J. King Saud Univ., Vol. **8**, *Agric. Sci.* (1), pp. 121-133, Riyadh (A.H. 1416 / 1996).

Teratogenicity and Embryotoxicity of 1,4-Benzoquinone and Wheat Flour Infested with Tenebrionid Beetles on Mice

I.H. El-Kashlan[1], Eglal Helal, Madeha Abd El-Hamid and M.A. Abdellatif

General Authority for Supply Commodities[1], Alexandria; and Department of Plant Protection, Faculty of Agriculture, University of Alexandria, Egypt

(Received 21/9/1413; accepted for publication 29/7/1414)

Abstract. Applying 1,4-benzoquinone orally to the mice induced toxic malformations, producing teratogenic and embryotoxic effects. The toxic symptoms are recorded for various parameters: maternal body weight, weight of uterus, number of fetus, resorption, body weight of fetus, crown length and skeletal abnormalities. The same symptoms were obtained when infested wheat flour with tenebrionid beetles was offered to the mice, which may lead to believe that the secretions of the defensive glands may cause the same harmful effects on mice as those treated with 1.4-benzoquinone.

Introduction

Teratogenicity is defined as the result of damage or death of certain cells of a developing animal organism. It is an acute toxicological phenomenon, requiring a short period of exposure to the teratogenic agent, and usually producing toxic symptoms within a short period thereafter. This behavior is different from carcinogenesis which is usually regarded as a chronic toxicity [1].

The teratogenicity and embryotoxicity of several chemicals have been evaluated by many authors on various experimental animals [2-9]. In his teratogenic studies, Baker [10] mentioned that all fetuses, live or dead, are counted, weighed, sexed, examined for external malformations. Subsequent studies of internal structures are followed. Fetuses should also be examined for internal abnormality and for skeletal structure. Brittelli *et al.* [11] measured the fetal toxicity by number of resorptions and live fetuses, fetal weights and crown-rump length. Cummings *et al.* [12] studied

developmental effect of methyl benzimidazole-carbamate exposure during early pregnancy. However, the teratogenic effect of benzoquinone compound has not been studied yet.

In this work 1,4-benzoquinone was applied as a teratogenic chemical that might induce embryotoxicity, due to producing mutations or malformations in germ cells or somatic tissue of the treated animals and their progeny. This chemical is known to be one of the important products secreted by the defensive glands of some tenebrionid beetles infesting stored products [13-16].

Also, mice kept feeding on heavily infested wheat flour with tenebrionid beetles for 3 months were subjected to the same tests of embryotoxic parameters, in a trial to evaluate the toxicity of the insect secretions on the mice and their progeny.

Materials and Methods

1) Chemical used

- Common name: Benzoquinone.
- Chemical name: 1,4-benzoquinone.
- Description: Yellow greenish color crystals, M.P. 115.7°, dissolved in ethylalcohol, diethyl ether or hot distilled water.
- Source: A standard 1,4-benzoquinone was obtained from Faculty of Science, Chemistry Department, Alexandria University.

2) Wheat flour used

Wheat flour infested with *Tribolium confusum, T. castaneum* and *Tenebrio molitor*, was used. Rate of infestation started with 90 insects/kg of wheat flour, then stored at 28°C and RH 50 - 60%. Six months later the experiment was carried out. The infested wheat was offered to the experimental mice, when insects have built up, a good population.

3) Animal tested

A pure strain of white swiss albino mouse *Mus musculus domesticus* L. was used. The strain named CD1-mice was obtained from the Naval Medical Research Unit (NAMRU-3) in Cairo. The average age of the experimental animals ranged between 2 and 3 months and the average weight was 23±2 gm. The animals were allowed at least two weeks to be adapted to the new environment, before experimentation. Ani-

mals were fed a prepared diet containing ground bread plus 10% milk and 40% water. All of these constituents were mixed together to make a slury prepared diet and water was provided.

