. *Satellite Association (SA)* : From the same

slide SA were scored. The SA involve a specific arrangement of acrocentric chromosomes (D.G.) with their satellites directed towards each other.

4. The Micronuclei : Induced by ziram was analysed from the chhromosomal preparation employed for chromosome aberration analysis. 1200 cells in interphase were analysed from each cullture.

The statistical significance of the results was got using the standard statistical procedure for comparison of sample proportions and means as given by Zar (1974).

RESULTS AND DISCUSSION

Regarding the mitotic index induced by ziram the data is presented in table 1. The data shows a slight increase in mitotic index at 0.1 µg dose when compared to 0.001, 0.01 µg dose.

Table 1 : Mitotic index observed in human leucocytes in culture when treated with ziram

Treatment	*Dose*	*Total no. of cells scored* (n_1)	*Dividing cells* (n_2)	*Mitotic index* $n_2/n_1 \times 100$	*S.E.* ±
DMSO	5%	12,000	820	6.83	0.153
Ziram	0.001 µg	25,000	1,130	4.52	0.131
Ziram	0.01 µg	20,000	928	4.64	0.148
Ziram	0.1 µg	22,000	1,380	6.27	0.163
Ziram	1.0 µg	-	cell death	-	-
Ziram	5.0 µg	-	cell death	-	-
Ziram	10.0 µg	-	cell death	-	-

The fungicide ziram did not inhibit or delay cell division. It slightly accelerated cell division at 0.1 µg/ml dose, and a few cells advanced into third mitotic division (M III). Newton and Lilly (1979) reported ziram causing leukaemia and increase in chromosomal aberrations.

Ziram in all the investigated doses, induced a number of aberrations, when compared with control. Data on the type and frequency of ab-

Table 2: Frequency of chromosomal aberrations observed in human leucocytes in culture following treatment with ziram

Treatment	*Metaphase scored*	*Number of methaphase with aberrations*		*Type of aberrations*					*Total abberations*		*Aberrations per cell*	*S.E.* ±
				G	*B*	*F*	*Dicentrics*	*Exchanges*				
		n	%						*n*	%		
5% DMSO	300	6	2.00	4	2	2	-	-	8	2.66	0.0266	1.04
Ziram												
0.001 µg	300	15	5.33	4	10	4	-	-	18	6.00	0.06	1.6
0.01 µg	300	40	13.33	4	20	20	2	-	46	15.33	0.15	2.32
0.1 µg	400	85	21.25	13	40	44	-	1	98	32.66	0.245	2.62
1.0 µg	-	Cell death	-	-	-	Cell death	-	-	-	-	-	-
5.0 µg	-	Cell death	-	-	-	Cell death	-	-	-	-	-	-
10.0 µg	-	Cell death	-	-	-	Cell death	-	-	-	-	-	-

G-Gaps B-Breaks F-Fragments

Table 3: Frequency of satellite association pattern observed in human leucocytes in culture when treated with ziram

Treatment	*Total cells scored*	*Types of satellite associations*							*Total number of associations*	*Association per cell*	*S.E* ±
		D-D	*D-G*	*G-G*	*2D-G*	*2G-D*	*2G-2D*	*3D*			
5% DMSO	300	-	-	-	-	-	-	-	-	-	-
Ziram											
0.001 µg	300	-	-	2	4	-	4	-	10	3.33	0.74
0.01 µg	300	4	4	3	3	-	4	4	22	7.33	1.08
0.1 µg	300	4	4	8	6	4	8	12	46	11.50	1.53

