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## Evaluation of Thimet 10-G for mutagenicity by 4 different genetic systems

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### Summary

The insecticide Thimet 10-G was tested for mutagenic activity by 4 different genetic systems. It was unable to induce gene mutation in *Salmonella*, transfection inhibition in *Mycobacterium*, micronuclei formation in mice, and sister-chromatid exchange (SCE) in human lymphocytes were evaluated. It caused in mice an increase in the ratio of normochromatic to polychromatic erythrocytes and in human lymphocytes a decrease in mitotic index and delay in cell cycle. The results indicate that the insecticide is not mutagenic in the 4 test systems used at present.

Thimet 10-G is a systemic and contact insecticide and acaricide used in most parts of India to protect primarily brassicas, coffee, cotton, field and root crops from sucking and biting insects, mites and certain nematodes. It is also used in maize and sugar beet against soil-dwelling pests. In plants and animals it is oxidised metabolically to give the sulphoxide and sulphone and their phosphorothioate analogues; as both the parent compounds and the oxidation products are readily hydrolysed, only a small portion of the sulphone results (cf. Worthing and Walker, 1983). Their behaviour in soils is similar but the sulphones can persist under some conditions (cf. Worthing and Walker, 1983). Its acute oral LD<sub>50</sub> in rats is 1-5 mg/kg.

The toxic action of organophosphorus insecticides is expressly to conjugate with the natural complement of cholinesterase enzymes in the body, thereby inactivating them. In tests carried out by

the Environmental Health Laboratory, Central Medical Department, American Cyanamid Company on Thimet 10-G, the investigators found that the cholinesterase levels of exposed workers were unaffected and in Japan also, no adverse effects were observed (Cyanamid Technical Information, 1980).

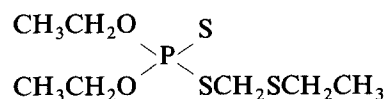
Mutagenic studies of Thimet 10-G in plant systems carried out by Pandita and Khoshoo (1984) found it to be clastogenic and turbagenic in *Allium cepa*. The study reported here provides information that the commercial grade of Thimet 10-G is not mutagenic in microbial, mice and human lymphocyte test systems.

### Materials and methods

Thimet 10-G used in this study is a technically prepared commercial grade obtained in the form of granules from Cyanamid India Ltd., Bombay. Its chemical name is *O,O*-diethyl *S*-(ethylthio-methyl) phosphorodithioate with a molecular weight of 259.4 dalton. Its structure is represented

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as



It has a low water solubility, approximately 50 ppm at room temperature. It is soluble in most of the solvents. In the present study, Thimet 10-G was dissolved in DMSO. Controls were also exposed to the same concentration of DMSO that was used for treatment. Thimet 10-G is stable at 25°C for 2 years and stability is optimum in the pH range 5–7.

#### *Ames Salmonella test*

*Salmonella typhimurium* strains TA98 and TA100 were obtained from Dr. B.N. Ames, Berkeley, CA. The test was carried out according to the procedure of Ames et al. (1975) and Maron and Ames (1983). Bacterial suspensions were grown in nutrient broth (Difco) with rotary shaking at 37°C in dark. They had a titer of about  $10^9$  viable cells per ml at the end of the incubation period. Before use in the mutation tests the cultures were refrigerated at 4°C. Mutation tests were performed by mixing 0.2 ml of the bacterial suspension with up to 0.2 ml of test material dissolved in DMSO (10  $\mu$ l) and phosphate-buffered saline with and without 0.5 ml of an S9 mix (10% S9) and incubated for 10 min at 37°C. To this mixture were added 2.5 ml of soft agar [containing 0.6% Difco agar, 0.2 ml of Vogel–Bonner 50-X salts (Vogel and Bonner, 1956), 0.2 ml of 0.5 mM L-histidine, 0.5 mM biotin and 0.025 ml of 20% glucose]. The mixture was stirred and poured over the surface of bottom agar for the detection of *his*<sup>+</sup> revertants. The plates were incubated in the dark at 37°C for 48 h. The pesticide was assayed in triplicate at each dose level. *N*-Methyl-*N*-nitro-*N*-nitroso-guanidine (NTG or MNNG or NG) was used for positive control.

