CHAPTER – III

POSSIBLE MECHANISMS FOR THE ANTI-IMPLANTATION ACTION AND IN LATE PREGNANCY OF CARBOFURAN IN ALBINO MICE
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INTRODUCTION

The mammalian ovary not only produces oocytes but also serves as a source of critical hormones necessary for the development and function of the female reproductive system. Successful reproduction in the female entails the highly coordinated and synchronous interactions of a wide array of complex processes, such as gametogenesis, sperm-ovum interaction, implantation, embryo development and parturition. These processes occur in various reproductive organs and are mediated by the sequence of biochemical events that are precisely regulated by a series of steroids and peptide hormones. The physiological events associated with the fertilization and pregnancy in mammals, including humans, of which relatively little is known about xenobiotics that disrupt these processes and mechanisms by which they mediate these effects are poorly understood (Matt et al., 1995).

Some insecticides have been reported to reduce the fertility and the cause of sterility in animals viz. chlordecone (Pinkston and Uphouse, 1988), DDT (Johanson et al., 1988, 1990) Endrin, Lindane and TCDD (Hassoun et al., 1995) dicofol (Jadaramkunti, 1999) and endosulfan (Hiremath, 2000). It has been reported that administration of methoxychlor in rats reduces the gain in the maternal body weight during gestation and also showed foetotoxic effects (Khera et al., 1978). Number of organophosphorus pesticides has also been reported to affect implantation, gestation and foetal growth (Fish, 1966; Khan, 1981; Nehez et al., 1986; Mahadevaswami, 2002). Recently, it has been reported that oral administration of carbamate fungicide mancozeb for 1-7 days of pregnancy caused complete inhibition of implantation
(Bindali and Kaliwal, 2002). It has been reported that the administration of carbofuran to rats for 7 to 19 days of gestation causes increased foetal mortality and decreased foetal body weight, but there is no significant change in these parameters at lower doses (Courtney et al., 1985). Earlier studies in animals exposed to carbofuran during gestation are limited and scanty. Studies in chapter I and II indicates that the carbofuran interrupts the estrous cycle, decreases the number of healthy follicles with concomitant increase in the number of atretic follicles and inhibits the ovarian compensatory hypertrophy in hemicastrated mice. Keeping the above points in view, the present investigation is undertaken to study the effect of carbofuran on implantation, its reversal by progesterone and on the later part of pregnancy in albino mice.
MATERIALS AND METHODS

Normal cycling virgin female Swiss albino mice were used for this experiment. Mice aged between 90-100 days old and weighing 22-25 g were selected for the experiments. The mice showing late proestrus or estrus in the evening were mated overnight with the proven fertile male. Next day the female mice were checked for vaginal plugs. Subsequently on that day the animal was designated as day one of the pregnancy. The maintenance of the animal is explained in chapter I. The body weight and smear were recorded daily in the morning before the administration of carbofuran. The doses were given below the LD₅₀ level of intoxication, according to the body weights. The mice were divided into five groups each, consisting of ten animals for the experiments.

Experiment I

Graded doses of 0.4, 0.7, 1 and 1.3 mg/kg body weight/d carbofuran were administered orally from days 1-7 of pregnancy. This experiment was designed to determine the effective dose of carbofuran on implantation in albino mice.

Experiment II

Temporal study was designed to find out the optimum period necessary to affect the implantation by using the effective dose of carbofuran based on the Iˢᵗ experiment.

Experiment III

The graded doses of progesterone from 4, 9 and 12 mg/kg body weight/d were administered subcutaneously along with 1.3 mg/kg body weight/d of
carbofuran from days 1-7 of pregnancy to study the efficacy of progesterone in the maintenance of implantation in carbofuran treated mice. All the mice were autopsied on the 8th day of pregnancy.

**Experiment IV**

Mice were treated with 1.3 mg/kg body weight/d carbofuran from days 1-7 of pregnancy and they were laparotomized under the light ether anaesthesia in semisterile conditions on the 8th day of pregnancy to note the number of implantations, as almost all the mice showed no implantations. Later progesterone 4, 9 and 12 mg/kg body weight/d was administered subcutaneously from days 8-15 of pregnancy to know the effect of progesterone in the anti-implantation activity of carbofuran in mice. The mice were autopsied on the 16th day of pregnancy.

**Experiment V**

The pregnant mice were laparotomized under mild ether anaesthesia under semisterile conditions on the 8th day of pregnancy to note the number of implantations. Mice having normal implantations were continued for the experiment. Graded doses of 0.4, 0.7, 1 and 1.3 mg/kg body weight/d carbofuran were administered orally from days 7-15 of pregnancy. This experiment was designed to determine the effective dose of carbofuran, which might cause abortion or foetal resorption in mice. The animals were autopsied on the 19th day of pregnancy.

**Experiment VI**

Graded doses of 0.4, 0.7, 1 and 1.3 mg/kg body weight/d carbofuran were administered orally from 7-15 days of pregnancy and animals were allowed to
deliver. The experiment was designed to know the effect of carbofuran on the length of gestation in mice.

In all the above mentioned experiments suitable olive oil treated control mice were maintained. The mice were autopsied by cervical dislocation and the ovaries, uterus, kidney, adrenals, liver, spleen, thymus and thyroid were dissected out, freed from the adhering tissue and weighed to the nearest milligram in single pan “Dhona” electrical balance. The number of foetuses, placental scars and corpora lutea were recorded in the experiments.
OBSERVATIONS

Experiment I

Anti-implantation activity of carbofuran in albino mice (Table 3.1; Graph 3.1, 3.2)

In this experiment, 0.4, 0.7, 1 and 1.3 mg/kg body weight/d of carbofuran is administered orally from 1-7 days of pregnancy. The results indicate that all the mated control mice are pregnant and show a mean number of 11.0 implantations at autopsy on the 8th day. The uterus shows normal implantation sites (Fig.1). The ovaries show a mean number of 11.6 corpora lutea and the pre-implantation loss is 5.17%. The mice exhibit a continuous diestrus phase of 7 days.

Treatment with 0.4 mg carbofuran from 1-7 days of pregnancy causes no inhibition of implantations. All the mated mice show a mean number of 9.8 implantation sites on the 8th day of pregnancy. The uterus shows normal implantation sites (Fig.2). The ovaries show a mean number of 11.1 corpora lutea as a result, the pre-implantation loss is 11.71%. There is no significant change in the number of implantation sites and corpora lutea when compared to that of the control mice. The mice exhibit a continuous diestrus phase of 7 days.

Treatment with 0.7 mg and 1 mg carbofuran from 1-7 days of pregnancy causes no inhibition of implantations. All the mated mice show a mean number of 8.1 and 3.3 implantation sites respectively on the 8th day of pregnancy. The uterus shows normal implantation sites (Figs. 3 & 4). The ovaries show a mean number of 10.5 and 10.2 corpora lutea as a result, the pre-implantation loss is 22.86 and 67.65% respectively. There is a significant decrease in the number of implantation sites when compared to that of the control mice. There is no significant change in
the corpora lutea when compared to that of the control mice. The mice exhibit a continuous diestrus phase for 7 days with 0.7 mg carbofuran treatment. However, the mice treated with 1 mg carbofuran shows 6.6 days of diestrus phase with a concomitant significant increase in the estrus phase of 0.4 days when compared to that of the control mice.

Treatment with 1.3 mg carbofuran from 1-7 days of pregnancy seems to be an effective dose as none of the mice show implantations. The uterus resembles that of the non-pregnant mice (Fig.5). The ovaries show a mean number of 10.3 numbers of corpora lutea as a result, the pre-implantation loss is increased to 100%. The mice exhibit 4.4 days of diestrus and 2.6 days of estrus phase.

The results of the present study indicate that the treatment with 0.4 and 0.7 mg carbofuran from 1-7 days of pregnancy causes no inhibition of implantations. There is a partial inhibition of implantations wherein 5 out of 10 mice are pregnant with 1 mg carbofuran treatment from 1-7 days of pregnancy. However, treatment with 1.3 mg carbofuran from 1-7 days of pregnancy causes complete inhibition of implantations (Table 3.1; Graphs 3.1, 3.2).

**Body weight (Table 3.2; Graph 3.3)**

The change in the mean body weight is 3.35 g in the control mice when compared to that of the initial body weight. Change in the mean body weight with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 3.10, 2.9, 2.20 and 1.50 g respectively. There is no significant change in the body weight with 0.4 and 0.7 mg carbofuran treatment. However, there is a significant decrease in the body weight with 1 and 1.3 mg carbofuran treatment when compared to that of the control mice.
Organs weight (Table 3.2 ; Graphs 3.3, 3.4)

Ovaries

The mean weight of the ovaries is 28.05 mg in the control mice. The mean weight of the ovaries with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 28.81, 29.87, 27.06 and 25.20 mg respectively. There is no significant change in the weight of the ovaries with 0.4, 0.7 and 1 mg carbofuran treatment. However, there is a significant decrease in the weight of the ovaries with 1.3 mg carbofuran treatment when compared with that of the control mice.

Uterus

The mean weight of the uterus is 507.06 mg in control mice. The mean weight of the uterus with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 502.49, 489.56, 477.83 and 437.46 mg respectively. There is no significant change in the weight of the uterus with 0.4, 0.7 and 1 mg carbofuran treatment. But, there is a significant decrease in the weight of the uterus with 1.3 mg carbofuran treatment when compared to that of the control mice.

Liver

The mean weight of the liver is 4.59 g in control mice. The mean weight of the liver with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 4.70, 4.51, 4.37 and 4.24 g respectively. There is no significant change in the weight of the liver with 0.4, 0.7 and 1 mg carbofuran treatment. However, there is a significant decrease in the weight of the liver with 1.3 mg carbofuran treatment when compared to that of the control mice.
Kidneys

The mean weight of the kidneys is 1.03 g in control mice. The mean weight of the kidneys with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 1.04, 1.01, 1.01 and 0.98 g respectively. There is no significant change in the weight of the kidneys of all the carbofuran treated mice when compared to that of the control mice.

Adrenals

The mean weight of the adrenals is 33.41 mg in control mice. The mean weight of the adrenals with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 35.43, 32.95, 33.23 and 31.91 mg respectively. There is no significant change in the weight of the adrenals of all the carbofuran treated mice when compared to that of the control mice.

Spleen

The mean weight of the spleen is 371.87 mg in control mice. The mean weight of the spleen with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 373.27, 368.79, 366.17 and 367.95 mg respectively. There is no significant change in the weight of the spleen of all the carbofuran treated mice when compared to that of the control mice.

Thymus

The mean weight of the thymus is 94.42 mg in control mice. The mean weight of the thymus with 0.4, 0.7, 1 and 1.3 mg carbofuran is 95.49, 97.20, 91.65 and 91.72 mg treatment respectively. There is no significant change in the weight of the thymus of all the carbofuran treated mice when compared to that of the control mice.
**Thyroid**

The mean weight of the thyroid is 8.79 mg in control mice. The mean weight of the thyroids with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment is 8.68, 8.92, 9.06 and 9.19 mg respectively. There is no significant change in the weight of the thyroid of all the carbofuran treated mice when compared to that of the control mice.

The results of the present study reveal that there is a significant decrease in the gain of the body weight with 1 and 1.3 mg carbofuran treatment. There is a significant decrease in the weight of ovaries, uterus and liver with 1.3 mg carbofuran treatment. However, there is no significant change in the weight of the kidneys, adrenals, spleen, thymus and thyroid of all the carbofuran treated mice (Table 3.2; Graphs 3.3, 3.4).

