

Effect of Methomyl on Fetal Development in Female Rats

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ABSTRACT

The environmental pollution by insecticides is most considerable due to their untoward effects on the production and reproduction in human and animals. The teratogenic potential of methomyl insecticide in rats was the aim of our study to assess. Pregnant rats were separated into four equal groups; the first one kept as a control group. The 2nd and 3th groups were orally administered methomyl on 6th through 15th of Gestation period at doses of 2 mg/kg b.wt. and 0.67 mg/kg b.wt ,daily corresponding to 1/10 and 1/30 LD50 methomyl, respectively. All pregnant rats were exposed to caesarean section on GD 21 and their fetuses were examined for morphological, visceral and skeletal abnormalities. Decreased maternal weight gain and fetal weight and significantly reduced the number of implantation sites, the number of live fetuses and increased the incidence of dead embryos and resorption sites at the same dose levels were recorded in groups exposed to methomyl at 2 mg /kg. b.wt.

The fetuses of dams rats exposed to methomyl at 2 mg/kg .b .wt., were significantly increased in the percentage of morphological, visceral and skeletal abnormalities. The retardation in growth of viable fetuses, hydrocephaly, an ophthalmia , lung hypoplasia, incomplete ossification of cranial bones, aplasia of metacarpals, metatarsals, phalanges and caudal vertebrae were the important fetal anomalies.

INTRODUCTION

FAO, 2003 recorded that the last several years, there have been widespread uses of potent substances that, although effective in their intended use, have also been suspected of being harmful to health. This environmental pollution that may adversely affect human fertility includes pesticides (**Cooper et al., 2010**).

Salih and Jaafar, 2013 mentioned that the use of pesticides to control pests in land and water has posed health hazards to live stock and wildlife. The environmental pollution by insecticides cause teratogenic effect, abortion and decreased fertility in experimental animals, malignant tumors, immunosuppressive action, (**Meeker et al., 2006**). **Sangha et al.,2013** ,recorded that pesticides may cause reproductive toxicity through direct damage to cells,.

Carbamates are the third major group of synthetic insecticides and they have frequently been used because of their relatively short life in the environment and fast action on the target pest worldwide for agriculture (**Kaur and Sandhir, 2006 and**

West and Marnett, 2006). They may be involved in oxidative stress through the generation of free radicals (**Ott *et al.*, 2007**). Methomyl is an oxime carbamate insecticide that controls a broad spectrum of insects (**WHO,1996 and Kidd and James 1991**). It is act as an endocrine disruptor , interference with the estrogen and/or androgen receptor and also genotoxic, capable of inducing structural and chromosomal aberration in mammalian cells and may cause reproductive toxicity through several different mechanisms (**Stamati *et al.*, 2007, Sangha et al., 2013 and Andersen et al.,2002**).

chronic administration of pesticide to females have been associated with reduced reproduction, spontaneous abortions, premature birth, low birth weight, developmental abnormalities and disruption of the hormonal function (**Dohle *et al.*, 2005 and Sharpe and Irvine, 2004**). The effects can be reversible, permanent or even trans generational, take place in the offspring.

Exposure to pesticides has been complicated in the reproductive disorders (**Garry, 2004 and Baskin *et al.*, 2001**), other birth defects (**Schreinemachers, 2003**); behavioral abnormalities and showed offspring sex ratios (**Mackenzie and Constanze, 2005 and Zala and Penn, 2004**). Since the female function can be compromised by exposure to toxic chemicals, the present study was conducted to assess the effect of exposure to methomyl at two different doses on reproductive function in female rats.

MATERIALS AND METHODS

Chemicals

Methomyl (ethomex®, *S*-methyl *N*-(methylcarbamoyloxy) thioacetimidate) is carbamate insecticide which introduced by Sigma -Aldrich. It was purchased from the market. It was diluted in saline and freshly prepared every week.

Animals:

Our study was carried out on thirty female albino rats weighing 190-200 gm. Rats were obtained from the National Institute of Ophthalmology, housed in metal cages, fed on basal diet and watered *ad-libitum*. They were left for two weeks for acclimatization before starting the experiment.