#### *Transfection test*

*Mycobacterium smegmatis* SN2 was taken as the host and the Mycobacteriophage I3 DNA was used as the transfecting material. Phage as well as host are slow growing organisms and were chosen

as test tools because of their use in our routine experimental work.

#### (a) *Preparation of Mycobacteriophage I3 DNA*

Intense care was taken during phage propagation to avoid any sort of contamination. Phage DNA was also isolated aseptically. *Mycobacterium smegmatis* SN2 was grown in Youmans medium containing 0.1% Tween-80 in culture shaken at 37°C for 12–15 h until the cell density reached  $5 \times 10^7$  cells per ml. To this was added CaCl<sub>2</sub> to give a final concentration of 1 mM. Phage was added at the multiplicity of infection of 5. After infection cells were grown for another 24 h and at the end 2 ml of chloroform were added per 200 ml culture. It was shaken for 15 min and left at rest for 30 min. Cells were pelleted by centrifugation in GSA cups at 8K for 20 min. The supernatant was collected and titrated. The phage from the lysate was pelleted in a Beckman ultracentrifuge type-30 rotor at 20000 rpm for 75 min. The phage pellet was suspended in a minimal volume of 10 mM Tris–HCl (pH 7) and 5 mM NaCl and centrifuged in a Sorvall HB-4 rotor at 8K for 10 min. The supernatant was collected and dialysed against a buffer of 10 mM Tris–HCl (pH 7), 10 mM NaCl and 5 mM MgCl<sub>2</sub> at 4°C for 24 h. DNAase treatment was given at the concentration of 1 mg per  $5 \times 10^{13}$  phages at 37°C for 45 min. The phage was loaded on to a 15–60% sucrose gradient and a run was made in a Beckman ultracentrifuge S-25.1 rotor at 20000 rpm for 90 min. The phage-specific band was collected and dialysed against a buffer of 10 mM Tris–HCl (pH 8), 50 mM NaCl, 1 mM EDTA at 4°C for 24 h. DNA from the phage was isolated very gently by a standard phenol, chloroform–isoamyl alcohol extraction and alcohol precipitation procedure (Maniatis et al., 1982). The precipitate was dissolved in a minimum volume of DNA dissolving buffer (10 mM Tris–HCl pH 7, 1 mM EDTA and 10 mM NaCl) and stored at 0°C. The optical density of DNA was measured at 260 nm and 280 nm. Protein contamination in DNA was evidently negligible because the ratio of  $A_{260}$  nm to  $A_{280}$  nm was more than 1.8.

#### (b) *Sensitization of mycobacterial cells*

*Mycobacterium smegmatis* SN2 cells were grown

in a stirred culture in a nutrient broth containing Tween-80 (0.3% v/v) for 12–14 h at 37°C to obtain a density of  $5 \times 10^7$  cells per ml. At this stage, glycine was added to the culture to a final concentration of 1.25%. The growth was continued for another 150 min before the cells were harvested by a Sorvall HB-4 rotor at 5K for 8 min. Cells were washed with cold diluting fluid (10 g of NaCl and 1 g of bactotryptone per liter) and suspended in a half volume of cold 50 mM CaCl<sub>2</sub>. They were kept ice-cold for another 60 min, centrifuged again and resuspended in one-tenth volume of cold 50 mM CaCl<sub>2</sub> to give a density of  $10^9$  cells per ml. These competent cells were used within a few hours for transfection assay.

