TERATOGENIC EFFECTS OF CARBOSULFAN IN FEMALE ALBINO RATS
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ABSTRACT
Teratogenic effects of carbosulfan in albino female rats (Rattus rattus) during organogenesis period (from the 6th to 15th day of gestation) was investigated. Three doses 18.5, 9.25 and 4.63 mg/kg b.wt. corresponding to 1/10, 1/20, 1/40 of the LD50, respectively, were used. The morphological changes revealed that, the alive foetus per dam was decreased with selected doses, while the percent post-implantation death, percent of implantation loss and the number of dead foetuses per dam were increased at the tested doses. Also, the mean foetal weights, the mean foetal crown-ramp length and the mean weight of placenta were reduced markedly. However, visceral examination of viable foetuses showed dilated renal pelvis, distended urinary bladder with urine, cleft palate, dilation of lateral ventricles and cerebral hemispheres (a symmetric). The most common skeletal malformations were wavy and shorting ribs, reduce or absence of sternbrae and absence of digits of fore- and hind-limbs and some coccygeal vertebrae. Also, cranial bone absent (skull incomplete ossification) was observed.

INTRODUCTION
The spread use of pesticides is usually connected with serious problems of pollution and health hazards. Male and female reproductive toxicity, foetotoxicity and teratogenicity are of special concern. Owing to pesticides methods of application as sprays, accidental and/or prolonged exposure to these agrochemical cause great public health concern and economic losses. Unfortunately, very little is known about the possible adverse long-term effects of chronic exposure to these chemicals on male fertility and their teratogenic effect in animals and human. The present investigation was initiated to study the teratogenic effects of carbosulfan as carbamate insecticide. Experiments were carried out on adult female pregnant rats as highly selected experimental animal model to perform such studies.

MATERIALS AND METHODS
1- Insecticides used:
Carbosulfan (Marshall) 25 % WP [2, 3-dihydro-2,2-dimethyl benzofuran-7-yl (dibutyl aminothio) methyl carbamate]. This compound introduced by FMC, Rhone-Pollence (U.S.A.).

2- Experimental animals:
Sexually mature female albino rats were obtained from the Organization of Biological and Vaccine (Helwan farm). The mature animals
having an average 170±10 gm body weight (2.5-3 months old) were periodically examined. The females were kept in hygienic conditions and introduced into the metallic cages for one month before being used and the food (23 % protein) and mated ad libitum.

3- Experimental procedure:

The acute oral toxicity [median lethal dose, (LD$_{50}$)] of tested compound to female albino rats was calculated according to Weil (1952). The remained females were paired with untreated males (1 male : 2 female) of proven fertility in a separate cage, usually in the early afternoon. In the next morning, a vaginal smear was stained with methylene blue (1 %) to verify first day of pregnancy. The female was considered mated when sperms plus cornified cells and leucocytes were found in the vaginal smears or vaginal plugs was detected, and that day is designed (zero-time) of pregnancy (Manson and Kang, 1989). In addition, the body weight was recorded daily for pregnant females which were proved to be in continuous oestrus and showed increased body weight.

At the 5th day of pregnancy, females were divided into four equal experimental groups of 10 females each and treated as follows:

- Group A: was orally administered 0.5 ml distilled water without any insecticidal treatment as control (untreated control).
- Groups B, C, D: were given carbosulfan at the dosage levels of 18.5, 9.25 and 4.63 mg/kg b.wt. (1/10, 1/20, 1/40 LD$_{50}$), respectively.

The tested materials were daily given by gavage from the 6th to 15th day of gestation period. This period (6th - 15th) was considered the time of maximum organogenesis. Both control (group A) and dosed pregnant females were kept under observation until the 20th day of gestation at which they were weighed, sacrificed, and dissected to investigate the effect of tested substance on the foetal development (morphological, visceral, and skeletal examination). The uterus was examined morphologically to estimate the resorption sites (Köple and Salewski, 1964). Foetuses were collected from each dam.

The method of Manson and Kang (1989) was used for morphological examinations. The evaluation of skeletal abnormalities was performed after clearing by using 95 % ethyl alcohol with potassium hydroxide and staining the skeleton by Mallisch's solution with alizarin red S stain (Staples and Schnell, 1964). For the visceral examination, the foetuses were preserved in Bouin's fixative and sectioned by free hand razor using the technique described by Wilson (1965).

Skeletal and visceral malformation of each fetuses were recorded as explained by Cook and Fairweather (1968) and Manson and Kang (1989).