Effect of methomyl on fetal development:

Male and female rats were housed for mating in the ratio of 2:1 during the dark period on the next morning, vaginal smears were obtained from female rats and inspected. The existence of spermatozoa in the vaginal smear was considered as GD 0 (**Paumgartten *et al.*, 1998**). Delivery in normal rat takes place on days 21-23 of gestation (**Rough,1968**).

After mating and ensuring successful conception,30 pregnant rats were distributed into three groups (n = 10).The first group served as control and received distilled water. The 2nd and 3th groups were orally received methomyl at doses of 2.0 and 0.67 mg/kg b.wt, respectively(1/10 and 1/30 of LD50). The vehicle and the insecticide were administered orally by gavage once daily on GD 6 through GD 15.

This period is considered as the critical period for the fetall development of female rats.

Morphological examination of the uteri and fetuses:

At GD 21, the dams were weighed, sacrificed and the laparotomy process was performed with exposure of the uterine horns. The uteri from each female were removed, and then the fetuses were separated. Numbers of implantation sites , viable, dead and resorbed fetuses were calculated. The live fetuses were dried on a bloating paper, weighed, measured and examined for external morphological abnormalities. Moreover, the placental weights were recorded.

Visceral examination of fetuses:

Two third of the fetuses from each dam were kept in Bouin solution for 1-2 weeks. Fetal sections were examined for any visceral malformations (**Hayes, 1986**).

Skeletal examination of fetuses:

The remaining fetuses were preserved in 95% ethanol solution for 7 days for dehydration. The abdominal wall of each dehydrated fetus was opened and the internal organs were removed and described by **Taylor (1986)**. The stained fetuses were kept in Mallsch's solution alone for 2 days. Different parts of the axial and appendicular skeleton of the stained fetuses were inspected under the dissecting binocular microscope for any anomalies.

Statistical analysis:

The results were subsequently analyzed following the statistical methods established by **Snedecor (1982)**

RESULTS AND DISCUSSION

Effect of methomyl on fetal development:

Acosta-Maldonado et al., 2009 said that pesticides cross the placental barrier and enter fetal circulation. Therefore, pesticides during pregnancy has a probability to induce some kind of structural anomalies in the fetuses. The embryonic period of rats (6-15 days) is when organogenesis takes place; therefore, this duration is one of high susceptibility to toxic materials. During this period, each organ or morphological trait has its own critical period of development.

Our investigation, indicated that the pregnant females of both the control and all experimental groups showed an increase in the body weight during the first 6 days of gestation before administration of methomyl. The body weight of rats of the control group continued to increase from 9th day to end of experiment, while those of methomyl exposed groups showed a less rate of increase in the body weight gain. On the 21st day, pregnant females of the control group exhibited high percentage of body weight gain .The rate of increase in body weight reduced as the dose of methomyl was increased. The dams exposed to 0.67 mg/kg b.wt showed slightly decreased in body weight gain ,while those, which were treated with methomyl at 2.0 mg/kg b.wt showed significantly decreased when compared with control group

(**Table 1**).The results could be explained by the fact that animals exposed to methomyl at 2.0 mg/kg b.wt had a lower number of viable fetuses as well as increased rates of post-implantation deaths (**Fig 1**). In agrrement with our results, (**Sangha et al. 2011 ,Farag et al. 2006 and Tian et al. 2005**),) who recorded a significant decrease in the body weight gain and body weight accompanied by lowered fetal weights and increased number of resorptions fetal weights in pregnant rats after treatment with endosulfan ,chlorpyrifos and dimethoate insecticide As expected, exposure to tested insecticide at dose of or 2.0 mg/kg b. wt had no considerable effect on the number of implantation sites (**Table 1**) as the exposure to the insecticide begins after implantation (GD 6). There was no resorption among the control group or in the group treated with methomyl at 0.67 mg /kg .b. wt from the GD 6 to GD 15. Oral administration of methomyl at 0.67 mg /kg.b.wt had no significant effect on the numbers of viable (11.6 ± 1.25) and dead (1.24 ± 0.611) fetuses, compared to (13.0 ± 0.73) and (0.13 ± 0.232), respectively in the control. Numbers of viable fetuses were found to be significantly decreased (1.202 ± 1.31) while the dead fetuses were significantly increased (0.79 ± 1.01 ,respectively) in dams administered of 2.0 mg/ kg .b. wt of methomyl (**Table 1**). It may be assumed that injury to cells and organ system due to insecticide exposure caused embryo lethality. The rates of resorbed fetuses maternally treated with methomyl at doses of 2.0 mg /kg.b.wt during the period of organogenesis was 83.77%,. (**Fig. 2**). Placental transfer of methomyl to fetuses during pregnancy may be the reason which led to the increased numbers of resorption in the treated groups which confirmed by **Dewan et al., 2013 and Saxena et al., 1981** . The dose (2.0 mg /kg.b.wt) of methomyl increased the percentage of post-implantation loss .It is well known that insecticides cross the placental barrier and can induce some changes in the development of placental structures. Drug or chemical-induced placental injury subsequently result in fetal growth retardation, resorption or teratogenicity (**El Ghareeb et al., 2015**). A dose-dependent decline in fetal weight and length was observed in all the experimental groups. This correlates well with the decrease in maternal weight. Decreases in fetal body weight and length are sensitive and precise indicators for growth retardation. The mean of fetal body weight and length on GD 21 were recorded in (**Table 1**) In the present study, treatment with methomyl at dose of 2.0 mg /kg.b.wt. caused fetal growth retardation indicated by reduction of both body (respectively) and length (3.54 ± 0.132 and 1.74 ± 0.199 cm, respectively) of the fetuses.