4) Mating of tested animals

Two female mice were provided with one sexually mature male. Each female was examined for the presence of a vaginal plug. The presence of a vaginal plug was counted at zero day of pregnancy. After mating, females were housed in groups, each group consists of five females, as a replicate.

5) Treatment of tested animals

a) Drug administration

On the sixth day of gestation, four groups of mice (5 mice each) were treated, with 10, 50, 100, 150 mg/kg by oral administration using a ball-ended dosing needle. The proposed doses of 1,4-benzoquinone were dissolved in 10 ml hot distilled water to get a final volume of 0.2 ml for each mouse. Treatment with 1,4-benzoquinone was accomplished at intervals of two days till the 18th day of gestation. The experiment included 5 mice as a control, treated with 0.2 ml hot distilled water.

b) Force-feeding of wheat flour infested with stored product insects

Female mice were fed wheat flour infested with stored product insects. Each experimental animal was fed three times a week on the prepared diet, at the ratio of 80 mg of wheat flour to 1 ml of hot water, following the same procedure as before. Mice were fed on infested diet for 3 months before mating. Control mice were fed on normal diet.

6) Recording the results

According to the method of Palmer [17], parameters recorded are:

a) General examination of pregnant mice

The control and treated pregnant mice were weighed before and after treatment throughout the gestation period. The number of live and dead pregnant mice was recorded.

b) Morphological examination of fetuses

Two days before expected delivery the uterus was opened and the fetuses were removed and examined. The number of live, dead, late and early resorptions of fetuses in the right and left horn were recorded. Fetuses were examined for any exter-

nal malformations. Then the body weight of fetuses and crown length (the length of fetus starting from nose till the end measured by a string) for all replicates were recorded.

c) Skeletal examination of fetuses

The fetuses were prepared for skeletal examination according to the method described by Varnagy [7]. At least two fetuses were stored in 4% formaline for 48 hours, then washed with water for one hour. Dehydration was completed by ethanol 90% for three days. After that fetuses were kept in 2% KOH for one day. Staining with Alizarin reds' stain was employed in aqueous solution, of which 2-3 drops were added to 100 ml KOH. The staining time was four days, then specimens were preserved in 87% glycerol for examination and photographing.

d) Statistical analysis

Statistical analysis was carried out according to Steel and Torrie [18]. Data were analyzed by factorial experimental for female parameters and fetuses. Chi square analysis was applied for skelton studies.

Results and Discussion

The present study describes the teratogenicity and embryotoxicity on mice of 1,4-benzoquinone and wheat flour infested with tenebrionid insects.

1) Body weight of maternal mice

The maternal weight of pregnant mice treated with different doses of 1,4-benzoquinone was measured at intervals of 2 days from the sixth day of gestation till the 18th day. From the presented data, reduction in body weight occurred at all doses used, compared with control.

The data showed that the lowest significant decrease of maternal weight gain occurred at concentrate 100 mg/kg compared with control mice and other doses (10, 50 and 150 mg/kg).

Feeding the non-pregnant mice on wheat flour infested with stored product insects for 3 months at the rate of initial infestation 90 insect/kg, caused a slight reduction in weight of mice, compared with the control mice. This retardation in growth rate may be due to toxic effects of the secreted chemicals by the tenebrionid insects. The effect of feeding on pregnant maternal mice, gain in weight, was reduced at the 6th day of gestation. However, the gain in weight increased significantly after that, but it was still low when compared with control.

2) Number of live and dead fetuses

The presented data pointed out that the number of live fetuses decreased significanlty at various treatment used with a significant increase in the number of dead fetuses. The largest number of dead fetuses was noticed at dose 150 mg/kg (all fetuses were died) compared with control mice. Such decrease appeared at all used doses compared with control mice. There was significant difference in number of featuses between right horn of uterus (R_H) and the left horn (L_H).