*(c) DNA treatment*

Thimet 10-G at concentrations of 0, 1, 2, 4, 8, 16 and 32 µg/ml was incubated with DNA (5 µg in 0.1 ml of 10 mM Tris-HCl pH 7 and 1 mM EDTA) at 37°C for 30 min.

*(d) Transfection assay*

The competent cells were transferred with chilled pipettes to chilled tubes and ice-cold transfecting DNA is then added by chilled pipettes. The transfection mixture consisted of 0.1 ml of sensitized cells ( $10^8$  cells) and 0.2 ml of 10 mM Tris-HCl (pH 7.4), 1 mM EDTA and 5 µg of DNA.

*(e) Bacterial pretreatment*

The pretreatment of sensitized bacterial cells was done by mixing them with various concentrations of Thimet 10-G (Table 2) and incubated at 37°C for 10 min.

*Schmid's micronucleus test*

Specific pathogen-free 4–6-week-old Swiss albino mice with a body weight range of 30–40 g were injected intraperitoneally with the test material to find the highest tolerable dosage. The results of the preliminary study showed that the maximal intraperitoneal dosage tolerated was 1.5 mg/kg body weight. The other three dosages were 0.05, 0.1 and 1 mg/kg body weight. All groups [3 mice for vehicle control and 6 mice (consisting of an equal number of males and females) for each concentration of the chemical] were given the

chemical in a standard volume of 1 ml/20 g body weight. The total dosages were given by intraperitoneal injection as 2 equal administrations separated by an interval of 24 h. DMSO concentration was maintained constant in all dosages administered. The vehicle control animals were treated in an identical manner with DMSO and sterile distilled water. Mitomycin C, administered by intraperitoneal injection, was used as a positive control to check the assay system. The mice were starved overnight before treatment.

They were killed, 6 h after the administration of the second dose, for the preparation of bone marrow smears. The stained smears were examined to determine the incidence of micronucleated cells in 1800 polychromatic erythrocytes and the ratio of normochromatic to polychromatic erythrocytes for each animal.

*Human lymphocyte sister-chromatid exchange assay*

Samples of 0.3 ml of heparinized whole peripheral blood from one healthy male donor were incubated at 37°C in dark-glass bottles containing 5 ml of TC-199 medium supplemented with 20% inactivated human AB serum, 0.1 ml phytohemagglutinin (Wellcome), streptomycin-penicillin and 5-bromodeoxyuridine (BrdU) at a final concentration of 10 µg/ml. Exposure to the test substance was made after 0 h and 48 h incubation. In one set of culture, Thimet 10-G at concentrations of 0, 1, 5, 10, 15, 20 and 40 µg/ml was introduced at 0 h of incubation. In the other set, the cultures after 48 h of incubation were centrifuged at 1000 rpm for 5 min, the supernatant was removed and the cells were incubated in the presence of BrdU in culture medium containing Thimet 10-G at concentrations of 0, 1, 5, 10, 15, 20 and 40 µg/ml. For metabolic activation 0.5 ml of S9 mix (10% S9) was also added. The test substance remained in contact with the lymphocytes until harvesting time. Colcemid was added at the final concentration of  $10^{-6}$  M, 2 h before harvesting. Flame-dried preparations were made, stained by a modified FPG technique (Perry and Wolff, 1974) described earlier by Pandita (1983a) and Pandita et al. (1983b). For scoring SCE frequency, 50 metaphases of second cycle from each culture were studied. For the kinetics of dividing cell populations, the method of Craige-Holmes and Shaw

(1976) was adopted. For finding the percentage of first, second and third cell cycle cells, 300 cells from each culture were studied.

## Results

The results of the frameshift reverse mutation test for the histidine requirement with *Salmonella typhimurium* are summarized in Table 1. Thimet 10-G did not increase the frequency of reversions over the untreated ones. Even in the presence of S9, the insecticide did not induce any reversions in *Salmonella typhimurium*.