**Experiment II**

**Temporal effect of carbofuran on implantation in albino mice (Table 3.3; Graphs 3.5, 3.6)**

Temporal study is conducted to find out the optimum period necessary to affect the implantations by using the effective dose of carbofuran based on dose experiment. The effective dose of 1.3 mg carbofuran was administered orally on the 3rd day and from days 1-3, 1-5 and 1-7 of pregnancy. The results indicate that all the mated control mice are pregnant and show a mean number of 11.0 implantations on the 8th day. The uterus shows normal implantation sites (Fig.6). The ovaries show a mean number of 11.6 corpora lutea as a result, the pre-implantation loss is 5.17%. These mice exhibit a continuous diestrus phase of 7 days.
Treatment with 1.3 mg carbofuran on the 3rd day and from days 1-3 of pregnancy causes partial inhibition of implantations wherein 8 out of 10 mice are pregnant on the 8th day with a mean number of 7.2 and 6.2 implantation sites respectively. The uterus shows normal implantation sites (Figs. 7 & 8). The ovaries show a mean number of 11.2 and 10.6 corpora lutea as a result, the pre-implantation loss is 35.71 and 41.51% with 1.3 mg carbofuran treatment on the 3rd day and from days 1-3 of pregnancy respectively. There is a significant decrease in the number of implantations. These mice exhibit a continuous diestrus phase of 7.00 and 5.60 days and estrus of 0.00 and 1.20 days respectively.

-Treatment with 1.3 mg carbofuran from 1-5 days causes partial inhibition of implantation wherein 3 out of 10 mice were pregnant on the 8th day with a mean number of 1.9 implantation sites. The uterus shows normal implantation sites (Fig.9). The ovaries show a mean number of 10.4 corpora lutea as a result, the pre-implantation loss is 81.61%. There is a significant decrease in the number of implantations. These mice exhibit a continuous diestrus phase of 4.70 days and estrus phase of 2.30 days.

-Treatment with 1.3 mg carbofuran from 1-7 days of pregnancy causes complete inhibition of implantations. None of the mice are pregnant on the 8th day. The uterus does not show any implantation sites and resembles that of non-pregnant mice (Fig.10). This indicates that the prolonged treatment of carbofuran completely inhibits implantations in mice. The ovary shows a mean number of 10.3 corpora lutea as a result, the pre-implantation loss is 100%. These mice exhibit a diestrus phase of 4.40 and estrus phase of 2.60 days.
The results of the present study indicate that the treatment with 1.3 mg carbofuran on the 3rd day of pregnancy causes partial inhibition of implantations wherein 8 mice out of 10 mice were pregnant. Treatment with 1.3 mg carbofuran from 1-3 days of pregnancy causes partial inhibition of implantations wherein 8 mice out of 10 mice are pregnant. However, treatment with 1.3 mg carbofuran from 1-5 days of pregnancy causes partial inhibition of implantations wherein only 3 mice out of 10 mice are pregnant. However, treatment with 1.3 mg carbofuran from days 1-7 causes complete inhibition of implantations in mice (Table 3.3; Graphs 3.5, 3.6).

Body weight (Table 3.4; Graph 3.7)

The change in the mean body weight is 3.35 g in control mice when compared to that of the initial body weight. Change in the mean body weights with 1.3 mg carbofuran treatment on the 3rd day and from 1-3, 1-5 and 1-7 days of pregnancy is 3.30, 2.90, 2.20 and 1.50 g respectively. There is no significant change in the body weight with 1.3 mg carbofuran treatment on the 3rd day and from days 1-3 of pregnancy. However, there is a significant decrease in the body weight with 1.3 mg carbofuran treatment from 1-5 and 1-7 days of pregnancy when compared to that of the control mice.

Organs weight (Table 3.4; Graphs 3.7, 3.8)

Ovaries

The mean weight of the ovaries is 28.05 mg in control mice. The mean weight of the ovaries with 1.3 mg carbofuran treatment on the 3rd day and from 1-3, 1-5 and 1-7 days of pregnancy is 27.75, 26.98, 25.96 and 25.20 mg respectively.
There is no significant change in the weight of the ovaries of all the treated mice when compared to that of the control mice.

**Uterus**

The mean weight of the uterus is 507.06 mg in the control mice. The mean weight of the uterus with 1.3 mg carbofuran treatment on the 3rd day and from 1-3, 1-5 and 1-7 days of pregnancy is 501.85, 488.72, 480.75 and 437.46 mg respectively. There is no significant change in the weight of the uterus with 1.3 mg carbofuran treatment on the 3rd day. However, there is a significant decrease in the weight of the uterus with 1.3 mg carbofuran treatment from 1-3, 1-5 and 1-7 days when compared to that of the control mice.

**Kidneys**

The mean weight of the kidneys is 1.03 g in control mice. The mean weight of the kidneys with 1.3 mg carbofuran treatment on the 3rd day and from 1-3, 1-5 and 1-7 days of pregnancy is 1.03, 1.04, 1.01 and 0.98 g respectively. There is no significant change in the weight of the kidneys of all the carbofuran treated mice when compared to that of the control mice.

**Adrenals**

The mean weight of the adrenal is 33.41 mg in control mice. The mean weight of the adrenals with 1.3 mg carbofuran treatment on the 3rd day and from 1-3, 1-5 and 1-7 days of pregnancy is 34.41, 34.67, 32.79 and 31.91 mg respectively. There is no significant change in the weight of the adrenals of all the carbofuran treated mice when compared to that of the control mice.
Liver

The mean weight of the liver is 4.59 g in control mice. The mean weight of the liver carbofuran treatment on the 3rd day and from 1-3, 1-5 and 1-7 days of pregnancy is 4.47, 4.72, 4.36 and 4.24 g with 1.3 mg respectively. There is no significant change in the weight of the liver with 1.3 mg carbofuran treatment on the 3rd day and from 1-3 and 1-5 days of pregnancy. However, there is a significant decrease in the weight of the liver with 1.3 mg carbofuran treatment from 1-7 days of pregnancy when compared to that of the control mice.

Spleen

The mean weight of the spleen is 371.87 mg in control mice. The mean weight of the spleen with 1.3 mg carbofuran treatment on the 3rd day and from 1-3, 1-5 and 1-7 days of pregnancy is 372.11, 369.53, 370.21 and 367.95 mg respectively. There is no significant change in the weight of the spleen of all the carbofuran treated mice when compared to that of the control mice.

Thymus

The mean weight of the thymus is 94.42 mg in control mice. The mean weight of the thymus with 1.3 mg carbofuran treatment on the 3rd day and from 1-3, 1-5 and 1-7 days of pregnancy is 93.58, 93.20, 91.81 and 91.72 mg respectively. There is no significant change in the weight of the thymus of all the carbofuran treated mice when compared to that of the control mice.

Thyroid

The mean weight of the thyroid is 8.79 mg in control mice. The mean weight of the thyroid with 1.3 mg carbofuran treatment on the 3rd day and from 1-3, 1-5 and
1-7 days of pregnancy is 8.57, 8.81, 9.1 and 9.19 mg respectively. There is no significant change in the weight of the thyroid of all the carbofuran treated mice when compared to that of the control mice.

The results of the present study indicate that the treatment with 1.3 mg carbofuran from 1-5 and 1-7 days of pregnancy causes a significant decrease in the body weight. Treatment with 1.3 mg carbofuran from 1-3, 1-5 and 1-7 days of pregnancy causes a significant decrease in the weight of the uterus. Treatment with 1.3 mg carbofuran from 1-7 days causes a significant decrease in the weight of the liver. There is no significant change in the weight of the ovary, kidney, adrenal, spleen, thymus and thyroid of all the carbofuran treated mice (Table 3.4; Graphs 3.7, 3.8).

Experiment III

Efficacy of progesterone in the maintenance of implantation in carbofuran treated albino mice (Table 3.5; Graphs 3.9, 3.10)

In this experiment 4, 9 and 12 mg of progesterone is administered subcutaneously along with 1.3 mg carbofuran from days 1-7 of pregnancy. The results indicate that in controls all the mated mice were pregnant on the 8th day with a mean number of 11.0 implantations. The uterus shows normal implantation sites (Fig.11). The ovaries show a mean number of 11.6 corpora lutea as a result, the pre-implantation loss is 5.17%. These mice exhibit a continuous diestrus phase of 7 days.

Treatment with 1.3 mg carbofuran from 1-7 days of pregnancy seems to be an effective dose as none of the mated mice are pregnant on the 8th day, thus causing
a complete inhibition of implantations (Fig. 12). The ovaries show a mean number of 10.3 corpora lutea as a result, the pre-implantation loss is 100%. These mice exhibit a continuous diestrus phase of 4.40 days and estrus phase of 2.60 days.

Treatment with 4, 9 and 12 mg of progesterone and 1.3 mg carbofuran from 1-7 days of pregnancy shows that none of the mated mice maintain the implantations on the 8th day. The uterus resembles that of non-pregnant mice (Figs. 13, 14, 15). Treatment with 4, 9 and 12 mg of progesterone along with 1.3 mg carbofuran from 1-7 days of pregnancy wherein the ovaries show a mean number of 10.5, 10.9 and 10.8 corpora lutea respectively, as a result, the pre-implantation loss is 100% in all the groups. Thus, progesterone is unable to maintain the implantations as the carbofuran may show the blostotoxic effect. The mice with 4, 9 and 12 mg progesterone and 1.3 mg of carbofuran treatment from 1-7 days of pregnancy exhibit a diestrus phase of 4.00, 4.20 and 4.50 days and estrus phase of 2.90, 2.80 and 2.50 days respectively.

The results of the present study indicate that the treatment with 4, 9 and 12 mg of progesterone and 1.3 mg carbofuran from 1-7 days of pregnancy are unable to counteract the effect of carbofuran and did not maintain implantations. This indicates that carbofuran may be blostotoxic (Table 3.5; Graphs 3.9, 3.10).

**Body weight (Table 3.6, Graph 3.11)**

The change in the mean body weight is 3.35 g in control mice when compared to that of the initial body weight. Change in the mean body weight is 1.50 g with 1.3 mg carbofuran treated mice. Change in the mean body weight with 4, 9 and 12 mg of progesterone treatment and 1.3 mg carbofuran treatment from 1-7
days of pregnancy is 2.10, 2.10 and 1.80 g respectively. There is a significant
decrease in the gain of the body weight of all the carbofuran treated mice when
compared to that of the control mice.

**Organs weight (Table 3.6; Graphs 3.11, 3.12)**

**Ovaries**

The mean weight of the ovaries is 28.05 and 25.20 mg in control and in mice
treated with 1.3 mg carbofuran. The mean weight of the ovaries with 4, 9 and 12 mg
of progesterone and 1.3 mg carbofuran treatment form 1-7 days of pregnancy is
24.13, 23.73 and 23.27 mg respectively. There is no significant change in the weight
of the ovaries in the treatment with 4 mg progesterone along with 1.3 mg carbofuran
treatment. However, there is a significant decrease in the weight of the ovaries with
9 and 12 mg of progesterone along with 1.3 mg carbofuran treatment from 1-7 days
of pregnancy when compared to that of the control mice.

**Uterus**

The mean weight of the uterus is 507.06 and 437.46 mg in control and in the
mice treated with 1.3 mg carbofuran. The mean weight of the uterus with 4, 9 and
12 mg of progesterone and 1.3 mg carbofuran treatment from 1-7 days of pregnancy
is 493.99, 448.19 and 438.52 mg respectively. There is a significant decrease in the
weight of the uterus of all the carbofuran treated mice when compared to that of the
control mice.

