Effect of methyl parathion formulation on estrous cycle and reproductive performance in albino rats

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The animals were injected intraperitoneally with graded doses of methyl parathion at 1.5 to 3 mg/kg body weight for 15 days from the day of estrus. Results indicated that the methyl parathion treatment showed irregular estrous cycles, affect the duration of each estrous cycle, proestrus and diestrus were significantly changed in 2.5 and 3 mg treatment groups. But there was no significant change in the number and duration of each estrous cycle, duration of proestrus and diestrus in 1.5 and 2 mg methyl parathion treatment groups. However, there was a significant decrease in the duration of estrus, while there was no significant change in the duration of metestrus in all methyl parathion treatment rats when compared with those of the corresponding parameters of the control. There was no significant effect on number of live pups on day 1 and 5 except in 3 mg methyl parathion treatment group where it was significantly decreased. There was no significant change in reproductive indices like pregnancy, parturition, live birth and viability in all the methyl parathion treatment rats except the viability index in the highest dose.

Some of the organophosphorus insecticides have been reported to reduce fertility and cause sterility in animals. In a multi-generation study conducted in rats, chlorfencinphos has been reported to produce effects on fertility, viability and lactation indices without producing any effect on gestation. On the other hand, crufomate has been found to cause prolongation of gestation and reduce lactation in female rats. Pasang and Kaliwal have reported that the administration of methyl parathion inhibits ovarian compensatory hypertrophy, affects estrous cycle and reduces the total number of healthy follicles in hemicastrated albino rats. There are no reports on the effect of methyl parathion on estrous cycle and reproductive performance in albino rats except that rats fed low dietary concentrations (30, 10, and 0 ppm) methyl parathion reduced the reproductive process. Therefore, the present investigation has been undertaken to study the effect of methyl parathion with higher concentrations and duration of treatment from days 1 to 15 on estrous cycle and reproductive performance in albino rats.

Chemical—Methyl parathion as metacid formulation of Bayer India Ltd. was used. The stock solutions were prepared in olive oil for intraperitoneal injection.(ip).

Animals—Normal cycling nulliparous rats of Wister strain were used. The rats breed were maintained in the animal house of the Post-graduate Department of Studies in Zoology, Karnataka University, Dharwad.

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Regularly cycling 70 to 90 days female albino rats weighing about 120-160 g were used. During the experiment animals were housed in individual cages with 12:12 hr L:D schedule at room temperature (26° ± 1° C). Food and water were made available ad libitum.

Stages of the estrous cycle were determined by the microscopic examination of the vaginal smears obtained in the morning between 1000-1100 hrs as described by Zarrow et al. The intraperitoneal LD₅₀ values were 8 mg/kg in female rats and 5.8 mg/kg in male rats. Hence, animals received methyl parathion in doses of 1.5, 2, 2.5 and 3 mg/kg body weight, ip for 15 days from the day of estrus. The animals in control group received olive oil. The quantity of solution injected into each animal never exceeded 2 ml/kg body weight/day. The estrous cycle of these animals was closely followed for three consecutive cycles and compared with that of control. The diestrus index was calculated as follows:

Diestrus index = No. of days with clear diestrus smear × 100 Total duration of treatment

To study the effect on reproductive performance the same rats which were used for estrous cycle were used. After an interval of 24 hr of the last treatment, the females were kept with untreated males in the ratio of 1:1. The presence of sperms in the vaginal smear or the presence of vaginal plug next morning indicated pregnancy and that day was designated as day 1 of pregnancy. The pregnant females were separated from males and housed in...
individual cages. The animals were observed for abortions and still-births by observing the vaginal blood smear. The litter size, number of live and dead pups, and their weights were recorded at birth. The number of pups, and their weights were recorded on day 1 and day 5. The reproductive indices viz, pregnancy, parturition, live-birth and viability were calculated. 8, 10

Pregnancy index = \( \frac{\text{Pregnancies}}{\text{Females mated}} \times 100 \)

Parturition index = \( \frac{\text{Deliveries}}{\text{Females mated}} \times 100 \)

Live-birth index = \( \frac{\text{No. of viable young once born}}{\text{Females mated}} \times 100 \)

Viability index = \( \frac{\text{No. of viable young once alive at 5 days}}{\text{No. of young once born}} \times 100 \)

Statistics—The mean and the standard errors were determined for each group and data were analysed using student’s t-test.

Estrous cycle—Control rats showed regular estrous cycle and the duration of each phases of estrous cycle was normal during the experimental period with methyl parathion treatment showed irregular estrous cycles and the number and duration of each cycle was significantly prolonged in 2.5 and 3 mg (high) concentration of methyl parathion treated groups when compared with those of control. There was a significant decrease in the duration of estrus, while there was no significant change in the duration of metestrus in all the methyl parathion treated rats. There was a decrease in the duration of proestrus with concomitant increase in the duration of diestrus as dosage of methyl parathion was increased, and it was significant in 2.5 mg \( (P < 0.05) \) and 3 mg \( (P < 0.001) \) methyl parathion treated rats. However, there was no significant change in the duration of proestrus and diestrus in 1.5 and 2 mg methyl parathion treated groups. Gradual increase of diestrus index was observed in all the methyl parathion treated groups (Table 1).

