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Teratogenic Effect of The Fungicides Benomyl and Carbendazim on Mice

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ABSTRACT

Benomyl and carbendazim are two plant systemic fungicides. The two compounds were administered via the dietary route to pregnant swiss Albino female mice at the doses of 250, 500, 1000, and 1500 mg/kg/day of each of the two compounds with or without corn oil from day 6 to day 17 of gestation. The results showed that benomyl at 1500 mg/kg and carbendazim at both 500 and 1000 mg/kg/day increased the number of resorbed fetuses. Besides, carbendazim at 500 mg/kg decreased the number of live fetuses. skeletal malformations were shown in fetuses of both benomyl at 500 mg/kg and carbendazim at 250 mg/kg doses where leg and hand phalanges bones were absent. Benomyl at 1000 mg/kg and carbendazim at 500 mg/kg, the leg and hand phalanges were completely absent, and the metacarpal and metatarsal bones were also absent, while retarded ossification of parietal and interparietal were induced. Parallel to these malformation the placental alkaline phosphatase activity was diminshed at the doses 1000 mg/kg of benomyl and 500 mg/kg of carbendazim.

INTRODUCTION

The widely used fungicide benomyl, methyl 1-(butylcarbamoyl) 2-benzimidazole Carbamate and its breakdown product, methyl 2-benzimidazole carbamate (MBC), was found to be mutagenic in Ames organisms (Lang et al., 1980). Benzimidazole carbamate was found to be teratogenic in rats when administered during the 14th-15th days of pregnancy (Delatour and Richard 1976). Its embryotoxic effect was quite strong when given during the first days of pregnancy (Ryabova and Novik, 1983).

Benomyl was embryotoxic and teratogenic in rats when given orally at 500 mg/kg in a single dose between the 19th and 30th day of pregnancy (Shtenberg and Torchinskii, 1972). However Shermon et al. (1975) reported that, feeding of as much as 0.50% benomyl to pregnant rats on days 6-15 of gestation did not affect the offspring of the embryonal development. Benomyl produced fetotoxicity but no teratogenic effects, when it was administered during organogenesis via the dietary route to pregnant rat (Kavlock et al., 1982).

Teratogenic activity of carbendazim was investigated by vergieva (1982). An increase in the mortality rate of fetuses was observed in female rats and rabbits where orally administered

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carbendazim at 20, 40 and 80 mg/kg/day from day 5 to 15 of gestation for rats or 40,80 and 150 mg/kg for rabbits.

The present study was initiated to evaluate the effect of dietary exposure to benomyl and carbendazim in the mice. Besides, the effect of two different types of diet and the effects on food consumption, maternal weight change on feed efficiency, on pregnant mice, the fetuses, and skeletal malformation were recorded, also the effect on placental alkaline phosphatase was determined.

MATERIALS AND METHODS

A. Tested fungicides: Commercial benomyl and carbendazim fungicides were provided by the ministry of Agric., Cairo.

B. Tested animals ; female mice were pen bred with males of the same strain of demonstrated fertility using one male per every twoi females. This procedure was carried out from 4 Pm to 8 Pm daily. Copulation was ascertained by the presence of a vaginal plag. This time was considered the first day of gestation. females were separated and housed in groups for treatments.

C. Treatment of animals : Administration of either benomyl or carbendazim was used on the maternal weight on 1st day of gestation. Treated animals recevied fresh prepared diet daily From day 6 to day 17 of gestation , benomyl or carbendazim doses incorporated in the diet were administered with or without 1% corn oil.

D. Histological studies: Fetuses from each litter were cleared and stained with Alizarin Red as described by Williams (1941).

E. Placental Alkaline Phosphatase: According to the methods described by Bessey et al (1946), and Dipietro & Zengerle, (1967), alkaline phosphatase activity was determined in placental preparations.