**Kidneys**

The mean weight of the kidneys is 1.03 and 0.98 g in control and in the mice
treated with 1.3 mg carbofuran. The mean weight of the kidneys with 4, 9 and 12
mg of progesterone and 1.3 mg carbofuran treatment from 1-7 days of pregnancy is 1.05, 1.02 and 1.03 g respectively. There is no significant change in the weight of the kidneys of all the carbofuran treated mice when compared to that of the control mice.

Adrenals

The mean weight of the adrenals is 33.41 and 31.91 mg in control and in mice treated with 1.3 mg carbofuran. The mean weight of the adrenals with 4, 9 and 12 mg of progesterone and 1.3 mg carbofuran treatment from 1-7 days of pregnancy is 32.95, 32.82 and 31.60 mg respectively. There is no significant change in the weight of the adrenals of all the carbofuran treated mice when compared to that of the control mice.

Spleen

The mean weight of the spleen is 371.87 and 365.95 mg in control and in mice treated with 1.3 mg carbofuran. The mean weight of the spleen with 4, 9 and 12 mg of progesterone and 1.3 mg carbofuran treatment from 1-7 days of pregnancy is 368.62, 367.19 and 366.12 mg respectively. There is no significant change in the weights of the spleen of all the carbofuran treated mice when compared to that of the control mice.

Liver

The mean weight of the liver is 4.59 and 4.24 g in control and in mice treated with 1.3 mg carbofuran. The mean weight of the liver with 4, 9 and 12 mg of progesterone and 1.3 mg carbofuran treatment from 1-7 days of pregnancy is 4.53, 4.56 and 4.32 g respectively. There is no significant change in the weights of the
liver of all the carbofuran treated mice. However, there was a significant decrease in the weight of the liver with 1.3 mg carbofuran treatment when compared to that of the control mice.

**Thymus**

The mean weight of the thymus is 94.42 and 91.72 mg in control and in mice treated with 1.3 mg carbofuran. The mean weight of the thymus with 4, 9 and 12 mg of progesterone and 1.3 mg carbofuran treatment from 1-7 days of pregnancy is 95.27, 93.02 and 93.64 mg respectively. There is no significant change in the weight of the thymus of all the carbofuran treated mice when compared to that of the control mice.

**Thyroid**

The mean weight of the thyroid is 8.79 and 9.19 mg in control and in mice treated with 1.3 mg carbofuran. The mean weight of the thyroid with 4, 9 and 12 mg of progesterone and 1.3 mg carbofuran treatment from 1-7 days of pregnancy is 9.17, 9.06 and 9.28 mg respectively. There is no significant change in the weight of the thyroid of all the carbofuran treated mice when compared to that of the control mice.

The results of the present study indicate that the treatment with 4, 9 and 12 mg of progesterone and 1.3 mg carbofuran from 1-7 days of pregnancy causes a significant decrease in the gain of the body weight and weight of the uterus. There is a significant decrease in the weight of the ovaries with 9 and 12 mg of progesterone and 1.3 mg carbofuran treatment from 1-7 days of pregnancy. However, there is no significant change in the weight of the kidneys, adrenals, liver,
spleen, thymus and thyroid of all the progesterone and carbofuran treated mice from 1-7 days of pregnancy (Table 3.6; Graphs 3.11, 3.12).

**Experiment IV**

**Implantation delay and nidation by progesterone in carbofuran treated albino mice (Table 3.7; Graphs 3.13, 3.14)**

In experiment I, II and III, it was shown that 1.3 mg carbofuran administered orally from 1-7 days of pregnancy caused inhibition of implantations. The implantations were not maintained when progesterone and carbofuran were administered concurrently. The question arises whether the inhibition of implantation by carbofuran might be due to several factors such as delayed transportation of blastocyst from the fallopian tube, blastotoxic, expulsion of the blastocyst from the uterus or an imbalance in the estrogen : progesterone ratio which might be the cause where the viable blastocyst is unable to implant. Hence, pregnant mice treated with 1.3 mg carbofuran from 1-7 days of pregnancy and laparotomized on the 8th day to note the number of implantations. Almost all mice show no implantations and were autopsied on the 16th day. Later, to other groups 4, 9 and 12 of progesterone is administered subcutaneously from 8-15 days to know the efficacy of progesterone on the anti-implantation activity of carbofuran in albino mice. The results indicate that in controls, all the mated mice were pregnant at laparotomy on the 8th day with a mean number of 10.3 implantations. The uterus shows normal embryos on the 16th day (Fig. 16). The ovaries show a mean number of 11.2 corpora lutea. The pre-implantation loss is 8.04%. Treatment with 1.3 mg carbofuran from 1-7 days of pregnancy shows no implantation sites at laparotomy on the 8th day.
From 8-15 days, no treatment is given and the mice show no implantations on the 16th day (Fig. 17). This indicates that the carbofuran inhibits implantations. The ovaries show a mean number of 11.0 corpora lutea as a result, the pre-implantation loss is 100%.

Treatment with 1.3 mg carbofuran from 1-7 days of pregnancy shows no implantation sites at laparotomy on the 8th day. From 8-15 days of pregnancy, 4, 9 and 12 mg of progesterone is administered and the mice show no implantation sites. The uterus resembled that of non-pregnant mice (Fig. 18, 19, 20). The ovaries show a mean number of 10.9, 11.0 and 10.6 corpora lutea respectively as a result, the pre-implantation loss is 100%.

The results of the present study indicate that the treatment with 1.3 mg carbofuran from 1-7 days of pregnancy and autopsied on the 16th day show no implantations. Treatment with 4, 9 and 12 mg of progesterone from 8-15 days of pregnancy in 1.3 mg carbofuran treated mice from 1-7 days of pregnancy, are unable to show delayed implantations. This indicates that carbofuran is blastotoxic and the viable blastocytes are not present in the uterus as the progesterone is unable to induce delayed implantation after the 8th day of pregnancy in mice. This indicates that carbofuran may be blastotoxic (Table 3.7; Graphs 3.13, 3.14).

**Body weight (Table 3.8; Graph 3.15)**

The change in the gain of the mean body weight is 7.25 and 1.60 g in control and in mice treated with 1.3 mg carbofuran respectively when compared to that of the initial body weights. Change in the gain of the mean body weight with 4, 9 and 12 mg of progesterone treatment from 8-15 days of pregnancy in 1.3 mg carbofuran
treated mice from 1-7 days of pregnancy is 1.80, 1.90 and 2.10 g respectively. There is a significant decrease in the gain of the body weight of all the carbofuran treated mice when compared to that of the control mice.

Organs weight (Table 3.8; Graphs 3.15, 3.16)

Ovaries

The mean weight of the ovaries is 27.94 and 24.78 in control and in mice treated with 1.3 mg carbofuran respectively. The mean weight of the ovaries with 4, 9 and 12 mg of progesterone treatment from 8-15 days in 1.3 mg carbofuran treated mice from 1-7 days of pregnancy is 27.34, 26.78 and 27.11 mg respectively. There is no significant change in the weight of the ovaries of all the carbofuran treated mice when compared to that of the control mice.

Uterus

The mean weight of the uterus is 2.33 g and 485.66 mg in control and in mice treated with 1.3 mg carbofuran respectively. The mean weight of the uterus with 4, 9 and 12 mg of progesterone from 8-15 days in 1.3 mg carbofuran treated mice from 1-7 days of pregnancy is 479.85, 477.29 and 472.04 mg respectively. There is a significant decrease in the weight of the uterus in all the carbofuran treated mice when compared with that of the control mice.

Kidney

The mean weight of the kidneys is 1.08 and 1.06 g in control and in mice treated with 1.3 mg carbofuran respectively. The mean weight of the kidneys with 4, 9 and 12 mg of progesterone treatment from 8-15 days in 1.3 mg carbofuran treated mice from 1-7 days of pregnancy is 1.07, 1.03 and 1.00 g respectively. There is no
significant change in the weight of the ovaries of all the carbofuran treated mice when compared to that of the control mice.

**Adrenal**

The mean weight of the adrenals is 33.76 and 34.29 mg in control and in mice treated with 1.3 mg carbofuran respectively. The mean weight of the adrenals with 4, 9 and 12 mg of progesterone from 8-15 days in 1.3 mg carbofuran treated mice from 1-7 days of pregnancy is 34.21, 32.08 and 31.51 mg respectively. There is no significant change in the weight of the adrenals of all the carbofuran treated mice when compared to that of the control mice.

**Liver**

The mean weight of the liver is 4.49 and 4.45 g in control and in mice treated with 1.3 mg carbofuran respectively. The mean weight of the liver with 4, 9 and 12 mg of progesterone from 8-15 days in 1.3 mg carbofuran treated mice from 1-7 days of pregnancy is 4.50, 4.41 and 4.44 g respectively. There is no significant change in the weight of the liver of all the carbofuran treated mice when compared to that of the control mice.

**Spleen**

The mean weight of the spleen is 372.47 and 375.04 mg in control and in mice treated with 1.3 mg carbofuran respectively. The mean weight of the spleen with 4, 9 and 12 mg of progesterone from 8-15 days in 1.3 mg carbofuran treated mice from 1-7 days of pregnancy is 374.74, 368.56 and 368.28 mg respectively. There is no significant change in the weights of the spleen of all the carbofuran treated mice when compared to that of the control mice.
Thymus

The mean weight of the thymus is 90.78 and 91.86 mg in control and in mice treated with 1.3 mg carbofuran respectively. The mean weight of the thymus with 4, 9 and 12 mg of progesterone from 8-15 days in 1.3 mg carbofuran treated mice from 1-7 days of pregnancy is 91.51, 89.90 and 87.98 mg respectively. There is no significant change in the weight of the thymus of all the carbofuran treated mice when compared to that of the control mice.

Thyroid

The mean weight of the thyroid is 8.55 and 8.81 mg in control and in mice treated with 1.3 mg carbofuran respectively. The mean weight of the thyroid with 4, 9 and 12 mg of progesterone from 8-15 days in 1.3 mg carbofuran treated mice from 1-7 days of pregnancy is 8.95, 9.27 and 9.32 mg respectively. There is no significant change in the weight of the thyroid of all the carbofuran treated mice when compared to that of the control mice.

The results of the present study indicate that the treatment with 4, 9 and 12 mg of progesterone from 8-15 days in 1.3 carbofuran treated mice from 1-7 days caused a significant decrease in the gain of the body weight and weight of the uterus. However, there is no significant change in the weights of the ovaries, kidneys, adrenals, spleen, liver, thymus and thyroid of all the carbofuran treated mice when compared to that of the control mice (Table 3.8; Graphs 3.15, 3.16).
Experiment V

Effect of carbofuran on pregnancy, body and organs weight in albino mice
(Table 3.9; Graphs 3.17, 3.18)

In this experiment, graded doses of 0.4, 0.7, 1 and 1.3 mg/kg body weight/d carbofuran is administered orally from days 7-15 of pregnancy. The results reveal that olive oil treated control mice show normal pregnancy. The mice show a mean number of 9.8 implantation sites on the 8th day. The ovaries show a mean number of 10.2 corpora lutea. The mean number of live foetuses is 9.5. The post-implantation loss and foetal survivality is 2.94 and 96.94% respectively.