Feeding of wheat flour infested with stored product insects significantly decreased number of live fetuses in right and left horns of uterus compared with control.

3) Weight of uterus, early and late resorption

Data indicated that in treated mice and at all doses used, reduced weight of uterus was obtained Fig. 1. No significant effect on early resorption of fetuses was observed, on the other hand late resorption was obvious at higher doses (100-150 mg/kg).

Fig. 1. A) Normal uterus., B,C) uterus growth retardation. (100 mg/kg).

Feeding experiments data revealed significant reduction in weight of uterus and number of early and late resorption in right and left horns of uterus compared with control.

4) Body weight and crown length of fetus

Effect of 1,4-benzoquinone on these aspects are recorded in Table 1. The largest effect on body weight of fetuses was observed on the treated mice (50, 100 and 150 mg/kg). These doses appeared the same decreae in the body weight of fetuses, Fig. 2. Dosage 10 gm/kg had no significant decrease in the body weight compared with control. There was a high significant reduction in fetal weight in positions 2,3 and 4 (0.46, 0.42 & 0.17 gm), but the reduction in position 4 was more obvious than in position 2 or 3. In addition, crown length at the previous doses was highly reduced for fetuses at the position 4.

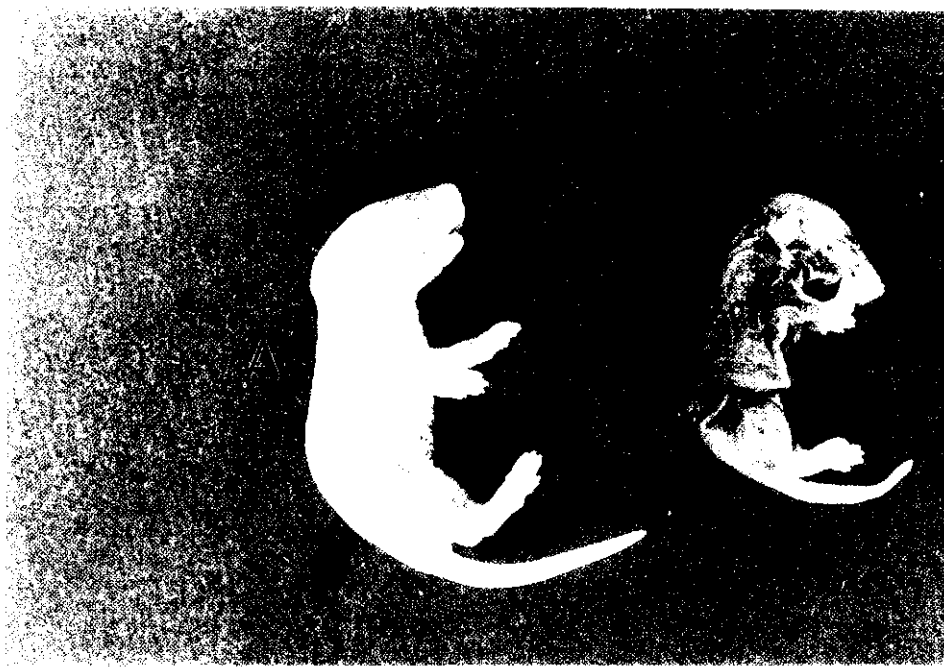

Fig. 2. A) Normal fetus., B) Fetus showing malformations, blood collection under skin, growth retardation of crown length and late resorption. (150 mg/kg).

Also, feeding on treated food reduced fetal weight and crown length compared with control. The highest reduction occurred at position 4 compared with positions 1, 2 & 3.

5) Hand and leg skeleton of fetus

1,4-benzoquinone caused significant effect in fetuses-skeletal malformations especially in its high doses. The effect could be summarized in four categories, (Figs. 3 to 6).