Reproductive performance—Administration of methyl parathion for 15 days to females prior to mating caused no significant change in the litter size, number and weight of the live pups on day 1 and day 5 in all the methyl parathion treated rats. However, there was a significant reduction in the number of live pups on day 5 with 3 mg level and weight of the live pups on day 5 with 2.5 and 3 mg levels in rats. The pregnancy, parturition and live-birth indices were not significantly affected in all the methyl parathion treated groups. There was no change in the viability index in rats treated with 1.5, 2 and 2.5 mg methyl parathion. However, significant \( (P < 0.05) \) effect was observed in viability index in rats treated with 3 mg of methyl parathion (Table 2).

Cyclic changes of the vaginal smear observed in the estrous cycle give a fair index of ovarian and uterine activities. In the present investigation administration of methyl parathion significantly decreased the number of estrous cycles with concomitant increase in the number of days in each estrous cycle with 2.5 and 3 mg methyl parathion treated rats. There was also a significant decrease in the duration of proestrus and estrus phases of estrous cycle with concomitant significant increase in the time spent in diestrus phase with 2.5 and 3 mg level. In hemicranastrated rats, administration (ip) of methyl parathion increased the period of diestrus phase by reducing the number of estrous cycles, inhibited the compensatory ovarian hypertrophy and reduced the number of healthy follicles. Similar observations were reported in mice and rats treated with different organophosphorous pesticides such as dimethoate, fenthion, malathion and sumithion. The irregularity in the estrous cycle with prolonged diestrus in methyl parathion treated rats at high dose is a result of either direct effect on the ovary or indirectly through the action on hypothalamus and/or pituitary remains to be determined.

The administration of methyl parathion to females prior to mating, caused significant reduction in the number of live pups on day 5 with 3 mg level and weight of the live pups on day 5 with 2.5 and 3 mg levels in rats. Litter size and survivability rate of pups were not affected significantly with 1.5 and 2 mg other exposure groups.

<table>
<thead>
<tr>
<th>Groups and treatment (mg/kg bw)</th>
<th>Number of cycles in 15 days</th>
<th>Days/ Cycle</th>
<th>Duration of each stage in 15 days treatment</th>
<th>Diestrus Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Control</td>
<td>3.00±0.00</td>
<td>5.00</td>
<td>2.50±0.34</td>
<td>7.83±0.30</td>
</tr>
<tr>
<td>(B) 1.5</td>
<td>2.16±0.31</td>
<td>6.94</td>
<td>1.83±0.31</td>
<td>4.16±0.16</td>
</tr>
<tr>
<td>(C) 2</td>
<td>2.20±0.40</td>
<td>6.82</td>
<td>1.80±0.73</td>
<td>0.50±0.22</td>
</tr>
<tr>
<td>(D) 2.5</td>
<td>1.40±0.24</td>
<td>10.71</td>
<td>1.20±0.37</td>
<td>9.33±0.71</td>
</tr>
</tbody>
</table>

P values: \( a < 0.05 \); \( b < 0.01 \); \( c < 0.001 \)
However, no effect on the litter size was observed in rats and mice treated with fenthion dimethoate and malathion organophosphates. However, a reduction in the weaning weight (weight of pups on day 21 of birth) has been reported in the newborns delivered from female rats given dermal application of crufomate. Increased mortality of pups has been reported after the administration of a number of organophosphate insecticides prior to and/or through the life in rats and mice. The higher mortality observed in pups could be attributed to reduced adaptability among pups to various environmental stresses. The significant mortality of pups also observed during present investigation is may be due to the inability of the pesticide to cause significant mortality of the pups and may be less toxic to embryos. The present investigation indicates that there was no significant change in reproductive indices like pregnancy, parturition, live birth and viability in all the methyl parathion treated rats. However, there was a significant decrease in the viability index at the highest dose of 3 mg/kg body weight. It has been well documented that the pregnancy parturition, live birth, viability and lactation indices are affected after the administration of organophosphate pesticides. A multigeneration study made by Anthony et al. using diethyl-1-(2,4-dichlorophenyl)-2-chlorovinylphosphate in rats revealed no effect on gestation but there were progressive effects on fertility, viability and lactation indices. Gowda and Sastry did not observe any toxic effect on different indices of reproduction in rats, maintained on basal diet containing an organophosphorus pesticide sumithion. Chlorfencinphos another organophosphate compound was however, found to produce progressive reduction in fertility, viability and lactation indices in rats. A slight effect was observed in viability index and lactation index in malathion treated rats. The significant reduction in the viability index observed in the present study could possibly be attributed to its direct toxic action on pups as observed in an organophosphate malathion treated rats.

References