RESULTS AND DISCUSSION

A. Pregnant Females:-

The results demonstrated that benomyl at 150 mg/kg/day decreased the number of pregnant females at necropsy in both diet groups , while benomyl at 1000, and 1500 mg/kg/day showed a different percentage of complete resorption. Benomyl also at 1000 mg/kg/day reduced weight gain in females. Carbendazim at 1500 mg/kg/day showed that, all females were not pregnant at necropsy in both diet groups, while 1000 mg/kg/day decreased the number of pregnantfe females. Females treated with carbendazim at 500 and 1000 mg/kg/day showed a complete resorption.

B. Feutses:

Benomyl at 1500 mg/kg/day increased the number of resorbed fetuses, and at 1000 mg/day decreased fetuses weight and length. Carbendazim at 500 and 1000 mg/kg/day decreased the number of resorbed fetuses photo (1,2) while at 500 mg/kg day decreased the mean weight , length, and the number of live fetuses. These findings are in agreement with the results suggested by Mercier-Parot (1976) who reported that parbendazole at the highest dose caused 100% resorption.

Benomyl at higher dose 1000 mg/kg/day reduced females weight gain. A similar result was obtained by Ogata, et al. (1978) who reported that the administration of thiabendazole decreased the weight gain of mice. Benomyl at the highest dose 1000 mg/kg/day reduced fetuses weight and length. This result in agreement with the results reported by Yoneyama, et al (1984). Carbendazim produced more severe effects on both dams and fetuses than benomyl. Thus the teratogenic potential of carbendazim might exceed that of benomyl. Styles and Carner (1974), reported that carbendazim is better absorbed from the gastrointestinal tract than the parent compound benomyl. This might explain the recorded differences.

Benomyl at 500 mg/kg/day caused the fetuses malformation where hand and leg phalanges bones were absent (Photo 3 & 4) which were associated with the absence of metacarpal and metatarsal bones (Photos 5 & 6). Also retarded ossification of parietal and interparietal was observed (Photo 7 & 8). On the other hand benomyl at 250 mg/kg /day did not cause any effect on the fetal skeleton. All doses of benomyl did not affect the rate of growth.

The results also indicated that carbendazim at 500 mg/kg/day showed fetuses with absent hand , leg phalanges bones, absent metacarpal and metatarsal bones and retarded ossification of parietal and interparietal. While 250 mg/kg/day caused effect on fetuses with absent hand and leg phalanges bones. Carbendazim at all doses did not cause any effect on weight gain. carbendazim at all doses induced skeletal malformations more than benomyl. On the other hand the induced skelatal malformation was dose dependent. These results are similar to those found by mercier-parot (1976), who reported that parbendazole caused dose-dependent anomalies. Khera et al. (1979) reported that thiabendazole increased incidence of anomalous in fetuses at the higher doses.

C. Placental alkaline phosphatase:-

Figure (1) indicated that benomyl at 250 and 500 mg/kg/day did not cause any effect on the activity of placental alkaline phasphatase, while at 1000 and 1500 mg/kg/day decreased the enzyme activity. Carbendazim at 250 mg/kg/day did not cause any effect, while at 500 and 1000 mg/kg/day decreased the enzyme activity.