The results of transfection of Mycobacteriophage I3 DNA in *Mycobacterium smegmatis* SN2 are summarised in Table 2. Mycobacteriophage I3 DNA (5 µg) gave 500 plaques within 18–24 h of incubation at 37°C. Molecular weight of Mycobacteriophage I3 DNA is approximately  $10^{-16}$  g and its transfection efficiency is  $10^{-8}$  (Pandita et al., 1983a). Transfection inhibition increased in linear fashion with the increase in insecticide concentration, as determined by the number of plaques formed.

To find whether the decrease in transfection was due to the presence of insecticide in the transfection assay, the insecticide was removed from the DNA by phenol, chloroform–isoamyl alcohol extraction and alcohol precipitation. This

DNA showed the same type of transfection as was shown by the untreated DNA in the transfecting assay.

Reduction in the number of plaques was found when the transfection was carried out immediately after the addition of phage DNA to the sensitized cells pretreated with insecticide. This showed that the decrease in transfection was not due to the mutation in transfecting DNA, and was due to incapacitation of bacterial cells because of insecticide toxicity.

The results of the Schmid's micronucleus test in mice are presented in Table 3. No increase of micronucleated polychromatic cells was observed but the ratio of normochromatic to polychromatic cells was affected. With the increased insecticide concentration, the ratio of normochromatic to polychromatic erythrocytes increased. A statistically significant ( $P < 0.05$ ) increase in the ratio of normochromatic to polychromatic erythrocytes was observed at the dosage of 0.2 mg/kg body weight. The response to Thimet 10-G treatment was identical in male and female mice.

The results with the human lymphocytes are summarized in Table 4. The insecticide did not affect the sister-chromatid exchange frequency but mitotic index and cell cycle duration were affected. The effect of insecticide in the presence of S9 was similar to that in its absence. The insecti-

TABLE 1  
REVERSIONS IN *Salmonella typhimurium* AFTER TREATMENT WITH THIMET 10-G

| Chemical concentration (µg/plate) | Revertants |          |           |             |              |          |           |             |              |
|-----------------------------------|------------|----------|-----------|-------------|--------------|----------|-----------|-------------|--------------|
|                                   | TA98       |          |           |             | TA100        |          |           |             |              |
|                                   | Plate I    | Plate II | Plate III | Mean ± S.D. | Plate I      | Plate II | Plate III | Mean ± S.D. |              |
| Control with DMSO (K)             | –          | 52       | 59        | 57          | 56 ± 3.60    | 200      | 190       | 204         | 204 ± 7.21   |
| Thimet 10-G                       | 0.5        | 60       | 53        | 61          | 58 ± 4.28    | 178      | 201       | 191         | 190 ± 11.53  |
|                                   | 1          | 46       | 53        | 51          | 50 ± 3.60    | 198      | 199       | 218         | 205 ± 11.26  |
|                                   | 5          | 49       | 43        | 52          | 48 ± 4.58    | 182      | 192       | 187         | 187 ± 5      |
|                                   | 10         | 45       | 32        | 40          | 39 ± 6.80    | 154      | 157       | 175         | 162 ± 11.53  |
|                                   | 25         | 10       | 9         | 17          | 12 ± 4.35    | 40       | 32        | 36          | 36 ± 4       |
|                                   | 50         | 6        | 1         | 5           | 4 ± 2.64     | 9        | 7         | 14          | 10 ± 3.60    |
|                                   | 100        | –        | –         | –           | –            | –        | –         | –           | –            |
| NTG (+Control)                    | 50         | 1900     | 1841      | 1924        | 1895 ± 43.86 | 1236     | 1321      | 1369        | 1308 ± 67.35 |