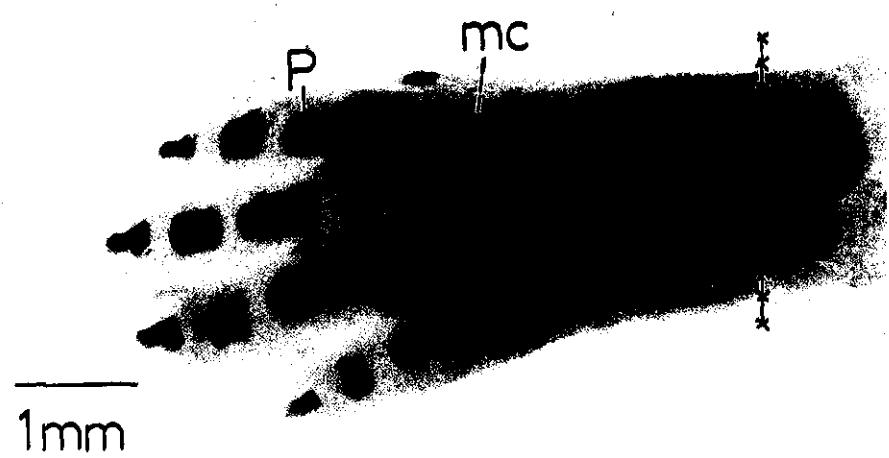

Fig. 3. Normal hand phalanges (P →), metacarpal bones (mc →) and ulna and Radius bones (*>).**

a) Hand phalanges bones are absent.
b) Leg phalanges bones are absent.
c) Hand and leg phalanges bones are absent.
d) Metatarsal and metacarpal bones are absent.

Feeding mice infested wheat flour induced slight skeletal malformations compared with mice treated with 1,4-benzoquinone.

In conclusion it could be mentioned that applying 1,4-benzoquinone orally to the mice may be toxic. These toxic symptoms are grouped and presented in Table 1. Various parameters under investigation were loss of the maternal body weight, weight of uterus, number of fetus, resorption, body weight of fetus and crown length.

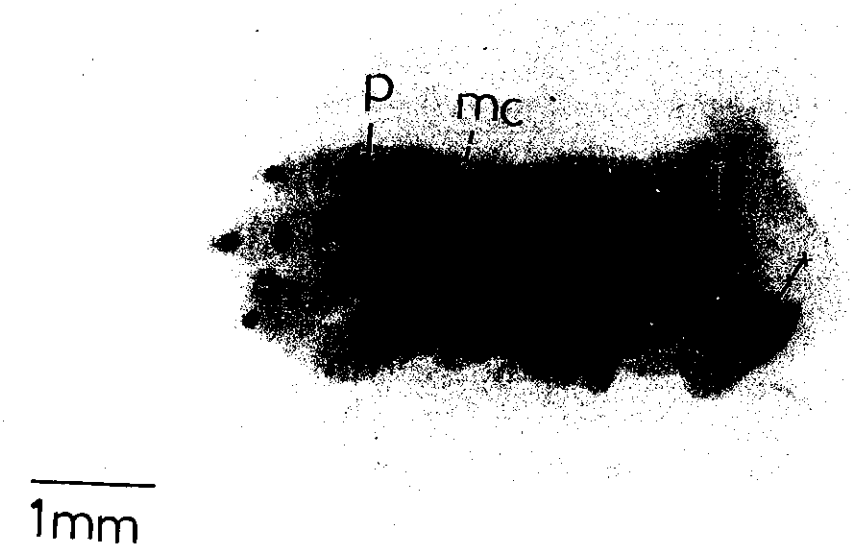

Fig. 4. Hand phalanges bones absent (p →), metacarpal bones (mc →) shorter and ulna bone shorter and Radius bone absent (* >) in mice treated with 100 mg/kg 1,4-benzoquinone .**