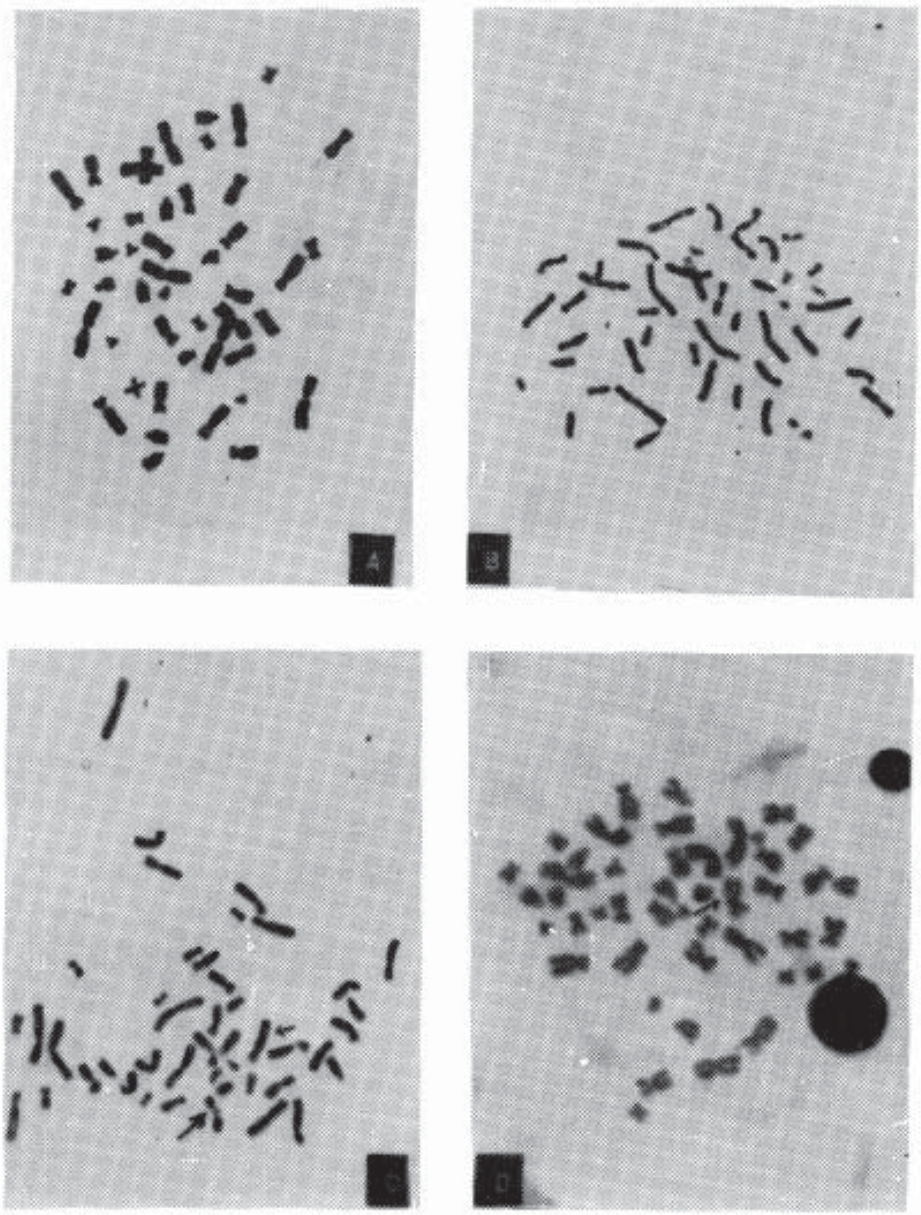

Fig. 1. Chromosme aberrations in human leucocytes in culture treatment with ziram
A) Normal - Diploid number of chromosomes (46)
B) A metaphase with arrow showing chromatid gap
C) A metphase showing chromatid gap(upper arrow), and chromatid break (lower arrow)
D) A metphase with arrow showing chromatid break