Treatment with 0.4, 0.7, 1 and 1.3 mg carbofuran from 7-15 days of pregnancy shows no inhibition of pregnancy of all the carbofuran treated mice. These mice show normal implantations at laparotomy on the 8th day with a mean number of 9.10, 8.00, 6.50 and 6.10 implantation sites respectively. The ovaries show a mean number of 10.1, 9.8, 8.8 and 7.7 corpora lutea with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment respectively. There is no significant decrease in the number of corpora lutea with 1.3 mg carbofuran treatment. There is no significant change in the corpora lutea with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment when compared with that of the controls. However, there is a significant decrease in the number of implantations and live foetuses in 1 and 1.3 mg carbofuran treated mice with a post-implantation loss of 4.55 and 5.19% and fetal survivality of 93.85 and 93.44%. There was no significant change in the number of implantations and live foetuses in 0.4 and 0.7 mg carbofuran treated mice with a post-implantation loss of 1.98 and 2.04% and fetal survivality of 97.80 and 97.50%. The mean weight of
foetuses is 1.24 g in control mice. The mean weight of fetus with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment from 7-15 days of pregnancy is 1.29, 1.24, 1.14 and 1.05 g respectively. There is a significant decrease in the fetal weight with 1.3 mg carbofuran treatment. However, there is no significant change in the fetus weight with 0.4, 0.7 and 1 mg carbofuran treatment from 7-15 days of pregnancy. The above results clearly indicate that carbofuran is not effective on the later part of pregnancy, as pregnancy is maintained in all the treated mice (Table 3.9; Graphs 3.17, 3.18).

**Body weight (Table 3.10; Graph 3.19)**

The change in the gain of the mean body weight is 8.67 g in control mice when compared to that of the initial body weight. Change in the gain of the mean body weight with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment from 7-15 days of pregnancy is 8.45, 7.85 and 7.63 g respectively. There is a significant decrease in the body weight in 1 and 1.3 mg carbofuran treatment. However, there is no significant change in the gain of the body weight in 0.4 and 0.7 mg carbofuran treated mice when compared to that of the control mice.

**Organs weight (Table 3.10; Graph 3.19, 3.20)**

**Ovaries**

The mean weight of the ovaries is 26.55 mg in control mice. The mean weight of the ovaries with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment from 7-15 days of pregnancy is 24.77, 25.58, 24.14 and 23.50 mg respectively. There is no significant change in the weight of the ovaries of all the carbofuran treated mice when compared to that of the control mice.
**Uterus**

The mean weight of the uterus is 3.46 g in control mice. The mean weight of the uterus with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment from 7-15 days of pregnancy is 3.44, 3.39, 3.20 and 3.06 g respectively. There is a significant decrease in the weight of the uterus in 1 and 1.3 mg carbofuran treatment. However, there is no significant change in the weight of the uterus in 0.4 and 0.7 mg carbofuran treated mice when compared to that of the control mice.

**Kidneys**

The mean weight of the kidneys is 1.13 g in control mice. The mean weight of the kidneys with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment from 7-15 days of pregnancy is 1.14, 1.14, 1.11 and 1.06 g respectively. There is no significant change in the weight of the kidneys of all the carbofuran treated mice when compared to that of the control mice.

**Adrenals**

The mean weight of the adrenals is 30.33 gm in control mice. The mean weight of the adrenals with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment from 7-15 days of pregnancy is 30.18, 29.26, 29.97 and 28.32 mg respectively. There is no significant change in the weight of the adrenals of all the carbofuran treated mice when compared to that of the control mice.

**Liver**

The mean weight of the liver is 4.57 g in control mice. The mean weight of the liver with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment from 7-15 days of pregnancy is 4.62, 4.51, 4.51 and 4.50 g respectively. There is no significant change
in the weight of the liver of all the carbofuran treated mice when compared to that of the control mice.

**Spleen**

The mean weight of the spleen is 385.17 mg in control mice. The mean weight of the spleen with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment from 7-15 days of pregnancy is 383.31, 380.12, 380.86 and 379.14 mg respectively. There is no significant change in the weight of the spleen of all the carbofuran treated mice when compared to that of the control mice.

**Thymus**

The mean weight of the thymus is 89.88 mg in control mice. The mean weight of the thymus with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment from 7-15 days of pregnancy is 88.85, 87.22, 87.76 and 86.84 mg respectively. There is no significant change in the weight of the thymus of all the carbofuran treated mice when compared to that of the control mice.

**Thyroid**

The mean weight of the thyroid is 9.17 mg in control mice. The mean weight of the thyroid with 0.4, 0.7, 1 and 1.3 mg carbofuran treatment from 7-15 days of pregnancy is 9.03, 9.27, 9.43 and 9.79 mg respectively. There is no significant change in the weight of the thyroid of all the carbofuran treated mice when compared to that of the control mice.

The results of the present study indicate that the treatment with 1 and 1.3 mg carbofuran from 7-15 days of pregnancy causes a significant decrease in the gain of the body weight and weight of the uterus. There is also a significant decrease in the
weight of the foetus with 1.3 mg carbofuran treatment. There is no significant change in the weight of the ovaries, kidneys, adrenals, liver, spleen, thymus and thyroid of all the carbofuran treated mice when compared to that of the control mice (Table 3.10; Graphs 3.19, 3.20).

Experiment VI

Effect of carbofuran on gestation length in albino mice (Table 3.11)

In this experiment, graded doses of 0.4, 0.7, 1 and 1.3 mg/kg body weight/d carbofuran is administered orally from 7-15 days of pregnancy. The control mice show a mean gestation length of 19.60 days. Treatment with 0.4, 0.7, 1 and 1.3 mg carbofuran show a mean gestation length of 19.80, 19.95, 20.40 and 20.50 days respectively. The results of the present study indicate that there is a dose related increase in the gestation length. The increase in the gestation length in all the carbofuran treated mice is found to be non significant (Table 3.11).


DISCUSSION

Effect of carbofuran on implantation in albino mice

Despite our understanding of the physiologic events associated with the fertilization, implantation and pregnancy in mammals including humans, relatively little is known about xenobiotics that disrupt these processes (Matt and Borzelleca, 1995). Implantation of blastocyst in the uterine endometrium is the basic feature of mammalian reproduction. It is the result of a complex series of interactive steps beginning with fixation of blastocyst in the uterus and ending with the formation of a definitive placenta (Weitlauf, 1994).

Crucial to successful implantation is the appropriate timing of the uterine endometrium at which blastocyst is capable of implanting only during a brief period of uterine receptivity, described as “implantation window”. Development of a receptive uterus, estrogen and progesterone play a key role in synchronizing the oviductal transport of the pre-implantation embryo (Finn, 1977). The absence of estrogen in the pregnant mouse at the time of implantation induces a state of dormancy of the embryo, and implantation is delayed. However, if the animal is maintained on progesterone, a single injection of a very small amount of estrogen can induce implantation (Yoshinaga, 1966; Psychoyos, 1973; Huet, 1987).

Inhibition of implantation by chemicals, hormones and antigo-adotropic compounds may be due to one or more mechanisms. The accelerated tubal transport or tube locking of the ova by estradiol and medroxy progesterone resulting in their degeneration or expulsion from the uterus due to the increased uterine motility.
(Whitney and Burdick, 1938; Greenwald, 1957, 1959, 1963; Davis, 1963; Banik and Pincus, 1964; Chang, 1966; Rudel and Kind, 1966). Harper (1964) and Pincus (1965) have reported that the chemicals may be antigonadotropic, inhibiting the LH surge necessary for ovulation which in turn inhibits the implantation. Antiestrogenic action of chemicals may interfere with decidualization of uterus, a pre-requisite for implantation (Duncan et al., 1962; Nelson et al., 1964; Duncan and Forbes, 1965; Prasad et al., 1965; Callantine et al., 1966; Prasad and Kalra, 1967). It has been suggested that chemicals may inhibit decidual cell response by blocking estrogen dependent enzyme activity and synthesis of DNA, RNA and proteins. The chemicals may be cytotoxic affecting the viability of the blastocyst (Segal and Nelson, 1958; Chang, 1964; Emmens, 1965). Antihistamine activity of chemicals may inhibit the release of histamine and subsequent decidualization, a pre-requisite for implantation (Duncan et al., 1962; Nelson et al., 1964; Duncan and Forbes, 1965; Prasad and Kalra, 1967; Schlough and Meyer, 1965). Chemicals, hormones and antigonadotropic compounds may cause hormonal imbalance particularly in the estrogen : progesterone ratio necessary for implantation (Cochrane and Meyer, 1957; Shelesnyak, 1960; Netting and Meyer, 1963; Callantine et al., 1966). Number of compounds has been known to have post coital antifertility activity. Anti progesterone (Banik and Pincus, 1964), anti LH serum (Hayashida and Yong, 1963; Munshi et al., 1972), ergo alkaloids such as ergot toxic and ergocornine (Shelesnyak, 1955; Shelesnyak, 1957; Kraicer and Shelesnyak, 1964) which are known to prevent nidation.
Therefore, the above reports suggest that, inhibition of implantation by chemicals, hormones and antigonadotropic compounds may be due to tubal locking, delayed transportation of the blastocyst, blastotoxic, expulsion of the blastocyst from the uterus, cessation of estrogen surge, antihistamine reaction, toxic uterine fluid or imbalance in estrogen : progesterone ratio in mice.

**Anti-implantation activity of carbofuran in albino mice**

The present study has revealed that the treatment with 0.4 and 0.7 mg carbofuran from 1-7 days of pregnancy caused no inhibition of implantation. Treatment with 1 mg carbofuran caused partial inhibition of implantation as only 5 out of 10 mice were showing implantations. However, treatment with 1.3 mg carbofuran seems to be an effective dose as none of the mated mice were pregnant thus, it clearly shows a complete inhibition of implantation with a 100% pre-implantation loss.

The data obtained in the temporal study revealed that treatment with 1.3 mg carbofuran from 1-7 days caused complete inhibition of implantation with pre-implantation loss of 100%. Treatment with 1.3 mg carbofuran on the 3rd day of pregnancy causes partial inhibition of implantations wherein 8 mice out of 10 mice were pregnant. Treatment with 1.3 mg carbofuran from 1-3 days of pregnancy causes partial inhibition of implantations wherein 8 mice out of 10 mice are pregnant. However, treatment with 1.3 mg carbofuran from 1-5 days of pregnancy causes partial inhibition of implantations wherein only 3 mice out of 10 mice are pregnant.
There was a significant decrease in the body weight with 1 and 1.3 mg carbofuran treatment. There was also a significant decrease in the weights of the ovaries, uterus and liver in 1.3 mg carbofuran treatment from 1-7 days of pregnancy. In temporal study, there was a significant decrease in the gain of the body weight in 1.3 mg carbofuran treatment for days 5 and 7, with a significant decrease in the weight of the uterus for 3, 5 and 7 days of 1.3 mg carbofuran treatment. The weight of the liver was also decreased in 1.3 mg carbofuran treatment from 1-7 days of pregnancy.

The organophosphate pesticides are reported to affect the reproduction i.e., implantation, gestation, foetal growth, abnormalities in mammals and development in birds (Khan, 1981; Fish, 1966; Budreau and Singh, 1973; Leybvich, 1973; Machin and McBride, 1989). Ambrose et al, (1970) have reported that an organophosphorus insecticide chlorfenciphos produce progressive effects on fertility, viability and lactation indices without producing any effects on gestation in rats. It has been reported that oral administration of bromophos increases post-implantation loss in mice (Nehaz et al., 1986). Bus and Gibson (1974) have reported that intraperitoneal injection of dicrotophos to pregnant mice resulted in no morphological anomaly. Recently, it has been reported that organophosphorus insecticides methyl parathion and dimithoate inhibit implantation in rats and mice (Soratur and Kaliwal, 1998; Mahadevaswami, 2002).