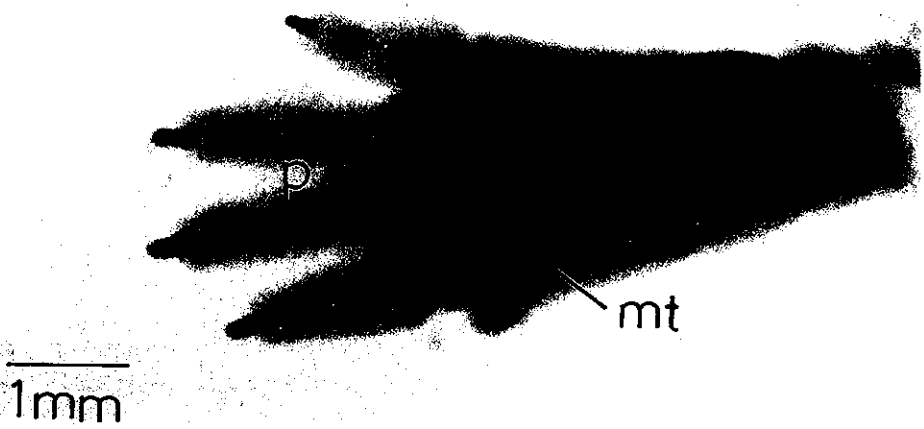

Fig. 5. Leg metatarsal bones absent (mt →) in mice treated with 150 mg/kg 1,4-benzoquinone .

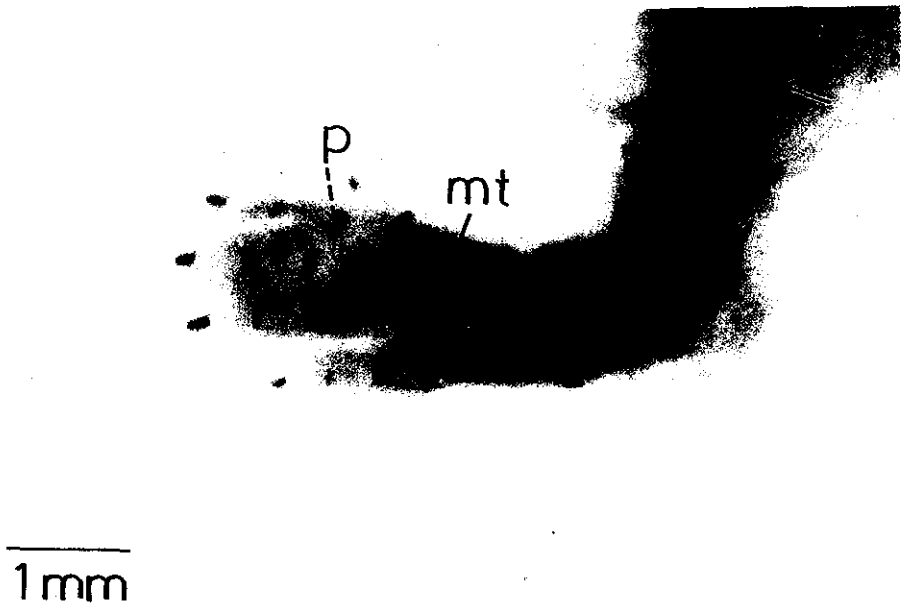

Fig. 6. Leg phalanges bones shorter (p →) and metatarsal bones shorter (mt →). Tibia and fibula (→) bones shorter and absent in mice treated with 150 mg/kg 1,4-benzoquinone .

The same symptoms were also obtained, when infested wheat flour with tenebrionid beetles was offered to the mice, which may lead to believe that the secretions of defensive glands may have the same harmful effect on mice as those treated with 1,4-benzoquinone. Several authors indicated that the defensive glands of tenebrionid beetles *T. confusum, T. castaneum* and *Tenebrio molitor* secrete a mixture of quinones and their derivatives 2-ethyl-1,4-benzoquinone, 2-methyl-1,4-benzoquinone and 2-methoxy-3-methyl-1,4-benzoquinone, [19-22].