The uterine development in mammals is the active cell proliferation and it is highly sensitive to chemical substances. A number from growth retardation to severe organ anomalies and functional defects have been reported to result from chemical exposure to embryos (**Garber, 1989**). The significantly decrease the weight gain of rats exposed to high dose of methomyl could indirectly related to the high incidence of resorptions and reduced litter weight. Pregnant dams administered methomyl at 0.67 and 2.0 mg/ kg.b.wt. showed fetuses with external malformations, with rates of

7.94 and 100%, respectively . (**Fig. 2**). showed growth retardation .Cross sections through the fetal body revealed a few visceral anomalies. Major anomaly noted in the head region was hydrocephaly (**Fig. 3**), represented by enlargement of the cerebral ventricles. The hydrocephaly was 12.79 and 100% in fetuses exposed to methomyl at 0.67 and 2.0 mg/ kg.b.wt, respectively. The retention of fluid in the brain is the reason for this abnormality. This is agreement with the results obtained by (**Sakata-Haga et al. 2004 and Sitarek 2004**) with other teratogenic agents. Unilateral an ophthalmia (**Fig. 4**) were also observed in fetuses of the treatment groups. **Iyer et al. 1999, Ozeki and Shirai ,1998 and Hoogenboom et al. 1991** also reported these congenital eye abnormalities in rats and mice. The fetuses exhibited pulmonary hypoplasia as seen by small-sized lungs of 2.0 mg /kg.b.wt. dose group. Fetuses of mothers treated with methomyl during the period of organogenesis have exhibited several skeletal alterations as compared with control ones (**Fig. 5**).Skeletal anomalies showed a dose related response. The percentages of fetuses with skeletal abnormalities were 0.00, 24 and 100 for the control and the 0.67 and 2.0 mg/ kg. b. wt. methomyl groups, respectively. Bones of the skull of 21 days old fetuses maternally treated with low dose of methomyl (0.67 mg/ kg.b.wt) from the 6th to the 15th day of gestation, showed mild degree of lack of ossification of frontal, parietal, squamosal and occipital bones of the skull (**Fig. 5**). Hypoplasia of the phalanges of fore and hind limbs and absence of caudal vertebrae were also recorded. The dams were administered methomyl at doses of 2.0 mg /kg.b.wt. revealed severe retardation of ossification of skull bones , hypoplasia of metacarpal and metatarsal bones , hypoplasia of the phalanges of fore and hind limbs and absence of caudal vertebrae of their fetuses .Moreover, less ossification of the bones of the pelvic girdle (ilium, ischium and pubis) and the bones of the hind limbs (femur, tibia and fibula) were also seen. The fore and hind limbs not only showed lower degree of ossification but also became shorter as compared with the control fetuses. The reduced ossification of fetal skeletons may be a reason for decrease in fetal weight. Similar relationship between reduced fetal body weights and retarded ossification of the skeleton has been reported by(**Murray et al. 1979**) following exposure to insecticide in rabbits and mice.