4- Statistical analysis:

Statistical significance between experimental and control values were calculated according to Dixon and Massay (1957). A difference was considered statistically significant at $P < 0.05$. 

4132
RESULTS AND DISCUSSION

The teratogenic effects of carbamate insecticide (carbosulfan) was investigated in pregnant rats as a mammalian system and hence, to other domestic animals exposed accidentally to this compound. The teratogenic effect was assessed by morphological, visceral and skeletal malformations of the obtained fetuses after oral administration of the pregnant dams at dose levels of 18.5, 9.25 and 4.64 mg/kg b.wt. of carbosulfan from the 6th to 15th day of gestation.

1- Acute oral toxicity (LD₅₀) of tested compound:

The median lethal dose (LD₅₀) of experimental substance to female albino rats was 185 mg/kg b.wt. (Weil, 1952).

2- Morphological changes of the uterus and foetuses:

The external examination of the uterus and foetuses of female rats treated with carbosulfan are obtained in Table (1). Results of the present investigation revealed that, oral administration of carbosulfan during organogenesis period (from 6th to 15th day of gestation), resulted in no significant differences in all parameters; while, an increase in the number of corpora lutea per dam, number of implantation sites per dam, number of dead fetuses/dam, number of resorption sites (early or late), percent pre-implantation loss/dam and percent post-implantation death/dam was observed. Contrast of these findings, the number of implantation sites/litter, number of corpora lutea/litter, number of alive fetuses/dam or litter, the mean fetal weight and the mean fetal crown-rump length (Plate, 1) were decreased. Moreover, the mean weight of placenta was increased at the lowest dose of carbosulfan.

Also, external examination revealed that, fetuses had oedematous foot and large subcutaneous haematous in several offspring of carbosulfan treated rats.

Such findings are incoincidence with the results of Soni and Bhatnager (1989), Hemeida et al. (1989) and Hasan et al. (1990).

Soni and Bhatnager (1989) reported that, the higher dose of phosphamidon (35 ppm) reduced the number of implantations and fetal weight. Also, Hemeida et al. (1989) mentioned that, oral administration of trichlorfon to pregnant female rats resulted in foetal resorption, growth retardation and death. Hasan et al. (1990) observed that treatment with methomyl from the 6th to 15th day of gestation caused early resorption of foetuses and decreased their weights.
Table (1): Effect of treatment with different concentrations of carboxsulfan (Marshal) on external morphological of uterus and foetuses in pregnant rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Treatments (mg/kg b.w.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>18.5</td>
</tr>
<tr>
<td>No. of corpora lutea per dam</td>
<td>7.5000±0.6368</td>
<td>8.3000±0.4230</td>
</tr>
<tr>
<td>No. of implantation sites per dam</td>
<td>7.4000±0.6360</td>
<td>8.1000±0.4333</td>
</tr>
<tr>
<td>No. of corpora lutea per litter</td>
<td>1.4685±0.3938</td>
<td>1.3460±0.1215</td>
</tr>
<tr>
<td>No. of implantation sites per litter</td>
<td>1.4685±0.3938</td>
<td>1.3100±0.1157</td>
</tr>
<tr>
<td>No. of dead foetuses per dam</td>
<td>0.1000±0.0999</td>
<td>0.6000±0.3999</td>
</tr>
<tr>
<td>No. of alive foetuses per dam</td>
<td>6.5000±0.8724</td>
<td>5.9000±0.7250</td>
</tr>
<tr>
<td>No. of alive foetuses per litter</td>
<td>0.9000±0.0999</td>
<td>0.9000±0.0667</td>
</tr>
<tr>
<td>No. of resorption sites-early</td>
<td>0.1000±0.0999</td>
<td>0.7000±0.4230</td>
</tr>
<tr>
<td>No. of resorption sites-late</td>
<td>0.4000±0.1633</td>
<td>0.9000±0.1795</td>
</tr>
<tr>
<td>% pre implantation loss per dam</td>
<td>1.0000±0.0000</td>
<td>1.5150±0.3436</td>
</tr>
<tr>
<td>% post implantation death per dam</td>
<td>3.0350±0.9116</td>
<td>5.1150±0.7274</td>
</tr>
<tr>
<td>Sex ratio</td>
<td>0.9830±0.2257</td>
<td>1.4330±0.4218</td>
</tr>
<tr>
<td>Mean foetal weight (gm)</td>
<td>3.8900±0.2310</td>
<td>3.6320±0.2594</td>
</tr>
<tr>
<td>Mean foetal crown rump length (cm)</td>
<td>3.4000±0.0650</td>
<td>3.3600±0.0897</td>
</tr>
<tr>
<td>Mean weight of placenta (gm)</td>
<td>0.4660±0.0191</td>
<td>0.4570±0.0330</td>
</tr>
</tbody>
</table>