Some of the chlorinated hydrocarbon pesticides also inhibit implantation and induce embryo toxicity (Huber, 1965; Uphouse, 1986; Johnson et al., 1988; Pinkston and Uphouse, 1988). Fuller and Draper (1975) have reported that
chlordecone on female reproductive activity are quite robust, disrupting fertility when treated prior to mating and on the day of mating (Uphouse, 1986). It has been suggested that the maternal effect of the pesticide methoxychlor independent of its potential embryo toxic activity (Cummings and Gray, 1987). It has been reported that ova viability and implantation are reduced by chlordecone. Recently, it has been reported that the chlorinated pesticide dicofol and endosulfan inhibit the implantation in rats and mice (Jadaramkunti and Kaliwal, 2001; Hiremath, 2000).

Mancozeb, a carbamate fungicide also inhibits implantation in mice (Bindali and Kaliwal, 2002). It has been reported that carbofuran causes foetal mortality and a decreased in the implantation rate in rats (Courtney et al., 1985). It has been revealed that the couples with habitual abortions may appear to have a problem with conception if the losses occur before the diagnosis of pregnancy. Accurate identification of such early losses is important for the correct classification of reproductive failure. Possible mechanisms by which a reproductive toxicant may produce early pregnancy loss including genotoxic effects on the gametes, alteration in the genital tract transport of early conceptus, impaired endometrial receptivity to the implanting blastocyst, and post-implantation abnormalities of development, hormone transport, or nutrition (Wilcox et al., 1988).

The present investigation clearly indicates that high dose and long term exposure of carbofuran may be ineffective in interrupting later part of the pregnancy. But, it inhibits implantation with 1.3 mg carbofuran treatment from 1-7 days of pregnancy in mice similar to that of mancozeb, dicofol, endosulfan and dimethoate treated rats and mice (Bindali and Kaliwal, 2001; Jadaramkunti and
Kaliwal, 2001; Hiremath, 2000; Mahadevaswami, 2002). Therefore, in the present study with high dose and long term treatment of carbofuran revealed that there is a complete inhibition of implantation, which may be due to tubal locking, degeneration of blastocyst, delayed transportation of blastocyst, blastotoxic, expulsion of the blastocyst from the uterus or an imbalance in the estrogen: progesterone ratio which are essential for normal implantation (Morris et al., 1967, 1996).

**Efficacy of progesterone in the maintenance of implantation in carbofuran treated albino mice**

The complex nature of the reproductive regulating processes allows for numerous target sites and accounts for the various mechanisms through which toxins operate to exert their adverse effects. In order for the uterus to become receptive, it must first be exposed to progesterone for 48 h followed by estrogen (Psychoyos, 1973). In addition to development of receptive uterus, estrogen and progesterone play a key role in synchronizing oviductual transport of the pre-implantation embryo. Exposure to xenobiotics that ultimately disrupt ovarian steroid secretion would indirectly result in inadequate uterine decidualization and receptivity (Matt and Borzelleca, 1995).

The data obtained in the present study has revealed that the treatment with 1.3 mg carbofuran from 1-7 days of pregnancy caused complete inhibition of implantation with a 100% pre-implantation loss. Treatment with 4, 9 and 12 mg progesterone along with 1.3 mg carbofuran from 1-7 days of pregnancy is unable to
maintain implantations but there was a complete inhibition of implantation with a 100% pre-implantation loss.

The body weight and weights of the ovaries and uterus were significantly decreased in all the treated groups. There was also a significant decrease in the weight of the liver with 1.3 mg carbofuran treatment from 1-7 days of pregnancy. However, there was a significant decrease in the weight of the adrenal with 12 mg progesterone along with 1.3 mg carbofuran treatment from 1-7 days of pregnancy.

Similar results have been reported with dicofol and endosulfan along with progesterone treated rats and mice (Jadaramkunti and Kaliwal, 1999a; Hiremath, 2000). It has been suggested that the administration of carrot seed extract which acts as a weak estrogen in rats. On the administration of carrot seed extract along with progesterone from 1-7 days of pregnancy maintains implantation and improves with increase in the doses of progesterone in rats (Kaliwal et al., 1987; Kaliwal, 1992).

The present study has indicated that progesterone is unable to maintain implantations in carbofuran treated mice. Recently, similar results also have been reported with organophosphorus pesticide dimethoate unable to maintain implantation with progesterone in mice (Mahadevaswami, 2002). It is well known that the slight increase in the estrogen : progesterone ratio affects the implantations (Yochim and Zarrow, 1960; Nutting and Meyer, 1963; Prasad et al., 1965). Therefore, the inhibition of implantation by carbofuran may be due to the tubal locking, degeneration of blastocyst from the uterus or causing an imbalance in the estrogen : progesterone ratio, since exogenous progesterone is administered to carbofuran treated mice were unable to maintain implantations.
Implantation delay and nidation by progesterone in carbofuran treated albino mice

The potential toxicologic insult that may occur in pre-implantation embryos is that the exposure must occur via uterine and tubal secretions, since the pre-embryos are freely floating during this stage of development. The uterine and tubal concentrations of many compounds are similar to those within the maternal serum. However, nicotine and DDT have been observed to be significantly higher in uterine fluid following maternal exposure (McLachlan et al., 1976). It is not unrealistic to suspect that other compounds also may preferentially concentrate in the uterine fluid compartment. Studies on rabbit blastocysts have indicated that the alkaline blastocyst cavity can concentrate acidic compounds, such as barbital (Fabro, 1973). Thus, considerations of tubal and uterine fluid concentrations of any suspected agent, as well as those that may be accumulated by the blastocyst should be considered when assessing xenobiotic exposure (Matt and Borzelleca, 1995).

The data obtained in the present experiment has revealed that treatment with 1.3 mg carbofuran from 1-7 days of pregnancy and autopsied on the 16th day caused complete inhibition of implantation with a 100% pre-implantation loss. The uterus showed no implantation sites. There was a significant decrease in the body and uterine weights of all treated groups. Treatment with 4, 9 and 12 mg of carbofuran treated mice from 1-7 days of pregnancy and autopsied on the 16th day have revealed that there are no implantations of all the treated mice with a 100% pre-implantation loss. The uterus showed no implantation sites. In the present study, it is revealed that carbofuran, which was administered from 1-7 days of pregnancy
caused a complete inhibition of implantation and has not maintained dormant blastocyst in a viable condition, as the exogenous treatment with 4, 9 and 12 mg of progesterone administered from 8-15 days of pregnancy to the carbofuran treated mice were unable to maintain implantations. This indicates that blastocysts were unable to retain the capacity to implant to the uterine wall and showed no implantations on the 16th day.

Recently, it has been reported that the treatment with dicofol from 1-7 days of pregnancy and exogenous treatment with progesterone from 8-15 days of pregnancy to rats unable to maintain implantations with a significant decrease in the uterine weight (Jadaramkunti, 1999a). Similar results also reported with endosulfan and dimethoate treated mice (Hiremath, 2000; Mahadevaswami, 2002). In contrast to the present results, Kaliwal et al., (1986) have stated that the carrot seed extract, which shows a weak estrogenic activity, was administered from 1-7 days of pregnancy showed no implantation sites on the 8th day but maintained dormant blastocysts in a viable condition. If exogenous progesterone was administered from 8-15 days of pregnancy to the carrot seed extract treated rats, all of them retained the capacity to implant and exhibited implantation on the 16th day.

The absence of estrogen in the pregnant mouse at the time of implantation induces a state of dormancy of the embryo, and the implantation is delayed. However, if the animal is maintained on progesterone, a single injection of very small amount of estrogen can induce implantation (Psychoyos, 1973; Yoshinaga and Adams, 1966; Huet and Dey, 1987). Induction of implantation in this delayed-implanting model is one of the most sensitive physiologic response of estrogen (Dey
and Johnson, 1986). Therefore, it is clear that carbofuran may affect the hormonal imbalance or blastotoxic thus affecting the implantation in mice. In addition, the effect of carbofuran on pre-implantation embryonic development and uterine receptivity are also important to maintain implantations. Estrogen receptor activation is required for ovarian progesterone secretion as well as uterine progesterone receptor production. Further investigation is required to determine if carbofuran alters uterine receptivity in the mouse via altered progesterone secretion and receptor number. Such changes could explain why mice treated with carbofuran along with progesterone were unable to maintain implantation.

Therefore, the anti-implantation activity of carbofuran that causes complete inhibition of implantation when administered from 1-7 days of pregnancy indicates that carbofuran may show blastotoxic effect. The exogenous progesterone administered was unable to show implantations. The graded doses of progesterone administered from 8-15 days of pregnancy to 1-7 days carbofuran treated mice were also unable to show implantation sties. This indicates that carbofuran may cause tubal locking, degeneration of blastocyst from the uterus or an imbalance in the estrogen : progesterone ratio as in the case of other organophosphorus and chlorinated pesticides (Leybovich, 1973; Johnson et al., 1988; Machin and McBride, 1989; Danielle et al., 1997; Soratur and Kaliwal, 1998; Jadaramkunti, 1999a; Hiremath, 2000; Mahadevaswami, 2002). However, further investigation is necessary to study the mechanism of action of carbofuran on implantation in mice.
Effect of carbofuran on pregnancy in albino mice

Pregnancy is a dynamic process with immense anatomic and physiologic changes that occur from fertilization to parturition. Any given agent may have vastly different effects depending on the stage of embryo and fetal development (Matt and Borzelleca, 1995). Pregnancy is a composite of integrated process where efforts of all endocrine glands mediated through the hypothalamo-hypophysial-ovarian and placental axis (Stock and Metcalf, 1994). It requires optimum quantities of ovarian hormones estrogens and progesterone, the secretion of which are maintained by pituitary, placental gonadotropins and luteotropic hormones. During gestation the placenta itself assumes responsibility to produce hormones of gestation. Anything interfering their secretion will result in the termination of gestation (Dickman and Hart, 1972; Glasser et al., 1972; Raziano et al., 1972). Maintenance of pregnancy by exogenous LH in hypophysectomised rats and its interruption in normal pregnant rats by using antiserum indicates that LH seems to be the main luteotrophic substance in the early part of gestation, particularly in rats and hamsters (Alloiteau and Bouhours, 1964; Moudgal, 1969; Loewit, 1970; Yang, 1973). The proponent of another view is that LH is the main luteotropin, which regulates progesterone production from the corpora lutea, essential for maintenance of pregnancy (Loewit et al., 1969; Yoshinaga, 1972). But studies of Morishige and Rothchild (1974) clarified this controversial issue to some extent by showing that the luteal progesterone secretion is sustained by prolactin through 7th day and by a placental luteotropin LH complex from 8 through 11 days. Therefore, it is concluded that luteal progesterone secretion maintained by prolactin through 7th day
whereas pituitary LH has successfully maintained pregnancy during the first half of
the pregnancy from 8\textsuperscript{th} day onwards. Progesterone during pregnancy begins on the
3\textsuperscript{rd} day with a first minor peak on the 4\textsuperscript{th} day, followed by the second major peak
between 13\textsuperscript{th} and 15\textsuperscript{th} days and terminates a day before parturation. The first rise of
progesterone secretion is due to prolactin and the second peak is due to the
lutenizing hormone and the third major peak is due to LH and prolactin (Uchid \textit{et al.},
1970; Pope and Rothchild, 1974)). Thus, though the progesterone is the main
hormone to maintain pregnancy, the synergistic action of estrogen and progesterone
is necessary for gestation. Any imbalance in the estrogen and progesterone ratio,
depending on the period of gestation, causes abortion in rats (Yochim and Zarrow,
1960).