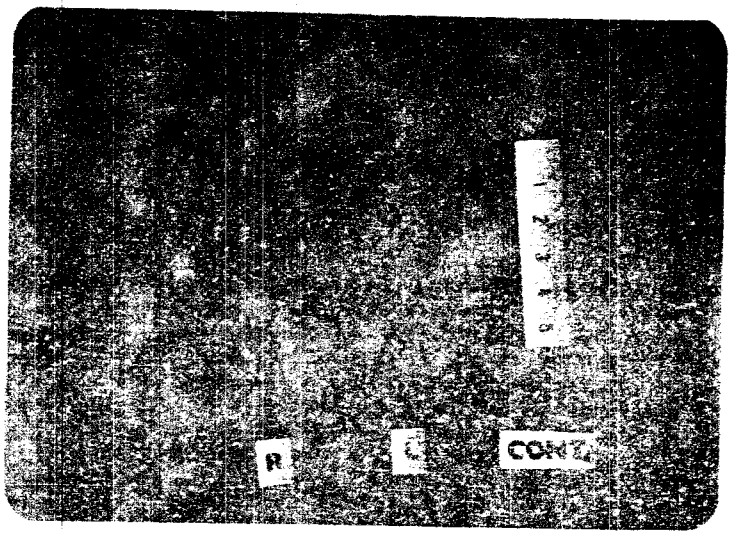

Photo (1): Normal fetuses (control).

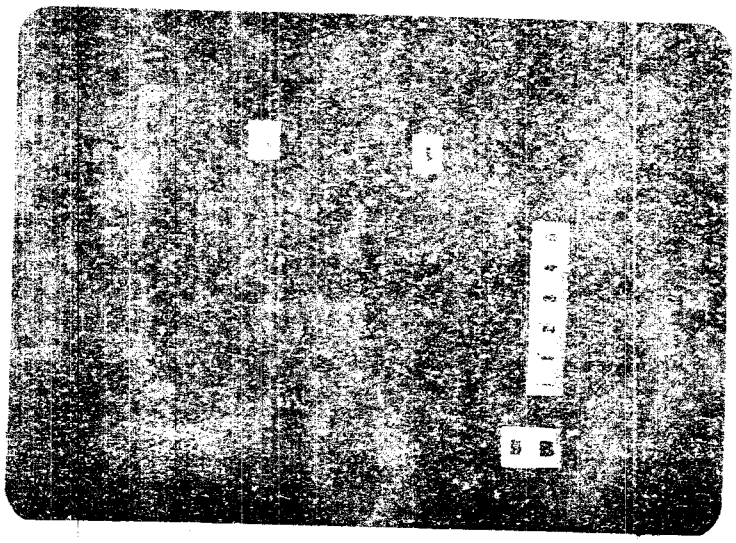

Photo (2): Fetuses resorbed in mouse uterus treated with 500mg/kg/day of carbendazim.

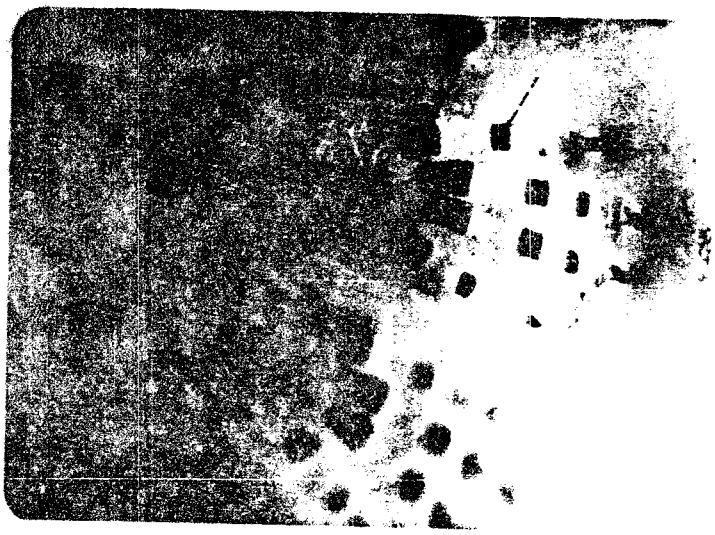

Photo (3): Normal fetuses. Hand phalanges(↙) and metacarpal bones(↙) (control).