Sherif (1991) mentioned that, an increase of the mean neonatal death and percentage of resorbed foetuses in Tamaron-treated rats (methimidaphos) was observed. While, the foetal weight and length were decreased. Mathur and Bhatnager (1991) noticed that, carbaryl tend to reduce litter size, increase the percentage of resorbed foetuses and to reduce fetal weight in female mice treated with the tested compound on day 8 or day 12 of pregnancy, or daily (as daily doses) from 6th-15th day of gestation.

Similar results were also reported by Abd El-Khalik et al (1993), Junaid et al (1995) and Farid (1997).

Abd El-Khalik et al. (1993) who showed that, early embryonic death, retardation of growth and increase the incidence of placenta weight were occurred in deltamethrin-treated female rats from 6th-15th day of gestation. Junaid et al. (1995) stated that, a decrease in fetal weight and crown-rump length was observed on chromium-treated female (through drinking water), on days 14-19 of pregnancy. Also, a significantly higher incidence of post-implantation loss was observed. Farid (1997) recorded that, buprofezin and diazathion caused decrease in the number of alive foetuses per dam, a
marked reduction in the mean of foetal weight and the mean of fetal crown-rump length and the mean weight of placenta during the organogenesis period, in pregnant rats.

3- Visceral examination:

Regarding visceral abnormalities induced by the oral administration of 18.5, 9.25 and 4.63 mg/kg b.wt. of carbosulfan, cross section in the head of foetuses obtained from treated dams showed cleft palate (Plate, 2), microcephalic, dilation of lateral ventricles and asymmetric cerebral hemispheres (Plate, 3).

Plate (1): Showing retardation in growth of rat foetuses obtained from dams given orally carbosulfan (control on the left).

Plate (2): Cross section in the head of rat foetuses showing cleft palate in head of rat foetuses obtained from dams treated with carbosulfan (control on the left).
Table (2) show the visceral examination of living foetuses obtained from female rats given carbosulfan at doses 18.5, 9.25 and 4.63 mg/kg b.wt., the most prominent anomalies were dilated renal pelvis with 10.2, 9.6 and 4.8 % at the investigated doses, respectively. The urinary bladder was abnormality, distended with urine (8.2, 3.8 and 2.4 %) at tested doses respectively, (Plate, 4). Also, dilated ureter (hydroureter) was observed (10.2, 5.8 and 4.8 %) at the experimental doses (Plate, 4).

Also, oral administration of carbosulfan to pregnant rats from 6th-15th day of gestation showed hypoplasia of heart and lung, liver and kidney, split of thymus and hydronephrosis.

Plate (3) : Cross section in the head of rat foetus showing hypoplasia of brain, cerebral hemispheres were unequal size (asymmetric) after oral administration of carbosulfan to the dams from 6th to 15th day of gestation.
A- Control, B & C = Treated.

Plate (4) : Showing distention of the urinary bladder with urine and dilated ureter (Hydroureter) after oral administration of carbosulfan to dams (control on the left).
| Dose (mg/kg b.w.) | Total no. of examined fetuses | Distend urinary bladder | Hydro-ureter | Dilated renal pelvis | Hydronephrosis | Hypoplasia of kidney | Hypoplasia of liver | Hypoplasia of lung | Split of thymus | Hypoplasia of heart | Cerbral hemispheres (asymmetric) | Ventrices | Dilation of lateral | Microcephalic | Cleft palate | Abdomen | Thorax | Head | Malignant | of | 
|------------------|-----------------------------|------------------------|--------------|---------------------|----------------|---------------------|---------------------|------------------|----------------|------------------|-------------------------------|-----------|-------------------|-------------|------------|--------|---------|--------|---------|
| 4.17             |                             |                        |              |                     |                |                     |                     |                  |                |                  |                                |           |                    |             |            |        |         |         |         |

15th day of gestation as compared to the control group.