The data obtained in the present study revealed that the treatment with 0.4, 0.7, 1 and 1.3 mg carbofuran from 7-15 days of pregnancy showed no inhibition at
the later part of pregnancy in all the carbofuran treated mice. These mice showed
normal implantations at laparotomy on the 8\textsuperscript{th} day and foetal survivality. These mice
showed normal foetuses as compared to that of the controls. There was a significant
decrease in the number of implantations and live foetuses in 1 and 1.3 mg
carbofuran treated mice. However, there was also a significant decrease in the
number of corpora lutea in 1.3 mg carbofuran treated mice. Treatment with 1.3 mg
carbofuran from 7-15 days of pregnancy caused a significant reduction in the foetal
weight. Treatment with 1 and 1.3 mg carbofuran from 7-15 days of pregnancy
caused a significant decrease in the gain of the body weight and uterus weight. In
the present study the gestation length was not changed significantly of all the carbofuran treated mice.

It has been reported that continuous exposure of methyl parathion at high doses produces maternal toxicity and decreases the foetal weight (Soratur and Kaliwal, 1998). The Food Machinery and Chemical (FMC) Corporation conducted several teratology studies with carbofuran in the rat and in the rabbit. In a dose-ranging study (IRDC, 1980a), 20 pregnant female Charles River CD rats were fed diets containing 0, 20, 60, 120, 160 or 200 mg/kg of carbofuran during gestation days 6 through 19. These dose levels corresponds to applied doses of 0, 10, 2.9, 58, 7.7 and 9.7 mg/kg/d assuming a body weight of 0.290 kg and food consumption rate of 0.014 kg/d. There was a 100 percent survival in all groups. Maternal body weight was decreased during the first two days of treatment in the 120, 160 and 200 mg/kg groups. Neonatal weight decreases were noted at the two highest dose groups. No other clinical signs were noted.

In a follow-up study (IRDC, 1981a), 40 pregnant female Charles River CD rats were fed 0, 20, 60 or 160 mg/kg in the diet during gestation days 6 through 19. One half of the foetuses were examined for visceral and skeletal abnormalities on the 20th day of gestation. Remaining dams delivered normally and the pups were mothered until the 21st day of lactation. There were also no clinical signs. Food consumption was normal in the 20 and 60 mg/kg groups but was reduced by 6 percent in the gain of the body weight and was significantly reduced in the 60 and 160 mg/kg groups. No effects were noted in the length of gestation, parturition, physical appearance of pups or any pup survival indices. Mean pup weights were
slightly reduced at 60 and 160 mg/kg but attributed to the reduction of maternal body weight.

Carbofuran was administered to 10-12 CD rats by gavage at 0, 0.5, 0.1, 0.5, 1.0, 3.0 or 5.0 mg/kg/day from days 7 to 19 of gestation showed a maternal toxicity at doses of 1, 3 and 5 mg/kg. Foetal toxicity was significant at 5 mg/kg level, this include reduced number of live foetuses per litter or increased foetal mortality or decreased foetal body weight. No significant changes in these parameters at lower doses were noted when compared to the controls (Courtney et al., 1985).

Twenty (Seven-month old) female New Zealand rabbits were administered 0, 0.12, 0.5 or 2.0 mg/kg/day carbofuran by oral gavage on day 6 through 18 of gestation caused reduction in the gain of the body weight during the treatment period, no differences in the number of fetuses, litter weight, or developmental or genetic abnormalities were observed in any of the carbofuran treated animals when compared to the control group. Three dams aborted towards the end of gestation period, one from each of the groups (IRDC, 1981b).

It has been reported that 2-chlorodibenzofuran a monochlorinated derivative or dibenzofuran administered to pregnant rats during days 9 to 11 of gestation showed no effects on the embryo-fetal growth (Usami et al., 1993) and similar study with 2-chlorodibenzofuran in pregnant mice from days 6-15 of pregnancy showed no significant difference in the number of live fetuses, fetal weight and mortality of implants (Usami et al., 1993). Nevagi and Kaliwal, (2001) have reported that high dose of dexamethosone does not affect implantation but affects the more advanced stages of pregnancy in rats. Oral administration of chlorpyrifos to rats from 6-15
days of gestation showed no effect on embryo/fetotoxic or teratogenic and did not adversely affect fertility, but effects on neonatal growth and survival were observed at a maternally toxic dose levels in one generation, this effect was not observed in the subsequent generation (Breslin et al., 1996). Three additional studies conducted by Ashry et al., (1994), Stanton et al., (1994), and Chanda et al., (1993) utilizing high dose of chlorpyrifos administered by oral gavage or subcutaneous injection, support the differential sensitivity between the dam and the foetus or weanling animal. It has been reported that the fertility and parturition indices were reduced in a dose dependent fashion, gestation index was not affected, viability and lactation indices were lightly reduced in high dose group, birth weight and crown-rump length of pups in high dose group were significantly less with no effect on average litter size following the administration of monocrotophos to rats (Adilaxmamma et al., 1994).

In the present study, data showed that there was no effect on gestation, similar results were reported in other chlorinated and organophosphorus insecticides, which had no effect on gestation (Ambrose et al., 1970; Adilaxmamma et al., 1994; Breslin et al., 1996; Hiremath, 2000; Mahadevaswami, 2002). Administration of lindane to pregnant rats increased the gestation length (Petrescu et al., 1978) but, no effects on gestation length in multigeneration study (Palmer et al., 1974). The mechanism of TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) endrin and lindane induced fetotoxicity are not known, although several mechanisms have been suggested for the foetotoxicity of TCDD (Poland et al., 1982; Couture et al., 1990; Hassoun et al., 1995). It has been reported that lindane dosage of 0.5 mg/kg/day
lengthened the gestation period, decreased the number of foetuses, increased the number of dead foetuses, and decreased the growth of the young and a dosage of 0.05 mg was without effect (Nayshteyn and Leybovich, 1971).

It has been suggested that methyl parathion administered to pregnant rats causes reduction in growth rate, increases the mortality and significantly reduces the foetal weight but not tertogenic anomaly (Kalow and Marton, 1961; Sortur and Kaliwal, 1998). It has been demonstrated that the carrot seed extract, which shows a weak estrogenic activity administered from 1-7 days onwards caused abortion in almost all rats and this can be reversed with the administration of progesterone from days 7-19 of pregnancy in rats (Kaliwal et al., 1984a, b).

In the present study there is a possibility that the inhibition of implantation may be due to affecting gonadotropin secretion, via the mechanism of central nervous system, as it was observed in the rats, following the administration of dithiocarbamates (Goldman et al., 1997). Goldman et al., (1990) have also reported that the insecticide chlordimeform may destroy endocrinologic homeostasis, by suppressing GnRH release. It has been suggested that toxic agents may act directly on the synthesis of gonadotropins and their secretion or indirectly by altering the pituitary cell responsiveness to GnRH or gonadal steroids thus affecting the hormonal imbalance causing inhibition of implantation. Both actions will result in the alterations of the levels of serum FSH and LH which may affect the implantations (Dickerson et al., 1992). It has been also reported that xenobiotics may impair reproductive function by direct insult to the cell populations within the pituitary which may affect the implantation. Any given compound may have vast
different effects depending upon the stages of the embryo and foetal development. For example, a particular toxic substance may be without effect during implantation, it may induce gross morphologic defects during early stages of embryogenesis, and during the foetal period it may have marked effects on the neurologic development. Therefore, investigation of the potential deleterious effects of a substance during pregnancy requires an exposure throughout the entire period of pregnancy (Matt and Borzellica, 1995).

The reason for the inhibition of implantation by carbofuran in the present study may be due to the hormonal imbalance in any of the stages in hypothalamo-hypophysial ovarian axis or by insensitizing the follicular receptors to the available gonadotropins or blastotoxic as suggested by the earlier workers mentioned above. But the exact nature, which causes hormonal imbalance in the inhibition of implantation by carbofuran, cannot be concluded from this study alone. Therefore, further study still remains to be explored to know the mechanism of action of carbofuran on implantation. There is significant decrease in the weight of the body, ovary, uterus and liver as there may be a suppression of food and water intake in high dose treatment. Hence, nutrition may have played a role in the gain of the body weight as nutritional deficiencies have been shown to alter the reproductive function and reduced rate of body weight gain in monocrotrophos, endosulfan and dimethoate treated rats and mice (Sanderson and Edson, 1964; Hellwig, 1968a; Gibel et al., 1973; Dikshith et al., 1988; Adilaxmamma et al., 1994; Hiremath, 2000; Mahadevaswami, 2002).
Indeed toxins acting through either direct or indirect mechanisms have been shown to interfere with reproductive function by acting at several levels of regulation, including the hypothalamus by causing changes in GnRH secretion and anterior pituitary gland by affecting the release of gonadotropin thereby, disrupting oogenesis or steroidogenesis or both (Gorospe and Reinhard, 1995). Thus, in the present study the exposure to carbofuran shows dual activity as it inhibits implantation but not pregnancy in mice. So each compound has different effects depending on the exposure. The author is fully aware of the conclusions of this study, which are not adequate to state the clear mechanism of action of carbofuran on pregnancy. Hence, further investigation is necessary.
SUMMARY

The present investigation is undertaken to study the effect of a carbamate pesticide carbofuran on implantation, graded doses of 0.4, 0.7, 1 and 1.3 mg/kg body weight/d carbofuran was administered orally from 1-7 days of pregnancy in albino mice. Olive oil was given to the control mice.

1. Pregnant mice treated with 0.4 mg carbofuran from 1-7 days of pregnancy causes no inhibition of implantation with a pre-implantation loss of 11.71%. In mice treated with 0.7 and 1 mg carbofuran caused partial inhibition of implantation with a pre-implantation loss of 22.86 and 67.65%. There were only 5 out of 10 mice were pregnant with 1 mg carbofuran treatment. However, treatment with 1.3 mg carbofuran seems to be an effective dose as none of the treated mice were pregnant, thus causing complete inhibition of implantation with a 100% pre-implantation loss and a concomitant significant decrease in the uterine weight when compared to that of the control mice.

2. There was a significant decrease in the gain of the body weight in 1 and 1.3 mg carbofuran treated pregnant mice. There was also a significant decrease in the weights of ovaries, uterus and liver with 1.3 mg carbofuran treatment from 1-7 days of pregnancy.

3. Based on the dose experiment it was concluded that 1.3 mg/kg body weight/d carbofuran was an effective dose and administered orally to mice on the 3rd day and for 3, 5 and 7 days of pregnancy. Olive oil was given to the control mice.
4. Treatment with 1.3 mg carbofuran on the 3\textsuperscript{rd} day and for 3 and 5 days of pregnancy caused partial inhibition of implantation with a pre-implantation loss of 35.71, 41.51 and 81.61\%. Treatment with 1.3 mg carbofuran on the 3\textsuperscript{rd} day of pregnancy causes partial inhibition of implantations wherein 8 mice out of 10 mice were pregnant. Treatment with 1.3 mg carbofuran from 1-3 days of pregnancy causes partial inhibition of implantations wherein 8 mice out of 10 mice are pregnant. However, treatment with 1.3 mg carbofuran from 1-5 days of pregnancy causes partial inhibition of implantations wherein only 3 mice out of 10 mice are pregnant. However, treatment with 1.3 mg carbofuran for 7 days caused complete inhibition of implantation in all the treated mice with 100\% pre-implantation loss.

5. There was a significant decrease in the gain of the body weight with 1.3 mg carbofuran treatment for 5 and 7 days and a significant decrease in the weight of the uterus for 3, 5 and 7 days of treatment. The weight of the liver was also decreased with 1.3 mg carbofuran treatment for 7 days of pregnancy.

6. Inhibition of implantation by carbofuran may be due to an imbalance in the estrogen : progesterone ratio. Based on this hypothesis 4, 9 and 12 mg progesterone was administered subcutaneously along with 1.3 mg carbofuran for 7 days of pregnancy to counteract the effect of carbofuran and to maintain the implantations.