The observer should be aware that the action of the toxic agent on the mother may affect the results of teratogenic studies and make interpretations more difficult, because of complex inter-relationship between lethal action upon the embryo, toxicity for the mother and teratogenic effect. In other words it is difficult to state if teratogenic effects may be induced directly or indirectly.

However, lack of information, and limited knowledge of the processes and mechanisms of teratogenic agents of other drugs necessitate the need for well organized research efforts of a long range character. Extensive and expensive programs of research must be undertaken, to reduce teratogenic problems which may face human beings in the future.

Table 1. Teratogenic effect of 1,4-benzoquinone and force feeding of treated wheat flour on maternal body weight and fetus of mice

Parameter / Doses mg/kg/ 2 days	Maternal body weight (gm) after 18 days from treatment	Weight of uterus (gm)	Number of fetus			Resorption		Mean of body weight of fetus (gm)	Mean of crown-length of fetus (cm)
			Total	Live	Dead	Early	Late		
Control	37.36[a]	9.49[a]	4.90[a]	4.90[a]	0.00[b]	0.00	0.00[bc]	0.82[a]	3.78[a]
10	21.75[c]	6.95[b]	3.20	3.10[b]	0.10[b]	0.00	0.10[bc]	0.74[a]	2.60[b]
50	23.43[b]	7.74[b]	3.27[b]	2.50[b]	1.00[b]	0.60	0.40[bc]	0.53[b]	1.70[c]
100	20.06[d]	6.21[b]	3.50[b]	0.80[c]	2.20[a]	0.50	1.00[b]	0.51[b]	2.12[bc]
150	21.76[c]	6.42[b]	3.00[b]	0.00	3.00[a]	0.60	1.40[a]	0.41[b]	1.66[c]
Rate of initial infestation insct/kg	**Maternal body weight (gm) after 18 days from treatment**	**Weight of uterus (gm)**	**Number of fetus**			**Resorption**		**Mean of body weight of fetus (gm)**	**Mean of crown-length of fetus (cm)**
			Total	**Live**	**Dead**	**Early**	**Late**		
Control	35.11[a]	9.16[a]	4.20[a]	4.20[a]	0.00	0.00	0.00	0.90[a]	2.78[a]
90	29.95*[b]	5.05[b]	3.00	2.50[b]	0.50	0.00	0.50	0.43[b]	1.66[b]

* Females fed treated wheat flour 3 months before mating.

References

[1] Bridges, B. "Environmental Genetic Hazards: The Impossible Problem." *EMS Newsletter*, 5, (1971), 13-15.

[2] Earl, F.L.; Miller E., and Van Loon E.J. "Reproductive, Teratogenic, and Neonatal Effects of Some Pesticides and Related Compounds in Beagle Dogs and Miniature Swine." *Pestic. Environ Continuing Controversy, Pap.* Inter-Am. Conf. Toxicol. Occup. Med. 8th. (1973), 145687 Y.

[3] Fraumeni, F.G. "Chemical in Human Teratogenesis and Transplacental Carcinogenesis." *Epidemiology Branch, National Cancer Institute, Bethesda, Maryland 20014., Supplement*, 53, No. 5, Part II, (1974), 807-812.

[4] Frederick, M.H.; Jawetz, Ernest and Goldfine, Alan. "Review of Medical Pharmacology." 5th (ed.) Chapter 19. *Histamine, Anthistamines serotonin*, (1974).

[5] Kennedy, G.L.; Arnold, D.W., and Calandra, J.C. "Teratogenic Evaluation of Lead Compounds in Mice and Rats." *Food Consmet Toxicol*, 13, No. 6 (1976), 629-632.

[6] Martin, D.H.; Lewis, R., and Tibbitts, F.D. "Teratogenicity of Fungicide Captan and Folpet in the Chick Embryo." *Bull. Environ. Contam. Toxicol.* 20, No. 2 (1978), 155-158. (C.F. *Chem Abst.* 89, 192078 p).