Table (2): Visceral examination of rat fetuses from dam given orally carbosulphan from the 6th to
4- Skeletal examination:

The skeletal examination of the remaining third of rat foetuses obtained from dams given orally carbosulfan (18.5, 9.25 and 4.63 mg/kg b.w.t.) are presented in Table (2). The most common abnormalities were incomplete ossification of the skull (cranial bone absent), 20.8, 23.1 and 19.2 %, at the three doses, respectively (Plate, 5).

Treatment of carbosulfan in pregnant rats induced wavy and shorting in ribs (Plate, 6). Reduced or absence of sternbrea were noticed (20.8, 19.2 and 11.5 %), at 18.5, 9.25 and 4.63 mg/kg b.w.t., respectively (Plate, 7). Moreover, absence of xiphoide was observed in foetuses of treated pregnant rats. This carbamate insecticide induced malformations in the fore and hind limbs (e.g. absence of digits).

Plate (5): Showing incomplete ossification (cranial bone absent) descended from dams treated with carbosulfan (control in the left).

Plate (6): Showing wavy and shorting ribs in rat foetuses obtained from dams given orally carbosulfan.
A = Control  B & C = Treated
Plate (7): Showing absence of sternbrae in rat foetuses obtained from dams treated orally with carboxulfan (control in the left).

Regarding to the malformations in vertebrae (absent of some coccygeal vertebrae) were 37.5, 26.9 and 23.1 % at the tested doses respectively. Also, hypoplastic, split off center and missing of sternal centers were noticed. Extra ossification center between the 13th thoracic arches and the 1st lumbar vertebrae was observed.

Several insecticides were shown to be highly teratogenic agents such as, trichlofon on rats (Hemeida et al., 1989), methomyl on rats (Hasan et al., 1990), and Tamaron on rats (Sherif, 1991).

Hemeida et al. (1989) stated that, oral administration of trichlorfon to pregnant rats caused foetal abnormalities. The most common visceral abnormalities were represented by cleft palate, microcephaly, cardiac hyperplasia and hypoplasia. The skeletal abnormalities include shorting of some ribs, absence of sternbrae and absence of phalanges of fore- and hind limbs.
Hasan *et al.* (1990) found that the smallest dose of methomyl in pregnant rats resulted in hyperplasia of heart and liver, corrugation of some ribs and absence of sessamoid bones of fore- and hind limbs. Sherif (1991) observed great abnormalities in skull, ribs, limbs and vertebral column in treated rats with Tamaron.

The present findings agree to great extent with data obtained by Koemiel and Abd-Allah (1991) who mentioned that oral administration of pregnant albino mice with carbaryl, methyl-parathion and fenvalerate, resulted in extra-rib, unossified proximal phalanges of foot and middle phalanges of hand which observed at 250 mg/kg of carbaryl. In addition, cervical-rib, unossified distal phalanges of hand and middle phalanges of foot were observed at 24 mg/kg of methyl-parathion. Otherwise, cervical-rib was observed only at 200 mg/kg of fenvalerate.

Such results are incoincidence with the results of Mathur and Bhatnagar (1991) on carbaryl in mice, Abd El-Khalik *et al.* (1993) on deltamethrin in rats, and Eckhoff *et al.* (1994) on 13-cis-retionic acid in rabbits. They mentioned that treatment with these pesticides increased the incidence of anomalous foetuses such as, reduced ossification, hypoplasia of the lungs and dilation of renal pelvis. Also, the most frequently observed malformations were eye defects (open eyes, micro- and excophthalmia), cardio megaly and haemorrhagic cerebral hemispheres in treated animals.

These observations were in agreement with description reported by Farid (1997) who reported that visceral examination of foetuses showed dilated renal pelvis and ureter and distended urinary bladder with urine. The most common skeletal malformations were wavy and shorting ribs, reduce or absence of stenbrae and absence digits of fore- and hind limbs.

Contrary to our results, are these previously reported by Abd El-Khalik (1993) who mentioned that no skeletal changes in foetuses recovered from deltamethrin-treated female rats were observed. Also, Srivastava and Riazada (1995) found that, there were no fetotoxicity/teratogenic effects of isoproturon (phenyl-urea, herbicide) at the tested dosage level. The tested material did not cause external, visceral and skeletal fetal observations.

The present study revealed that carbosulfan produced foetotoxicity among litters of treated rats which evidenced by decreased mean foetal weight, mean foetal crown-rump length and mean weight of placenta.

A number of large molecular potent teratogenic agents are though to affect the foetuses by acting on placenta, thus interfering with embryo nutrition without being able to reach of foetuses because of their large molecules (Williams, 1982).