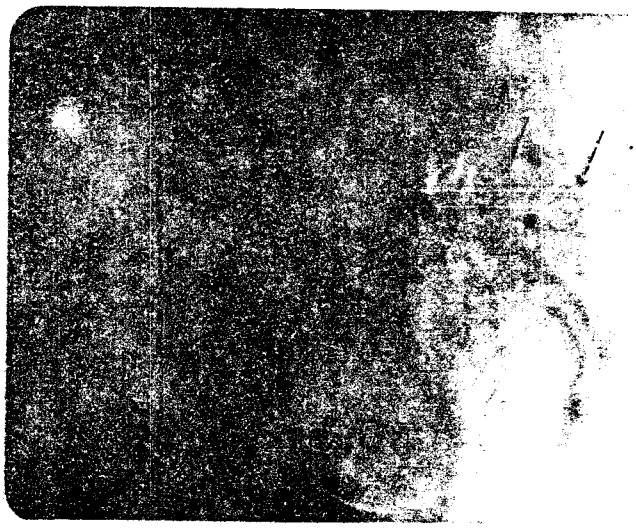

Photo (4): Fetuses treated with 500mg/kg/day of benomyl. Hand phalanges bones absent(↙) and also metacarpal bones absent.(↙)

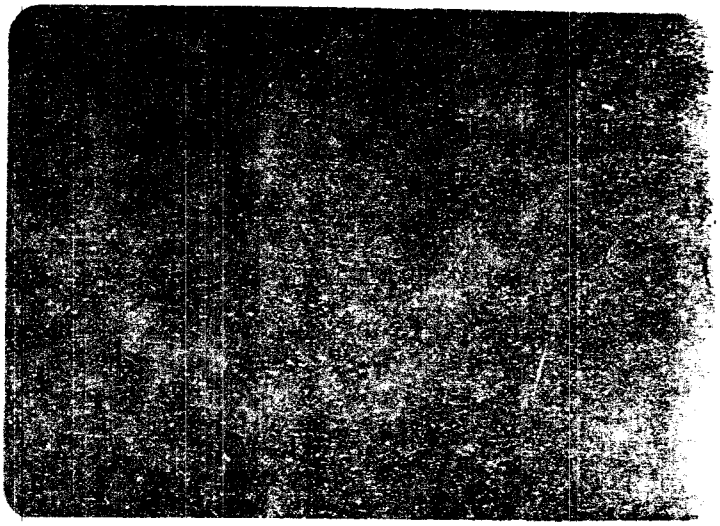

Photo (5): Normal fetuses. Leg phalanges ( ) and metatarsal bones ( ) (control).

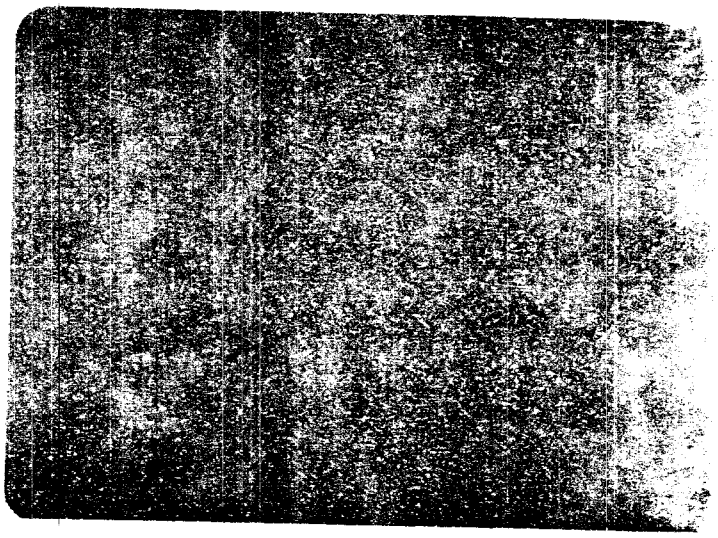

Photo (6): Fetuses.with 500mg/kg/day of benomyl. Leg phalanges bones absent( ) and also metatarsal bones absent( ).