Andrews and Gray,(1990) and Welsch and Morgan (1985) recorded incomplete ossification of most bones may be related to the effect of insecticide on calcium metabolism and/or bone morphometric by reducing the supply of calcium and magnesium ions to the growing fetus thereby inducing retardation in the bone development Moreover, the delay in ossification of the skeletal system may be associated to the delay in fetal growth, as indicated by the reduced fetal weights in the high dose group of the present study. have also related poor ossification of the skeletal system to growth retardation of the fetuses.

CONCLUSION

In conclusion, the occurrence of morphological, visceral and skeletal malformations in fetuses exposed to methomyl was dose dependent and was more severe in the group exposed to the dose of 2.0mg/ kg. b. wt. Accordingly, exposure to methomyl during pregnancy should be avoided or if necessary, their doses must be decreased.

Table 1: Showing the effect of methomyl on the body gain and fetal morphological changes in pregnant rats (n = 10 pregnant rats)

| Parameters | Control | Methomyl | |
|---------------------------------|------------------------|------------------------|--------------------------|
| | | 0.67mg/kg .b.wt | 2.0mg/kg.b.wt |
| body weight gain/100 g | 24.033±3.223 | 21.1±5.12 | 7.232±1.311* |
| Total No of viable fetuses | 130.99 | 116 | 12.02 |
| No. of viable fetuses | 13.0±0.73 (99.24%) | 11.6±1.246 (90.71%) | 1.202±1.31** (9.17%) |
| No. of dead fetuses | 0.13±0.232 (0.756%) | 1.24±0.611 (9.1%) | 0.79±1.01 (6.11%) |
| Number of resorbed fetuses | 0.0±0.00 (0.00%) | 0.0±0.00 (0.00%) | 10.99±1.04** (83.77%) |
| fetal body weight (g) | 4.01±0.201 | 3.37±0.218 | 1.11±0.22** |
| fetal body length (cm) | 4.23±0.101 | 3.54±0.132 | 1.74±0.199** |
| Morphological malformations (%) | 0.00 | 7.94 | 100.0 |
| Skeletal abnormalities% | 0.00 | 24.0 | 100.0 |
| Hydrocephaly % | 0.00 | 12.791 | 100.0 |

Significantly different from control group at *p<0.05 and **p<0.01, methomyl

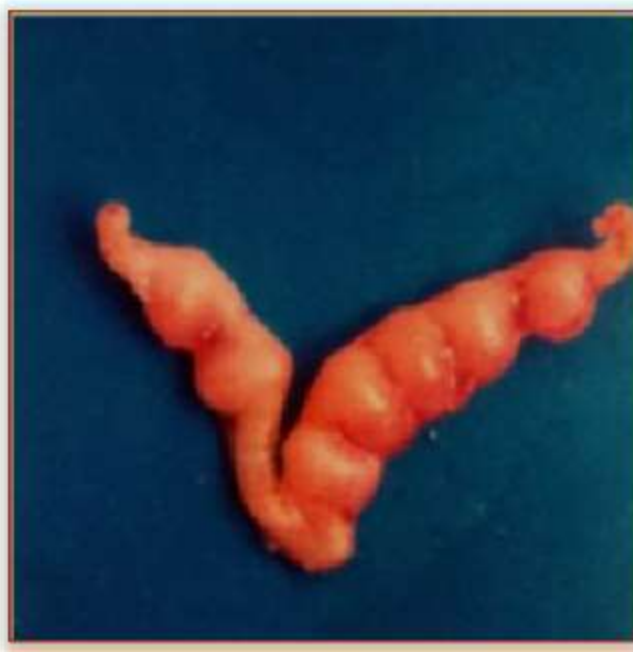


Fig. 1: Complete resorption in pregnant rats administered orally methomyl at 2.0 mg/ kg.b.wt



(A)



(B)

Fig. 2: Growth retardation (A) Control and (B) Malformed in a fetus obtained from pregnant rat administered orally methomyl at 2.0 mg/ kg.b.wt.