7. Mice treated with 4, 9 and 12 mg progesterone along with 1.3 mg carbofuran for 7 days of pregnancy was unable to maintain the implantations as a result there was a 100\% pre-implantation loss.
8. There was a significant decrease in the gain of the body and weights of the ovaries and uterus of all the carbofuran treated groups. There was also a significant decrease in the weight of the liver with 1.3 mg carbofuran treatment for 7 days of pregnancy. However, there was a significant decrease in the weight of the adrenal with 12 mg progesterone along with 1.3 mg carbofuran treatment for 7 days of pregnancy.

9. Treatment with 4, 9 and 12 mg progesterone from 8-15 days of pregnancy in 1.3 mg carbofuran treated mice from days 1-7 of pregnancy were failed to show any implantation sites with 100% pre-implantation loss.

10. There was a significant decrease in the gain of the body weight and uterus weight in all the treated groups when compared to that of the control pregnant mice.

11. Treatment with 0.4, 0.7, 1 and 1.3 mg/kg body weight/d carbofuran to later part of the pregnant mice from days 7-15 showed no inhibition of pregnancy. The mice showed normal foetuses. There was a significant decrease in the number of implantations and live foetuses in 1 and 1.3 mg carbofuran treated mice. There was a significant decrease in the foetal weight with 1.3 mg carbofuran treatment in pregnant mice.

12. There was a significant decrease in the gain of the body weight and uterus weight with 1 and 1.3 mg carbofuran treatment from 7-15 days of pregnancy.

13. Treatment with 0.4, 0.7, 1 and 1.3 mg/kg body weight/d carbofuran from days 7-15 of pregnancy showed dose related increase in the gestation length in
mice. However, the increase in the gestation length was not significant when compared with that of the control mice.

The above results clearly suggest that carbofuran inhibits implantation, which may be due to tubal locking, expulsion of the blastocyst from the uterus, blastotoxic or an imbalance in the estrogen : progesterone ratio which is essential for normal implantation. However, carbofuran is not more effective in later part of the pregnancy. Each compound has different effect depending on the exposure time and dose at which it has been exposed. The author is fully aware that the conclusions of this study are not adequate to understand the clear mechanism of action of carbofuran on the implantation and pregnancy in albino mice. Hence, further investigation is necessary to support the findings.
EXPLANATION TO PHOTOGRAPHS

Effect of carbofuran on implantation in albino mice

Fig. 1. Uterus of the control mouse showing normal implantations (Mean weight = 507.06 mg, mean number of implantations = 11.0). At autopsy – Diestrus.

Fig. 2. Uterus of the pregnant mouse treated with 0.4 mg / kg body weight / d carbofuran from days 1-7 showing normal implantations (Mean weight = 502.49 mg, mean number of implantations = 9.8). At autopsy – Diestrus.

Fig. 3. Uterus of the pregnant mouse treated with 0.7 mg / kg body weight / d carbofuran from days 1-7 showing normal implantations (Mean weight = 489.56 mg, mean number of implantations = 8.1). At autopsy – Diestrus.

Fig. 4. Uterus of the pregnant mouse treated with 1 mg / kg body weight / d carbofuran from days 1-7 showing normal implantations (Mean weight = 477.83 mg, mean number of implantations = 3.3). At autopsy – Diestrus.

Fig. 5. Uterus of the pregnant mouse treated with 1.3 mg / kg body weight / d carbofuran from days 1-7 showing no implantations like a non-pregnant mouse (Mean weight = 437.46 mg, mean number of implantations = 0). At autopsy – Diestrus.

Im – Implantation
EXPLANATION TO PHOTOGRAPHS

Temporal effect of carbofuran on implantation in albino mice

Fig. 6. Uterus of the control mouse showing normal implantations (Mean weight = 507.06 mg, mean number of implantations = 11.0). At autopsy – Diestrus.

Fig. 7. Uterus of the pregnant mouse treated with 1.3 mg / kg body weight / d carbofuran on day 3 of pregnancy showing normal implantations (Mean weight = 501.85 mg, mean number of implantations = 7.2) At autopsy – Diestrus.

Fig. 8. Uterus of the pregnant mouse treated with 1.3 mg / kg body weight / d carbofuran from days 1-3 of pregnancy showing normal implantations (Mean weight = 488.72 mg, mean number of implantations = 6.2) At autopsy – Diestrus.

Fig. 9. Uterus of the pregnant mouse treated with 1.3 mg / kg body weight / d carbofuran from days 1-5 of pregnancy showing normal implantations (Mean weight = 480.75 mg, mean number of implantations = 1.9) At autopsy – Diestrus.

Fig. 10. Uterus of the pregnant mouse treated with 1.3 mg / kg body weight / d carbofuran from days 1-7 of pregnancy showing no implantations like a non-pregnant mouse (Mean weight = 437.46 mg, mean number of implantations = 0). At autopsy – Diestrus.

Im – Implantation
EXPLANATION TO PHOTOGRAPHS

Efficacy of progesterone in the maintenance of implantation in carbofuran treated albino mice

Fig. 11. Uterus of the control mouse showing normal implantations (Mean weight = 507.06 mg, mean number of implantations = 11.0). At autopsy – Diestrus.

Fig. 12. Uterus of the pregnant mouse treated with 1.3 mg / kg body weight / d carbofuran from days 1-7 of pregnancy showing no implantations like a non-pregnant mouse (Mean weight = 437.46 mg, mean number of implantations = 0). At autopsy – Diestrus.

Fig. 13. Uterus of the pregnant mouse treated with 1.3 mg / kg body weight / d carbofuran along with 4 mg progesterone from days 1-7 of pregnancy showing no implantations like a non-pregnant mouse (Mean weight = 493.99 mg, mean number of implantations = 0). At autopsy – Diestrus.

Fig. 14. Uterus of the pregnant mouse treated with 1.3 mg / kg body weight / d carbofuran along with 9 mg progesterone from days 1-7 of pregnancy showing no implantations like a non-pregnant mouse (Mean weight = 448.19 mg, mean number of implantations = 0). At autopsy – Diestrus.

Fig. 15. Uterus of the pregnant mouse treated with 1.3 mg / kg body weight / d carbofuran along with 12 mg progesterone from days 1-7 of pregnancy showing no implantations like a non-pregnant mouse (Mean weight = 438.52 mg, mean number of implantations = 0). At autopsy – Diestrus.

Im – Implantation
EXPLANATION TO PHOTOGRAPHS

Implantation delay and nidation by progesterone in carbofuran treated albino mice

Fig. 16. Uterus of control mouse showing normal foetuses (Mean weight = 2.33 g, mean number of implantations = 10.3). At autopsy – Diestrus.

Fig. 17. Uterus of the pregnant mouse treated with 1.3 mg / kg body weight / d carbofuran from days 1-7 of pregnancy and autopsied on day 16 showing no implantations like a non-pregnant mouse (Mean weight = 485.66 mg, mean number of implantations = 0). At autopsy – Diestrus.

Fig. 18. Uterus of the pregnant mouse treated with 1.3 mg / kg body weight / d carbofuran from days 1-7 and 4 mg/kg body weight/d progesterone from days 8-15 of pregnancy showing no implantations like a non-pregnant mouse (Mean weight = 479.85 mg, mean number of implantations = 0). At autopsy – Diestrus.

Fig. 19. Uterus of the pregnant mouse treated with 1.3 mg / kg body weight / d carbofuran from days 1-7 and 9 mg/kg body weight/d progesterone from days 8-15 of pregnancy showing no implantations like a non-pregnant mouse (Mean weight = 477.29 mg, mean number of implantations = 0). At autopsy – Diestrus.

Fig. 20. Uterus of the pregnant mouse treated with 1.3 mg / kg body weight / d carbofuran from days 1-7 and 12 mg/kg body weight/d progesterone from days 8-15 of pregnancy showing no implantations like a non-pregnant mouse (Mean weight = 472.04 mg, mean number of implantations = 0). At autopsy – Diestrus.

Im – Implantation
Table 3.1. Effect of carbofuran on implantation in albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg/d)</th>
<th>No. of mice mated</th>
<th>No. of mice pregnant at autopsy</th>
<th>Implantations</th>
<th>Corpora lutea</th>
<th>% pre-implantation loss</th>
<th>Duration (days) mean ± SEM</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>Mean</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>10</td>
<td>10</td>
<td>110</td>
<td>11.0 ± 0.75</td>
<td>116</td>
<td>11.6 ± 0.54</td>
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<tr>
<td>II</td>
<td>0.4</td>
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<td>10</td>
<td>98</td>
<td>9.8 ± 0.55</td>
<td>111</td>
<td>11.1 ± 0.46</td>
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<tr>
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<td>0.7</td>
<td>10</td>
<td>10</td>
<td>81</td>
<td>8.1 ± 0.41*</td>
<td>105</td>
<td>10.5 ± 0.34</td>
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<tr>
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<td>5</td>
<td>33</td>
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<td>102</td>
<td>10.2 ± 0.36</td>
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<td>V</td>
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<td>0</td>
<td>0</td>
<td>0*</td>
<td>103</td>
<td>10.3 ± 0.52</td>
</tr>
</tbody>
</table>

* Significant P < 0.05 compared to control

\[
\text{% pre-implantation loss} = \frac{\text{Total number of corpora lutea} - \text{Total number of implantations}}{\text{Total number of corpora lutea}} \times 100
\]
Table 3.2. Effect of carbofuran on body and organs weight in early pregnant mice

* Significant $P < 0.05$ compared to control

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg/d)</th>
<th>No. of mice</th>
<th>Body weight gain (g)</th>
<th>Relative weight/100g body weight; (mean ± SEM)</th>
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<td></td>
<td></td>
<td>Ovaries (mg)</td>
<td>Uterus (mg)</td>
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<td>Control</td>
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<td>3.35 ± 0.18</td>
<td>28.05 ± 0.98</td>
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<tr>
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<td>0.4</td>
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<td>28.81 ± 1.12</td>
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<tr>
<td>III</td>
<td>0.7</td>
<td>10</td>
<td>2.90 ± 0.23</td>
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<tr>
<td>IV</td>
<td>1</td>
<td>10</td>
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<td>27.06 ± 0.74</td>
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<tr>
<td>V</td>
<td>1.3</td>
<td>10</td>
<td>1.50 ± 0.17*</td>
<td>25.20 ± 1.23</td>
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</table>

* Significant $P < 0.05$ compared to control
Table 3.3. Temporal effect of carbofuran on implantation in albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Duration of treatment (days)</th>
<th>No. of mice mated</th>
<th>No. of mice pregnant at autopsy</th>
<th>Implantations</th>
<th>Corpora lutea</th>
<th>% pre-implantation loss</th>
<th>Duration (days); mean±SEM</th>
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<tr>
<td>I</td>
<td>Control</td>
<td>10</td>
<td>10</td>
<td>110</td>
<td>116</td>
<td>5.17</td>
<td>7.00 ± 0.00</td>
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<td>72</td>
<td>112</td>
<td>35.71</td>
<td>7.00 ± 0.00 0.00</td>
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<td>8</td>
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<td>10</td>
<td>3</td>
<td>9</td>
<td>104</td>
<td>81.61</td>
<td>4.70 ± 0.21 2.30 ± 0.30*</td>
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<tr>
<td>V</td>
<td>7</td>
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<td>0</td>
<td>103</td>
<td>100.00</td>
<td>4.40 ± 0.21 2.60 ± 0.22*</td>
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</table>

