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# Effect of Ziram, Thiram, and Dithane M-45 on Bone Marrow Cells of Mice-Assessed by Micronucleus Test

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Micronucleus test is an extensively used protocol to assess the mutagenicity of environmental chemicals. This was developed by Schmid and his co-workers (Matter and Schmid, 1971; Ledebur and Schmid, 1973). The micronucleus test is simple, quick and as sensitive as the chromosome aberration analysis. It is based on the principle that during anaphase, acentric chromatid and chromosome fragments lag behind, where as centric elements move towards the spindle pole. After telophase both the undamaged chromosomes and the centric fragments give rise to the daughter nuclei. The lagging elements are transferred into one or several secondary nuclei, which are as a rule much smaller than the main nucleus, and therefore called micronucleus (Schmid, 1973). The clastogenic effect of various chemicals is measured by micronucleus test. Erythrocytes are two types, the younger ones are polychromatic erythrocytes (PCE), stain bluish and the older, the normo chromatic erythrocytes (NCE) which stain reddish. A few hours after the completion of last mitosis the erythroblasts expel their nucleus for unknown reasons and the micronucleus alone remains in the cytoplasm of the Polychromatic erythrocytes, and they are easily recognisable. Erythrocyte micronucleus represents the consequence of chromosomal aberrations induced during preceding mitotic division of erythrocytes (Matter and Grauwiler, 1974).

## MATERIALS AND METHODS

The following were the three different doses of the fungicides tested.

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| | |
|---|---|
| Ziram | - 25, 50 and 100 mg/kgbw |
| Thiram | - 250, 500 and 1000 mg/kgbw |
| Dithane M-45 | - 250, 500 and 1000 mg/kgbw |
| Test animal | - Swiss albinomice about 25 grams weight |
| Solvent used | - 5% DMSO (dimethyl sulfoxide) |

Four mice about 25 gms weight were exposed to the test chemical for 30 and 60 hours duration. Three different doses of the fungicide were made as fine suspension in 5% DMSO, fresh each time. The chemical was injected as a single dose once, Schmid (1975) had administered a second dose, 24 h after the first administration, the second dose was not administered in the present study. A solvent control with the vehicle alone was also run.

Micronucleus (MN) slides were prepared using slightly modified method of Schmid (1975). In this method instead of fetal calf serum of Schmids' method, 5% bovine albumin was used as suspending medium (Seetha Rama Rao et al 1983).

5% bovine albumin solution was made by dissolving 5 grams of bovine albumin powder (E.Merck) in phosphate buffered saline solution. This was done by adding the powder little by little to the solvent and mixing it thoroughly each time, so as to avoid clumping of protein. To this clear solution, two drops of 1% sodium azide were added as preservative.

Phosphate buffered saline was prepared by dissolving NaCl, 8g; Kcl, 0.2g; $KH_2PO_4$, 0.2g and $NaHPO_4$, 1.15g in one litre of deionised water. The pH was adjusted to 7.

After the treatment schedule, mice were killed by cervical dislocation. The femur bones were separated and cleaned well with gauze paper. The upper end of the femur bone was cut till a small opening was visible. About 0.5 ml of bovine albumin was taken into the syringe, the needle was inserted into the opening of the bone and the marrow was flushed out into a clean, dry centrifuge tube. This was repeated to ensure that the marrow was removed completely. The contents were aspirated well to get a homogeneous cell suspension. The contents were made upto 3 ml and centrifuged at 1000 rpm for 5 minutes. The supernatant was discarded and the cell button was disturbed by gentle tapping and mixed well in a minimum quantity of bovine albumin.

A drop of the cell suspension was placed over a clean dry slide and spread with another slide (spreader), holding it at 45 angle (Schmid, 1975). The slides were air dried and fixed in absolute methanol for 5 minutes.

The slides were stained for 20 minutes in 5% Giemsa solution in 0.01 M phosphate buffer (0.71 grams of $Na_2HPO_4$, 0.6 grams of $KH_2PO_4$ in one litre of distilled water). The pH was adjusted to 6.8. The slides were washed in running tap water, air dried and mounted with DPX as mountant (Schmid, 1975; Chaubey et al, 1975).

The slides were scored at 1000 x magnification under bright field in oil and at least 1000 polychromatic cells were enumerated for each animal.

Statistical analysis was done using Zar 1974 method.