TABLE 2  
EFFECT OF THIMET 10-G ON THE TRANSFECTION

| Group | Material           | Concentration<br>( $\mu\text{g}/\text{ml}$ ) | Transfection of<br>the pesticide treated<br>phage I3 DNA |                            | Transfection of<br>the phenol extracted<br>pesticide<br>treated phage<br>I3 DNA |                            | Transfection in<br>pesticide pre-<br>treated bacte-<br>rial cells |                            |
|-------|--------------------|--|--|----------------------------|---|----------------------------|---|----------------------------|
|       |                    |  | Number of<br>plaques                                     | Transfection<br>efficiency | Number of<br>plaques  | Transfection<br>efficiency | Number of<br>plaques  | Transfection<br>efficiency |
| 1     | Control (K)        | 0  | 500  | $10^{-8}$                  | 489   | $9.7 \times 10^{-9}$       | 506   | $1.01 \times 10^{-8}$      |
| 2     | Thimet 10-G        | 1  | 501  | $1.002 \times 10^{-8}$     | 490   | $9.8 \times 10^{-9}$       | 500   | $10^{-8}$                  |
| 3     | Thimet 10-G        | 2  | 490  | $9.8 \times 10^{-9}$       | 481   | $9.6 \times 10^{-9}$       | 480   | $9.6 \times 10^{-9}$       |
| 4     | Thimet 10-G        | 4  | 440  | $8.8 \times 10^{-9}$ *     | 478   | $9.5 \times 10^{-9}$       | 410   | $8.2 \times 10^{-9}$ *     |
| 5     | Thimet 10-G        | 8  | 380  | $7.6 \times 10^{-9}$ *     | 482   | $9.6 \times 10^{-9}$       | 295   | $5.9 \times 10^{-9}$ *     |
| 6     | Thimet 10-G        | 16   | 149  | $2.98 \times 10^{-9}$ **   | 485   | $9.7 \times 10^{-9}$       | 70  | $1.4 \times 10^{-9}$ **    |
| 7     | Thimet 10-G        | 32   | 30   | $6 \times 10^{-9}$ **      | 467   | $9.3 \times 10^{-9}$       | 6   | $0.12 \times 10^{-10}$ **  |
| 8     | Thimet 10-G        | 64   | 0  | $10^{-10}$ (nil)           | 473   | $9.4 \times 10^{-9}$       | 0   | $10^{-10}$ (nil)           |
| 9     | NTG<br>(+ Control) | 32   | 250  | $5 \times 10^{-9}$         | 220   | $4.4 \times 10^{-9}$       | 302   | $6.04 \times 10^{-9}$      |

Difference from control (K) is statistically significant according to the *t*-test.

\*  $P < 0.05$ ; \*\*  $P < 0.001$ .

cide concentration below  $10 \mu\text{g}/\text{ml}$  introduced at 0 h was noninhibitory for cell transformation. With the increase in insecticide concentration there was a decrease in mitotic index and delay in cell

transformation. Increase in insecticide concentration resulted in a significant decrease ( $P < 0.001$ ) in mitotic index in comparison with the control. A significant difference ( $P < 0.001$ ) between the cells

TABLE 3  
TREATMENT SCHEDULE, NUMBER OF MICRONUCLEATED POLYCHROMATIC ERYTHROCYTES AND THE RATIO OF NORMOCHROMATIC TO POLYCHROMATIC ERYTHROCYTES IN BONE-MARROW SMEARS AFTER INTRAPERITONEAL ADMINISTRATIONS OF THIMET 10-G TO MICE