* Significant P < 0.05 compared to control

% pre-implantation loss = \( \frac{\text{Total number of Corpora lutea} - \text{Total number of implantation}}{\text{Total number of corpora lutea}} \times 100 \)
Table 3.4. Temporal effect of carbofuran on body and organs weight in early pregnant mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Duration of treatment (days)</th>
<th>No. of mice</th>
<th>Body weight gain (g)</th>
<th>Relative weight/100g body weight; (mean ± SEM)</th>
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<tr>
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<td>Ovaries (mg)</td>
<td>Uterus (mg)</td>
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<td>Control</td>
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<td>3.35 ± 0.18</td>
<td>28.05 ± 0.98</td>
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<td>Day 3 only</td>
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<td>10</td>
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<td>5</td>
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<td>25.96 ± 0.50</td>
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<td>10</td>
<td>1.50 ± 0.17*</td>
<td>25.20 ± 1.23</td>
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* Significant P < 0.05 compared to control
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<tr>
<th>Groups</th>
<th>Treatment (mg/kg/d)</th>
<th>No. of mice mated</th>
<th>No. of mice pregnant at autopsy</th>
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<th>Corpora lutea</th>
<th>% pre-implantation loss</th>
<th>Duration (days) mean ± SEM</th>
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<td>Mean</td>
<td>Total</td>
<td>Diestrus</td>
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<td>Control</td>
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<td>Carbofuran (1.3)</td>
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<td>0*</td>
<td>103</td>
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<td>Carbofuran (1.3)</td>
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<td>10.5 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>+ Progesterone (4 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Carbofuran (1.3)</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0*</td>
<td>109</td>
<td>10.9 ± 0.48</td>
</tr>
<tr>
<td></td>
<td>+ Progesterone (9 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Carbofuran (1.3)</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0*</td>
<td>108</td>
<td>10.8 ± 0.44</td>
</tr>
<tr>
<td></td>
<td>+ Progesterone (12 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant P < 0.05 compared to control

% pre-implantation loss = \( \frac{\text{Total number of Corpora lutea} - \text{Total number of implantation} \times 100}{\text{Total number of corpora lutea}} \)
Table 3.6. Effect of progesterone on body and organs weight in carbofuran treated early pregnant mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg/d)</th>
<th>No. of mice</th>
<th>Body weight gain (g)</th>
<th>Relative weight/100g body weight; (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ovaries (mg)</td>
<td>Uterus (mg)</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>10</td>
<td>3.35 ± 0.18</td>
<td>28.05 ± 0.98</td>
</tr>
<tr>
<td>II</td>
<td>Carbofuran (1.3)</td>
<td>10</td>
<td>1.50 ± 0.17*</td>
<td>25.20 ± 1.23*</td>
</tr>
<tr>
<td>III</td>
<td>Carbofuran (1.3) + Progesterone (4 mg)</td>
<td>10</td>
<td>2.10 ± 0.18*</td>
<td>24.13 ± 1.12</td>
</tr>
<tr>
<td>IV</td>
<td>Carbofuran (1.3) + Progesterone (4 mg)</td>
<td>10</td>
<td>2.10 ± 0.20*</td>
<td>23.73 ± 1.10*</td>
</tr>
<tr>
<td>V</td>
<td>Carbofuran (1.3) + Progesterone (4 mg)</td>
<td>10</td>
<td>1.80 ± 0.20*</td>
<td>23.27 ± 0.80*</td>
</tr>
</tbody>
</table>

* Significant P < 0.05 compared to control
Table 3.7. Implantation delay and nidation by progesterone in carbofuran treated albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg/d)</th>
<th>No. of mice mated</th>
<th>No. of mice pregnant at</th>
<th>No. of implantations at</th>
<th>Corpora lutea</th>
<th>% Post implantation loss</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Laparotomy</td>
<td>Autopsy</td>
<td>Total</td>
<td>Mean</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>103</td>
<td>10.3 ± 0.20</td>
</tr>
<tr>
<td>II</td>
<td>Carbofuran (1.3)</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>Carbofuran (1.3)</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Days 1-7 +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Progesterone (4mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Days 8-15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Carbofuran (1.3)</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Days 1-7 +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Progesterone (9 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Days 8-15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Carbofuran (1.3)</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Days 1-7 +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Progesterone (12 mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Days 8-15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant P < 0.05 compared to control

% pre-implantation loss = \( \frac{\text{Total number of implantations} - \text{No. of foetuses}}{\text{Total number of corpora lutea}} \times 100 \)
Table 3.8. Effect of graded doses of progesterone on body and organs weight in carbofuran treated albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg/d)</th>
<th>No. of mice</th>
<th>Body weight gain (g)</th>
<th>Relative weight/100g body weight; Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ovaries (mg)</td>
<td>Uterus (mg)</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>10</td>
<td>7.25 ± 0.21</td>
<td>27.94 ± 0.91</td>
</tr>
<tr>
<td>II</td>
<td>Carbofuran (1.3)</td>
<td>10</td>
<td>1.60 ± 0.16*</td>
<td>24.78 ± 1.11</td>
</tr>
<tr>
<td>III</td>
<td>Carbofuran (1.3)</td>
<td>10</td>
<td>1.80 ± 0.20*</td>
<td>27.34 ± 0.72</td>
</tr>
<tr>
<td></td>
<td>Days 1-7 +</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Progesterone (4 mg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Carbofuran (1.3)</td>
<td>10</td>
<td>1.90 ± 0.23*</td>
<td>26.78 ± 0.65</td>
</tr>
<tr>
<td></td>
<td>Days 1-7 +</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Progesterone (9 mg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Days 8-15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Carbofuran (1.3)</td>
<td>10</td>
<td>2.10 ± 0.18*</td>
<td>27.11 ± 0.67</td>
</tr>
<tr>
<td></td>
<td>Days 1-7 +</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Progesterone (12 mg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Days 8-15</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant P < 0.05 compared to control
Table 3.9. Effect of carbofuran on pregnancy in albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg/d)</th>
<th>Number of mice</th>
<th>Corpora lutea</th>
<th>Pregnant at Laperotomy</th>
<th>% Post implantation loss</th>
<th>% Foetal survival</th>
<th>Foetal weight (g/100g b. wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pregnant at Laperotomy</td>
<td>Autopsy</td>
<td>Abortion/resorption</td>
<td>Total</td>
<td>Mean</td>
<td>Total</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>102</td>
<td>10.2</td>
<td>± 0.47</td>
</tr>
<tr>
<td>II</td>
<td>0.4</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>101</td>
<td>10.1</td>
<td>± 0.53</td>
</tr>
<tr>
<td>III</td>
<td>0.7</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>98</td>
<td>9.8</td>
<td>± 0.47</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>88</td>
<td>8.8</td>
<td>± 0.53</td>
</tr>
<tr>
<td>V</td>
<td>1.3</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>77</td>
<td>7.7</td>
<td>± 0.58*</td>
</tr>
</tbody>
</table>

Values represent the mean ± SEM
* Significant P < 0.05 compared to control

% Post implantation loss = \( \frac{\text{Number of implantation} - \text{Number of foetuses}}{\text{Number of corpora lutea}} \) \times 100

% Foetal Survival = \( \frac{\text{Number of foetuses}}{\text{Number of implantations}} \) \times 100
Table 3.10. Effect of carbofuran on body and organs weight in pregnant albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg/d)</th>
<th>No. of mice</th>
<th>Body weight gain (g)</th>
<th>Relative weight/100g body weight; (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ovaries (mg)</td>
</tr>
<tr>
<td>I</td>
<td>Control</td>
<td>10</td>
<td>8.67 ± 0.14</td>
<td>26.55 ± 0.73</td>
</tr>
<tr>
<td>II</td>
<td>0.4</td>
<td>10</td>
<td>8.45 ± 0.14</td>
<td>24.77 ± 1.13</td>
</tr>
<tr>
<td>III</td>
<td>0.7</td>
<td>10</td>
<td>8.06 ± 0.17</td>
<td>25.58 ± 0.75</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>10</td>
<td>7.85 ± 0.24*</td>
<td>24.14 ± 0.78</td>
</tr>
<tr>
<td>V</td>
<td>1.3</td>
<td>10</td>
<td>7.63 ± 0.27*</td>
<td>23.50 ± 0.92</td>
</tr>
</tbody>
</table>

* Significant P < 0.05 compared to control
Table 3.11. Effect of carbofuran on gestation length in albino mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg/d)</th>
<th>Number of mice</th>
<th>Gestation length (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>10</td>
<td>19.60 ± 0.16</td>
</tr>
<tr>
<td>II</td>
<td>0.4</td>
<td>10</td>
<td>19.80 ± 0.13</td>
</tr>
<tr>
<td>III</td>
<td>0.7</td>
<td>10</td>
<td>19.95 ± 0.28</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>10</td>
<td>20.40 ± 0.26</td>
</tr>
<tr>
<td>V</td>
<td>1.3</td>
<td>10</td>
<td>20.50 ± 0.34</td>
</tr>
</tbody>
</table>
Graph 3.1. Effect of carbofuran on implantations and corpora lutea in albino mice

Graph 3.2. Effect of carbofuran on percent pre-implantation in albino mice
Graph 3.3. Effect of carbofuran on body, kidney and liver weights in early pregnant albino mice

Graph 3.4. Effect of carbofuran on organs weight in early pregnant albino mice
Graph 3.5. Temporal effect of carbofuran on implantations and corpora lutea in albino mice

Graph 3.6. Temporal effect of carbofuran on pre-implantation loss in albino mice
Graph 3.7. Temporal effect of carbofuran on body, kidney and liver weights in early pregnant albino mice

Graph 3.8. Temporal effect of carbofuran on organs weight in early pregnant albino mice
Graph 3.9. Effect of carbofuran and progesterone on implantation and corpora lutea in early pregnant albino mice

Graph 3.10. Effect of carbofuran and progesterone on pre-implantation loss in early pregnant albino mice
Graph 3.11. Effect of carbofuran and progesterone on body, kidney and liver weight in early pregnant mice

Graph 3.12. Effect of carbofuran and progesterone on the organs weight in early pregnant albino mice
Graph 3.13. Effect of carbofuran and progesterone on implantation and corpora lutea in delayed implantation in albino mice

Graph 3.14. Effect of carbofuran and progesterone on pre-implantation loss in delayed implantation in albino mice
Graph 3.15. Effect of carbofuran and progesterone on body, kidney and liver weight in delayed implantation in mice

Graph 3.16. Effect of carbofuran and progesterone on the organs weight in delayed implantation in albino mice
Graph 3.17. Effect of carbofuran on number of implantation, corpora lutea, live foetuses and foetal weight in albino mice

Graph 3.18. Effect of carbofuran on % post-implantation loss and % foetal survival rate in albino mice
Graph 3.19. Effect of carbofuran on body, uterus, kidney and liver weight in pregnant albino mice

<table>
<thead>
<tr>
<th>Treatment (mg/kg body weight / d)</th>
<th>Ovanes (mg)</th>
<th>Adrenals (mg)</th>
<th>Spleen (mg)</th>
<th>Thymus (mg)</th>
<th>Thyroid (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.4 mg</td>
<td>0.7 mg</td>
<td>1.1 mg</td>
<td>0.1 mg</td>
<td>0.3 mg</td>
</tr>
</tbody>
</table>

Graph 3.20. Effect of carbofuran on the organs weight in pregnant albino mice

<table>
<thead>
<tr>
<th>Treatment (mg/kg body weight / d)</th>
<th>Ovaries (mg)</th>
<th>Adrenals (mg)</th>
<th>Spleen (mg)</th>
<th>Thymus (mg)</th>
<th>Thyroid (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.4 mg</td>
<td>0.7 mg</td>
<td>1.1 mg</td>
<td>0.1 mg</td>
<td>0.3 mg</td>
</tr>
</tbody>
</table>