Sternberg (1979) mentioned that, the foetuses may react poorly to toxic substances absorbed in the maternal blood, in part, due to that foetal liver is poorly developed in terms of its detoxifying ability.

The placenta plays an important role for developing foetuses as it provides nutrition and hormonal regulation and transfers metabolic waste products. Accumulation of toxic compounds may alter placental function and impair embryonic and foetal development. The placenta may be directly involved in many instances of early spontaneous abortions, foetal death and intrauterine growth retardation (Faulk, 1981 and McIntyre & Faulk, 1983).
Harbison (1975) mentioned that embryo and foetuses are highly susceptible to cytotoxic agents, because they constantly have groups of cells in the growth phase. Acute toxic doses may cause cellular death and result in foetal death. Survivors may show symptoms damage to systems that contain cells in cycle and with a short cycle time.

The decrease in placenta weight associated with the decrease in fetal weight in carbosulfan-treated females rats was observed. This findings strongly suggest that the teratogenic effects of carbosulfan may be attributed, at least in part, to the effect of the compound on placenta. There is a risk to the development of foetus if the mother is exposed to high concentrations of the toxic agents during pregnancy, because this agent could penetrate the placental barrier and accumulate in the fetal tissue (Junaid et al., 1995).

Finally, these findings may emphasize to the fact that sublethal doses of a given toxic agent might inflict toxic hazards and serious disorders on man and his domestic animals.

REFERENCES


Weil, C.S. (1952). Tables for convenient calculation of median effective dose (LD50 and ED50) and instruction in their use. Biometrics, 8 : 249-263.


التأثيرات التشوهية لمركب الكاريسولفان على أجنحة القفزور البيضاء
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- كلية الزراعة - جامعة الزقازيق - قسم وقاية النبات
- المعمل المركزي للسيدات - مركز البحوث الزراعية - الدقي الجيزة

تم دراسة تأثير مركب الكاريسولفان على أجنحة القفزور البيضاء - حيث أخذت عدد 40 من إناث القفزور البيضاء الحوالى وقسمت إلى أربعة مجموعات متساوية (كل مجموعة عشرة قفزور)

1- المجموعة الأولى: عولمت بمجموعة مكعبة ضادة
2- المجموعة الثانية: عولمت بمركب الكاريسولفان بالتركيز 4.6 مجم/كم من وزن الجسم (و هو تعادل 1/4 من LD50) و ذلك من اليوم السادس إلى اليوم الخامس عشر من الحمل (فترة تكون الأعضاء) و ذلك عن طريق الني المعيدي.
3- المجموعة الثالثة: عولمت بالتركيز 9.2 مجم/كم من وزن الجسم من المركب (و هو تعادل 1/4 من LD50) و ذلك بنفس الطريقة.
4- المجموعة الرابعة: حيث عولمت بالتركيز 18.5 مجم/كم من وزن الجسم من نفس المركب (و هو تعادل 1/10 من LD50) وذلك بنفس الطريقة السابقة.

في اليوم الثامن من الحمل تم استخراج الأجنحة من أرحام إناث القفزور البيضاء الحوالى و كذلك تم فحص النبض و الرحم حيث تم أخذ الأجسام الصفراء و الأجنة المدمضة ثم بعد ذلك أجرت الفحوصات المورفولوجية للأجنحة من حيث النشوبات الخارجية ثم قسمت بعد ذلك الأجنحة إلى قسمين (2/3، 1/3) حيث أجرى فحص الأحشاء الداخلية ل 3 لجننة الفئران أما ال 1/3 الباقى فاجرى له فحص الهيلك العظمى.

وقد أدت معاملة إناث القفزور البيضاء الحوالى بمركب الكاريسولفان باكون من 1/10 من الجرعة التصفية المسمى (50 LD50) إلى

1- نقص عند الأجنحة الحبيبة، زيادة عدد الأجنحة الممتدة بعد الغرس و زيادة عدد الأجنحة الممتدة,
2- لوحظ نقص واضحاً في وزن و طول الأجنحة بالإضافة إلى نقص وزن المشيمة
3- لوحظ نقص الأحشاء الداخلية للأجنحة لوحظ انخفاض كل من الحوض الكلي و الحالب و اندماد نسيج النيتي في الاحشاء الداخلي
4- عند الفحص البصري ظهور غياب بعض عظام القفص و وجود تغيرات في الضفوع و حدوث فشل في طول الضفوع

4143