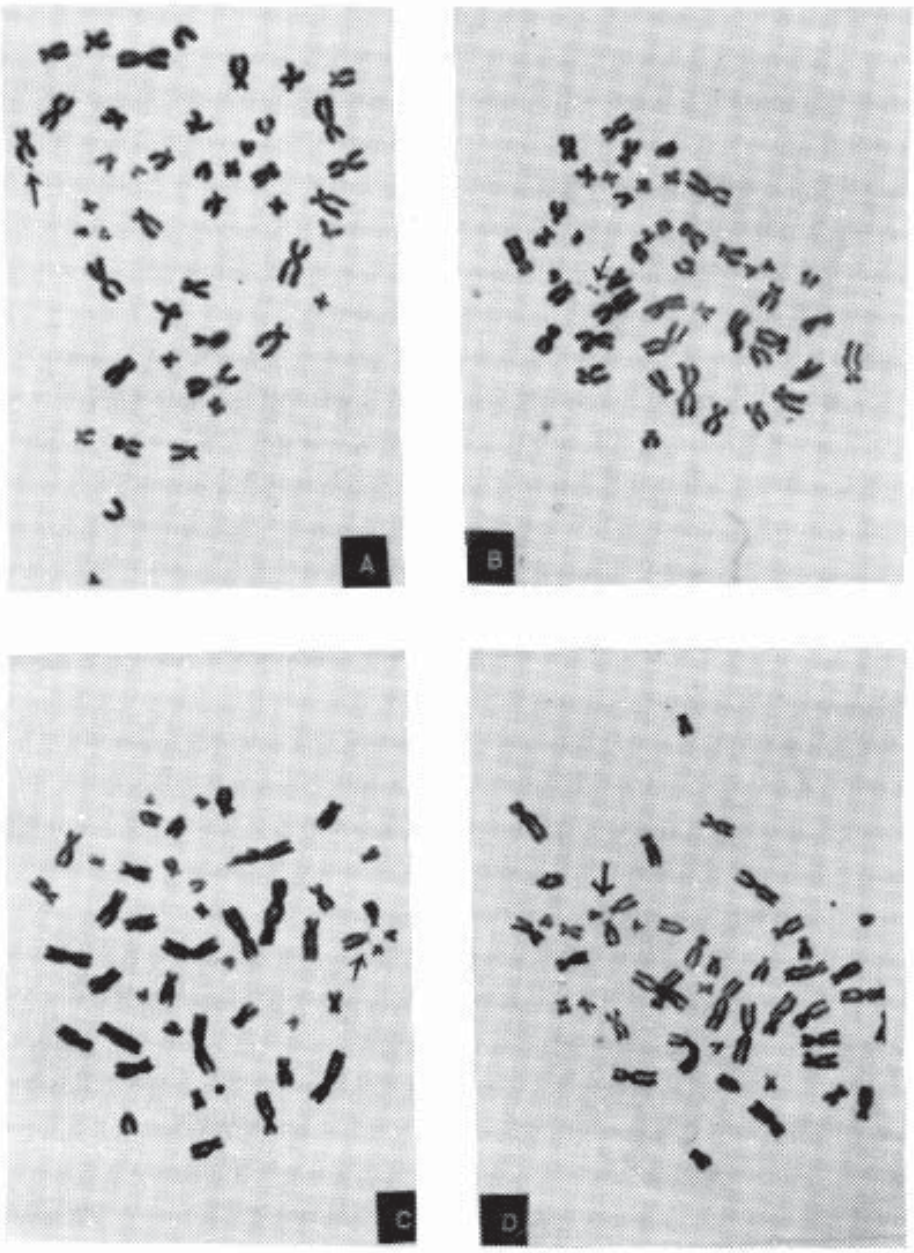

Fig. 2. Chromosome aberrations in human leucocytes in culture on treatment with ziram
A) A metaphase showing (top arrow) a minute (a very small fragment), (middle arrow) big fragment and (bottom arrow) satellite association
B) A metaphase showing double minute (left arrow) and satellite association (right arrow)
C) A metaphase showing fragment (bottom arrow), and satellite association (top arrow)
D) A metaphase showing chromatid gap (bottom arrow) and satellite association (top arrow)

errations is presented in table 2. At doses 1µg and above complete cell death occured.

Ziram induced both chromatid and chromosome type aberrations. Endoreduplication, dicentrics and aneuploidy were also recorded. A high frequency of satellite association was observed and was found to be dose related and statistically significant (Table 3). Among the chromatid aberrations single acentric fragments predominated. Breaks at centromere were also recorded forming two telocentric chromosomes. Among the chromosome type aberrations paired acentric fragments were recorded. A direct and strong correlation exists between the concentration of ziram and the frequency of aberrant metaphases. The aberrations are presented in figures 1 and 2.

The frequency of micronucleus induced by ziram is presented in table 4 which shows a significant increase at 0.1 µg dose when compared to control.

Table 4 : Frequency of micronuclei induced by ziram

Treatment	*Dose*	*Interphase cells with micronuclei per 1200*		
		n	%	*SE* ±
DMSO	5%	6	0.50	0.111
Ziram	0.001	6	0.50	0.111
Ziram	0.010	10	0.83	0.143
Ziram	0.100	30	2.50	0.246
Ziram	1.00	cell death	-	-
Ziram	5.00	cell death	-	-
Ziram	10.00	cell death	-	-

Chromosome type of aberrations arises in G_1 stage and chromatid type in S and G_2 stages (Pilinskaya, 1971). In our studies the entire cell cycle of first mitosis was observed in leucocytes in culture and in this case the chromatid type aberrations were predominant. So we can assume that S and G_2 periods of cell cycle are more sensitive to the action of ziram. Our results in this regard are in good agreement with Pilinskaya's (1971) results in human lymphocytes in culture.

The satellite association has been considered as a predisposing factor for non disjunction (Hansson, 1970). Ziram induced high frequency of satellite association at 0.1 µg dose. The increased structural aberrations in metaphases and micronuclei in interphase cells at the same concentration and sampling time may indicate the micronuclei result from chromatid and chromosome fragment and lagging chromosomes.

From the results of our present study it may be concluded that ziram is mutagenic *in vitro* and *in vivo* studies also have to be carried out to confirm the mutagenicity of ziram.

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