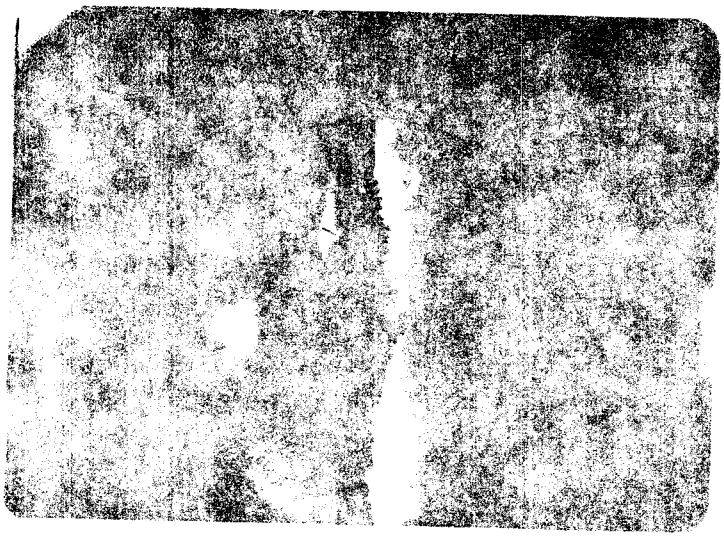

Photo (7): Normal Fetuses. Ossification of parietal(←) and interparietal(←) (control).

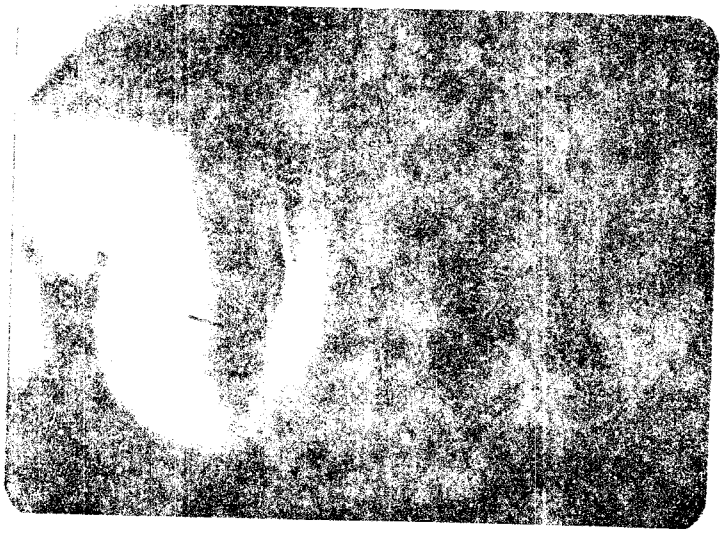

Photo (8): Fetuses treated with. Retarded ossification of parietal(←) and interparietal(←).