| Group | Material   | Number of<br>animals<br>in each<br>treat-<br>ment<br>group | Dosage per<br>administra-<br>tion (mg/kg<br>body weight) | Total<br>dosage<br>(mg/kg<br>body<br>weight) | Number of micronucleated<br>cells per 1800 poly-<br>chromatic erythrocytes<br>per mouse |           | Ratio of normochromatic<br>to polychromatic ery-<br>throcytes |               |
|-------|--|--|--|--|---|-----------|---|---------------|
|       |  |  |  |  | Mean  | Range     | Mean  | Range         |
| 1.    | Sterilized<br>dist. H <sub>2</sub> O<br>with DMSO<br>(vehicle<br>controls) | 3  | -  | -  | 1.19  | 0- 4.02   | 1.70  | 0.51- 3.40    |
| 2.    | Thimet 10-G  | 6  | 0.05   | 0.1  | 1.29  | 0- 4.00   | 1.78  | 0.58- 4.10    |
| 3.    | Thimet 10-G  | 6  | 0.10   | 0.2  | 1.18  | 0- 4.05   | 2.02 *  | 0.69- 6.25 *  |
| 4.    | Thimet 10-G  | 6  | 1.00   | 2  | 1.27  | 0- 3.78   | 3.74 *  | 0.89- 7.13 *  |
| 5.    | Thimet 10-G  | 6  | 1.50   | 3  | 1.23  | 0- 3.85   | 5.64 **   | 1.36-12.46 ** |
| 6.    | Mitomycin C<br>(+ K)   | 6  | 5.00   | 10   | 78.75   | 35-130.00 | 21.95   | 4.12-49.65    |

Difference from control (K) is statistically significant according to the *t*-test.

\*  $P < 0.05$ ; \*\*  $P < 0.001$ .

TABLE 4

FREQUENCY OF SCEs PER CELL, MITOTIC INDEX AND CELL CYCLE WITH DIFFERENT CONCENTRATIONS OF THIMET 10-G INTRODUCED IN CULTURE AT 0 h AND 48 h

| Chemical concentration ( $\mu\text{g/ml}$ ) | 0 h               |               |            |        |         | 48 h              |                   |            |        |         |
|---|-------------------|---------------|------------|--------|---------|-------------------|-------------------|------------|--------|---------|
|   | SCE/cell $\pm$ SD | Mitotic index | Cell cycle |        |         | SCE/cell $\pm$ SD | Mitotic index     | Cell cycle |        |         |
|   |                   |               | I (%)      | II (%) | III (%) |                   |                   | I (%)      | II (%) | III (%) |
| 0 (K)                                       | 7.49 $\pm$ 1.25   | 4.98          | 10         | 27     | 63      | 7.79 $\pm$ 0.49   | 5.10              | 13         | 38     | 49      |
| DMSO only (K)                               | 8.05 $\pm$ 0.93   | 5.12          | 7          | 32     | 61      | 8.21 $\pm$ 0.71   | 4.95              | 8          | 34     | 58      |
| 1   | 7.89 $\pm$ 0.84   | 4.93          | 8          | 28     | 64      | 8.05 $\pm$ 0.70   | 4.91              | 6          | 25     | 69      |
| 5   | 7.95 $\pm$ 0.53   | 4.65          | 8          | 27     | 65      | 7.86 $\pm$ 1.12   | 4.65              | 11         | 29     | 60      |
| 10  | 8.13 $\pm$ 1.01   | 4.08 *        | 21 *       | 52 *   | 27 *    | 7.96 $\pm$ 0.98   | 4.03              | 13         | 60 *   | 27 *    |
| 15  | 7.58 $\pm$ 0.96   | 3.71 *        | 53 **      | 34 **  | 13 **   | 8.15 $\pm$ 0.67   | 2.98 *            | 20 *       | 69 *   | 11 *    |
| 20  | 7.12 $\pm$ 1.32   | 1.01 **       | 65 **      | 32 **  | 3 **    | 7.87 $\pm$ 1.01   | 0.79 **           | 28 *       | 66 *   | 6 *     |
| 40  | -                 | No division   | -          | -      | -       | -                 | Very low division | -          | -      | -       |

Difference from (K) group statistically significant according to Student's *t*-test.

\*  $P < 0.05$ ; \*\*  $P < 0.001$ .

in first (I), second (II), and third (III) cycle division metaphases in cultures with insecticide concentration above 10  $\mu\text{g/ml}$  was seen when compared with those untreated. With the increase in the insecticide concentration there is a proportionate increase in first and second cycle metaphase.