[7] Varnagy. L. "Preliminary Toxicity and Teratogenicity Testing of Captan in Japanese Quail Embryos." *Acta Vet. Aced. Sci. Hung.*, 29, No. 2 (1981), 193-196.

[8] Varnagy, L.; Imre R.; Fancsi, T., and Hadhazy, A. "Teratogenic Studies of Captan in Japan Quail and Pheasant Embryos." *Acta Vet. Aced. Sci. Hung.*, 29, No.4 (1984), 383-388.

[9] Rogers, J.N.; Barbee R.; Burkhead, L.M.; Rushin, A.E., and Navlock, R.J. "The Mouse Teratogen Dinocap has Lower A/D Ratio and is not Teratogenic in the Rat and Hamster." *Teratogenic*, 37, No. 6 (1988), 553-559. (C.F. *Chem. Abst.* 109. 50064 p).

[10] Baker, J.B.E., "Principles for the Testing of Drugs for Teratogencity." *Report of a WHO Scientific Group*. Geneva, No. 364, (1967).

[11] Mavis, B.R.; Rudolfculik, L.D.O., and Favyerweather, W.F. "Skin Abosorption of Hexafluoroacetone: Teratogenic and Lethal Effects in the Rat Fetus." *Toxicology and Applied Pharmacology* 47, (1979), 35-39.

[12] Cummings, A.M.; Ebron-mccoy, M.T.; Rogers, J.M.; Barbee, B.D., and Harris, S.T. "Developmental Effect of Methyl Benzimidazo-lecarbamate Following Exposure During Early Pregnancy." *Fundamental and Applied Toxicology*, 18, (1992), 288-293.

[13] Schildknecht, H., and Weis, H. "Mitteilung Uber Insektenabwehrstoffe." VI. Uber Das Fluchtigh Sekret Vom to Tehkafer (*Blaps martisaga*) *Z. Naturf.*, 15, No.6 (1960), 200.

[14] Eisner, T. and Meinwald, J. "Defensive Secretions of Arthropods." *Science*, 153 (1966), 1341-1350.

[15] Ladisch, R.K.; Ladisch, K.S.L., and Howe, P.M.H. "Quinoid Secretions in Grain and Flour Beetles." *Nature*, 215, (1967), 939-940.

[16] Ganem, N.F. "Factors Responsible for the Carcinogenicity of Flour Infested with the Weevil" *T. castaneum* Using Swiss Albino Mice as Biological Test Animals." *Ph. D. Thesis*. Faculty of Science, Alexandria University (1990).

[17] Palmer, A. K. "Regulatory Requirements for Reproductive Toxicology Theory and Practice." In: *Developmental Toxicology*. C.A. Kimmel and J. Buelk-sam (Eds.), New York: Raven Press. 1981.

[18] Steel., R.G.D. and Torrie, J.H. *Principles and Procedures of Statistics A Biometrical Approach*. 2nd ed. Singapore: McGraw-Hill International Company, 1981.

[19] Loconti, J.D. and Louis, M.R. "Composition of the Odorous Secretion of *Tribolium castaneum*." *Ann. of Entomol. Soc. of America*, 46, No.2 (1953), 281-289.

[20] Tseng, Y.L.; Davidson, A., and Menzer, R.E. "Morphology and Chemistry of the Odoriferous

Gland of the Lesser Meal Worm, *Alphitobius diaperinus." Ann. Entomol. Soc. Am.*, 64, (1971), 425-430.

[21] Tschinkel, W.R. "6-Alky-1,4-naphthoquinones from the Defensive Secretion of the Tenebrionid Beetle, *Argoporis alutacea." J. Insect. Physiol.*, 18, (1972), 711-722.