Standard error is calculated using the formula

$$SE = \frac{PQ}{n_1} + \frac{PQ}{n_2}$$

where $$P = \frac{n_1 P_1 + n_2 P_2}{n_1 + n_2}$$ (Probability of happening)

$n$ = size of sample 1
$n_2$ = size of sample 2
$p_1$ = Probability of sample 1
$p_2$ = Probability of sample 2
$Q$ = 100 - P (Probability of not happening)

$$Z = \frac{P_1 - P_2}{SE}$$

**RESULTS AND DISCUSSION**

From the table it was inferred that ziram at higher doses significantly increased the incidence of micronuclei in polychromatic erythrocytes over and above that observed in control group. There was a significant increase in the frequency of micronuclei with increase of time. The increase was found to be 2 to 3 fold depending on the dose (Fig.I).

Ziram was thus found to be effective in inducing micronuclei at all the durations and doses tested.

Thiram from the table was found to be insignificant in inducing micronuclei at 250 mg/kgbw dose both at 30 h and 60 h treatment. With 500 amd 1000 mg dose the incidence of micronuclei was significant both at 30 h and 60 h treatment.

Dithane M-45 was not significant in inducing micronuclei at 250 and 500 mg doses both at 30 h and

**Table 1.** Frequencies of micronuclei observed in the bone marrow cells of mice treated with ziram, thiram and dithane M-45.

| Fungicide | Dose (mg/kg) | Micronuclei | | | | | |
|---|---|---|---|---|---|---|---|
| | | 30 hrs | | | 60 hrs | | |
| | | PCE cells with MN* | MN (%) | SE± | PCE cells with MN* | MN (%) | SE± |
| Control | | | | | | | |
| DMSO | 5% | 10 | 0.25 | 0.08 | 12 | 0.3 | 0.086 |
| Ziram | 25 | 11 | 0.28 | 0.08 | 33 | 0.83 | 0.143 |
| | 50 | 28 | 0.70 | 0.13 | 61 | 1.53 | 0.193 |
| | 100 | 40 | 1.00 | 0.16 | 72 | 1.80 | 0.210 |
| DMSO | 5% | 10 | 0.25 | 0.08 | 12 | 0.3 | 0.086 |
| Thiram | 250 | 13 | 0.33 | 0.09 | 15 | 0.38 | 0.096 |
| | 500 | 34 | 0.85 | 0.15 | 48 | 1.20 | 0.172 |
| | 1000 | 45 | 1.13 | 0.17 | 56 | 1.40 | 0.185 |
| DMSO | 5% | 10 | 0.25 | 0.08 | 10 | 0.25 | 0.078 |
| Dithane M-45 | 250 | 12 | 0.30 | 0.09 | 12 | 0.30 | 0.086 |
| | 500 | 16 | 0.40 | 0.10 | 14 | 0.35 | 0.093 |
| | 1000 | 22 | 0.55 | 0.12 | 23 | 0.58 | 0.119 |

* Data obtained from 4000 polychromatic erythrocytes (PCE); four mice (1000 PCE analysed from each)

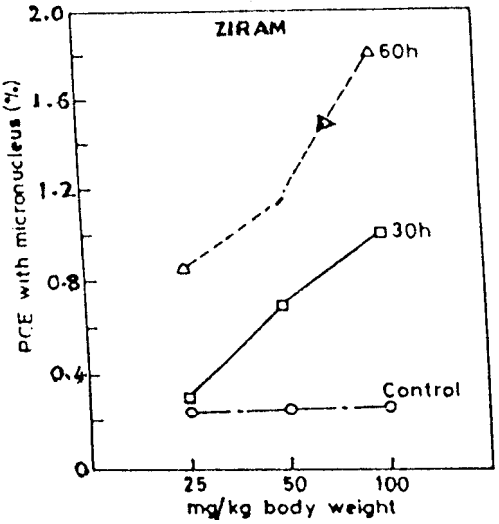
**Table 2.** Z Values for the comparison of two sample proportions for the data.