Insecticide introduction after 48 h of incubation also had no effect on SCEs, but mitotic index and cell cycle were drastically affected. At an insecticide concentration of 10  $\mu\text{g/ml}$  and above, a decrease in mitotic index and an increase in first and second cycle metaphases resulted. This indicates that the insecticide causes mitotic depression.

## Discussion

Organophosphorus insecticides represent a major class of agricultural chemicals. They have many structural similarities with naturally occurring compounds. Mohn (1973), Wild (1975) and Pandita (1983b) reported that a number of organophosphorus insecticides induce mutagenesis in microorganisms. The present observations reveal that Thimet 10-G, an organophosphate insecticide, is unable to induce mutagenesis in *Salmonella typhimurium*.

Carefully isolated deoxyribose nucleic acid generally consists of a homogenous population of molecules, each of which contains the same sequence of nucleotides. Since it has been shown that a change (deletion, addition or alteration) of only one base or base pair of transfecting nucleic acids may result in a loss of infectivity (Fiers and Sinsheimer, 1962; Kamen, 1969; Marwin and Schaller, 1966; Schuster and Schramm, 1958; Strack and Kaiser, 1965; Young and Sinsheimer, 1965), transfection assays are a very sensitive probe for the fidelity of a phage nucleic acid sequence. Transfection is also inhibited by base mutation, cleavage of DNA, nicks, loss of cohesive ends, interaction with histones, spermidine and nalidixic acid etc. (Benzinger, 1978). Thimet 10-G was not effective in inhibiting transfection. Present investigations indicate that the Thimet 10-G had no direct effect on DNA, as the transfection efficiency was not affected.

Some of the organophosphate insecticides such as Dursban, Gardona, and Metasystox-R have been reported to increase the frequency of micronuclei and the ratio of normochromatic to polychromatic erythrocytes in the mammalian systems in vivo (Ames and Fahmy, 1982, 1985; Pandita, 1983b). Present observations reveal that Thimet 10-G did not increase the frequency of micro-

nuclei, but enhanced the ratio of normochromatic to polychromatic erythrocytes in the mouse. This suggests that the insecticide is not a clastogen, but somehow disturbs the cell cycle in the mammalian system *in vivo*, which may be due to the high toxicity of the insecticide.

The FPG stain technique (Latt, 1973; Perry and Wolff, 1974) facilitates the study of the kinetics of dividing cell populations and the detection of the exchange between sister chromatids (Craig-Holmes and Shaw, 1976). Latt and Schreck (1980) proved SCEs to be a highly sensitive indicator for assessing potential mutagens and carcinogens. Some of the organophosphorus insecticides have been reported to induce SCEs and delay the cell cycle (Chen et al., 1982; Pandita, 1983b). The present investigations revealed that Thimet 10-G did not induce SCEs. It did cause a decrease in the mitotic index and delay in the cell cycle. This supports the hypothesis that Thimet 10-G is not a direct genotoxin in human lymphocytes.

The pesticide Thimet 10-G was not effective (a) in inducing mutations in *Salmonella* with and without metabolic activation, (b) in inhibiting transfection in *Mycobacterium smegmatis*, (c) in inducing micronuclei in mouse bone marrow cells *in vivo* and (d) in inducing an increase in the frequency of sister-chromatid exchanges *in vitro*. It was able to induce (a) an increase in the ratio of normochromatic to polychromatic erythrocytes in mouse, and (b) decrease in mitotic index and delay in cell cycle in cultured human lymphocytes.

Present observations support that Thimet 10-G is highly toxic to bacterial and mammalian test systems when administered at the concentrations at which the pesticide proved mutagenic in plant systems (Pandita and Khoshoo, 1984). The subtoxic doses of the pesticide did not prove to be mutagenic in the most sensitive test systems applied at present. The pesticide at subtoxic doses cannot be classified as genotoxic.

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