[22] Markarian, H.; Florentine, G., and Pratt, Y. "Quinone Production of Some Species of *Tribolium." J. Insect. Physiol.*, 24, No. 12 (1978), 785-790.

الثأثير التشوهي الجنيني لـ ١ ، ٤ بنزوكينون والدقيق المصاب بخنافس الفصيلة Tenebrionidae على الفئران

إبراهيم هاني القشلان[1]، إجلال هلال، مديحة عبدالحميد، محمد عباس عبداللطيف
الهيئة العامة للسلع التموينية[1] وقسم وقاية النبات، كلية الزراعة،
جامعة الإسكندرية، الإسكندرية، مصر

(قُدم للنشر في ١٤١٣/٩/٢١هـ؛ وقبل للنشر في ١٤١٤/٧/٢٩هـ)

ملخص البحث. تم دراسة تأثير كل من الـ ١ ، ٤ بنزوكينون والدقيق المصاب بمعدل ٩٠ حشرة لكل كجم على الأمهات والأجنة داخل الرحم للفئران السويسرية البيضاء لمعرفة التأثيرات التشوهية والجنينية لكلا المعاملتين. وقد سجلت التأثيرات الناتجة من المعاملة بـ ١ ، ٤ بنزوكينون عن طريق الفم تبعًا لمقاييس خاصة وهي وزن الأمهات الحوامل ووزن الرحم وعدد الأجنة داخل الرحم والادمصاص المبكر والمتأخر ثم وزن الأجنة وطولها التاجي. ثم سجلت أيضًا التغيرات غير الطبيعية للهيكل العظمي. وقد سجلت أيضًا المقاييس السابقة نفسها لطريقة المعاملة بالدقيق المصاب بخنافس الفصيلة Tenebrionidae والتي تفرز مادة ١ ، ٤ بنزوكينون من غددها الدفاعية وذلك للمقارنة بين المعاملتين.

وقد وجد أن التركيزات المرتفعة لـ ١ ، ٤ بنزوكينون (١٠٠، ١٥٠مجم/ كجم) قد أحدثت تأثيرات تشويهية جنينية في وزن الأمهات وعدد الأجنة داخل الرحم (الميت والحي) والادمصاص المبكر والمتأخر للأجنة بداخل الرحم. لم تكن الفروق معنوية إذا ما قورنت بغير المعامل. كذلك أحدثت تأثيرات في وزن الرحم نفسه. أما عن الأجنة فقد ظهرت تشوهات خارجية فيها عند استعمال التركيزات المنخفضة (١٠، ٥٠مجم/ كجم) كذلك انخفض وزن الجنين عن الوزن الطبيعي. وبقياس الطول التاجي للأجنة وجد أنه أقل عن الطول الطبيعي أيضًا.

أما عن التشوهات التي أحدثتها طريقة المعاملة بالـ ١ ، ٤ بنزوكينون عن طريق الفم في الهيكل العظمي فقد أحدثت التركيزات المرتفعة (١٠٠، ١٥٠مجم/ كجم) تشوهات في الأطراف (الأيدي والأرجل) عن التركيزات المنخفضة (١٠، ٥٠مجم/ كجم).

وقد درست المقايس السابقة نفسها لطريقة المعاملة بالدقيق المصاب بخنافس الفصيلة Tenebrionidae فوجد أن هناك فروقًا واضحة بين طريقتي المعاملتين حيث وجدت فروق معنوية في طريقة المعاملة بـ ١ ، ٤ بنزوكينون عن طريق الفم بين المعامل والكنترول (غير المعامل) وكانت هذه الفروق أقل بكثير عند استخدام طريقة المعاملة بالتغذية على الدقيق المصاب.

ويمكن القول إن التغذية على الدقيق المصاب بخنافس الفصيلة Tenebrionidae قد أحدثت تأثيرات تشوهية جنينية في الأمهات الحوامل وفي الهيكل العظمي لأجنة الفئران السويسرية البيضاء.