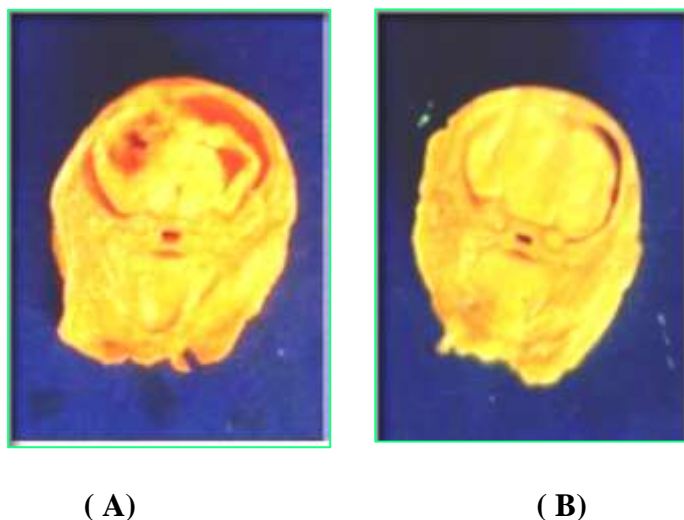


Fig. 3: Hydrocephaly in a fetus (A) Control and (B) Malformed obtained from pregnant rats exposed to methomyl at 2.0 mg/ kg.b.wt.

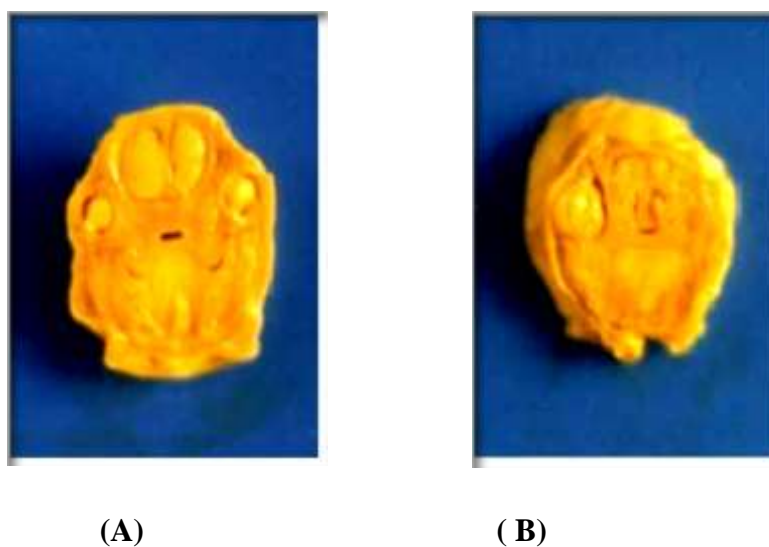


Fig. 4: Unilateral anophthalmia in a fetus (A) Control and (B) Malformed obtained from pregnant rats administered methomyl at 2.0 mg /kg.b.wt

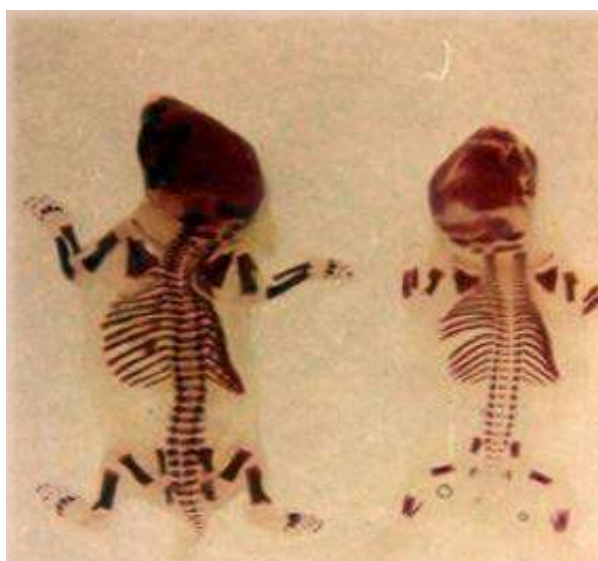


Fig. 5: Skull ossification, hypoplasia of metacarpal and metatarsal bones, hypoplasia of fore limb and hind limb phalanges and absence of caudal vertebrae in a fetus obtained from pregnant rats administered to methomyl at 2.0 mg/ kg.b.wt

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