| Fungicide | Comparison between | 'Z' values | |
|---|---|---|---|
| | | 30 h | 60 h |
| Ziram | C X $D_1$ | 0.22 | 0.32 |
| | C X $D_2$ | 3.21** | 0.61 |
| | C X $D_3$ | 4.44*** | 6.80*** |
| | $D_1$X $D_2$ | 2.83** | 3.475*** |
| | $D_1$X $D_3$ | 4.26*** | 3.9*** |
| | $D_2$X $D_3$ | 1.25 | 1.01 |
| Thiram | C X $D_1$ | 0.626 | 0.601 |
| | C X $D_2$ | 3.8*** | 4.670*** |
| | C X $D_3$ | 4.755*** | 2.075* |
| | $D_1$X $D_2$ | 3.143** | 4.252*** |
| | $D_1$X $D_3$ | 4.30*** | 5.02*** |
| | $D_2$X $D_3$ | 1.25 | 0.769 |
| Dithane M-45 | C X $D_1$ | 0.43 | 0.43 |
| | C X $D_2$ | 1.18 | 0.82 |
| | C X $D_3$ | 2.13* | 2.16* |
| | $D_1$X $D_2$ | 0.79 | 0.125 |
| | $D_1$X $D_3$ | 1.77 | 1.87 |
| | $D_2$X $D_3$ | 1.04 | 1.60 |

* P < 0.05

** P < 0.01

*** P < 0.001



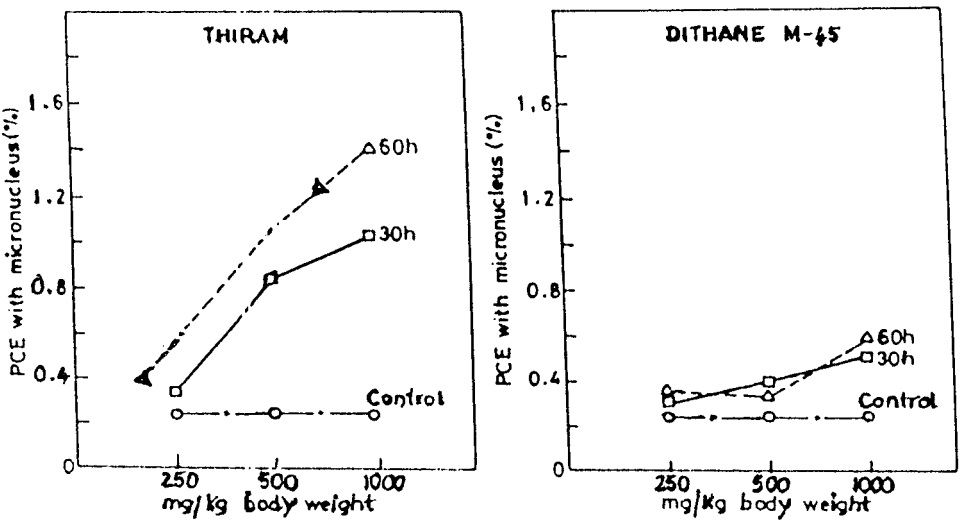




**Figure 1. Frequency of micronuclei (%) observed in PCE cells of mouse treated with various funqicides**

60 h treatment. Dithane M-45 significantly induced micronuclei only with 1000 mg/kgbw.

The frequency of micronuclei when treated with different doses of ziram, thiram and dithane M-45 showed a significant dose related response both at 30 h and 60 h. The incidence was still more at 60 h indicating the duration dependent response.

The frequency of micronuclei in 5% DMSO (solvent control) treated mice showed no significant increase, suggesting the failure of DMSO to induce any chromosomal damage in the bone marrow cells of mice. In the present study the PCE cells with micronuclei increased with dose and duration which may be related to the toxic action of the metabolic byproducts of the three carbamate fungicides tested.

**REFERENCES**


Chaubey R C Chauhan P S and Sundaram K (1975) Proceedings of the symposium on mutagenicity, carcinogenicity and teratogenicity of chemicals (Dept. of Atomic Energy, Govt. of India) p.75.

Matter B E and Schmid W (1971) Trenimon induced chromosomal damage in bone marrow cells of six mammalian species. Evaluated by micronucleus test. Mutat Res 12: 417.

Matter B E and Grauwiler J (1974) Micronuclei in mouse bone marrow cells, a simple in vivo model for the evaluation of drug induced chromosomal aberrations. Mutat Res 23: 239.

Schmid W (1973) Chemical mutagen testing on in vivo somatic mammalian cells. Agents and action 3: 77.

Schmid W (1975) The micronucleus test, Mutat Res 31: 9.

Seetharama Rao K P Abdul Rahiman M and Koranna S P (1983) Bovine albumin as a substitute of foetal calf serum in the micronucleus, Presatellite meeting, XV Congress of GENETICS, Madras-28.

Zar J H (1974) Biostatistical analysis, Prentice Hall Inc.