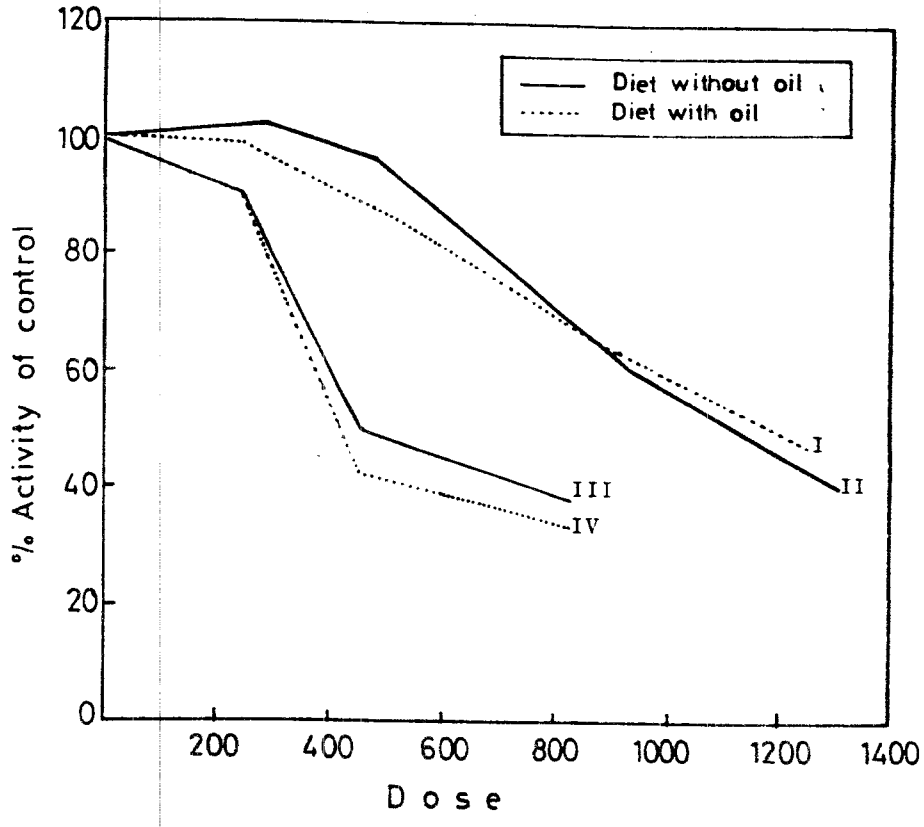

Fig.(1) : In vivo inhibition of placental alkaline phosphatase activity of mice treated with benomyl. (I , II)and carbendazim (III, IV) .

Thus the higher doses of benomyl and carbendazim which caused a complete resorption had decreased the activity of alkaline placental phosphatase. Carbendazim was more effective as inhibitor of the enzyme activity than benomyl. These findings were in agreement with Georgiev and Mirkova (1975) and Mirkova (1976) who reported that the fungicide bectfungin decreased the activity of placental thermostable alkaline phasphatase.

The present data supports the use of placental alkaline phosphatase as a paramater to predict the hazard of exerting fetuses malformation.

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الملخـــص

البيونوميـــل والكارباندازيـم مبيدات فطريان جهازيان ،ولقد اعطيـت هذه المركبات عن طريق الفـم لحيوانات التجارب الفئـران الصغيره بالجرعـات ٢٥٠ ، ٥٠٠ ، ١٠٠٠ ، ١٥٠٠ ملجرام / كجم / يوم مـن كل من هذان المركبيـن في وجـود اوعـدم وجـود زيت الذره مـن اليـوم السادس الى السابع عشـر من الحمـل . ولقد اوضحت النتائج ان البيونوميـل بمعدل ١٥٠٠ ملجرام / كجم والكاربانـدازيـم بمعدل ٥٠٠ ، ١٠٠٠ ملجرام /كجم / يوم أدت الى زيادة عدد الاجنه الممتصه وبجانب ذلك وجد ان الكارباندازيم بمعـدل ٥٠٠ ملجرام / كجم ينقص من عدد الاجنه الحيه ولقد ادى كل من البيونيل بمعدل ٥٠٠ ملجرام / كجم والكارباندازيم بمعدل ٢٥٠٠ ملجرام / كجم الى غياب عظام الارجـل واليـد اما البيونوميل بمعـدل ١٠٠ ملجرام / كجـم والكارباندازيم بمعدل ٥٠٠ملجرام / كجم قد ادى الى الغيـاب الكامل لعظام اليـد والارجل ،وكذلك غيـاب عظـام المشط في اليـد والقدم وكذلك عظام الجمجمـه وظاهره عـدم التعظـم كانت واضحـه . واحداث التشوهات كان متوازيـه مع التأثير على نشاط انزيم الفوسفاتيز في البلاسنتا خصوصا على ١٠٠٠ ملجـرام / كجم من البيونوميـل ، ٥٠٠ ملجرام / كجم من الكاربانـدازيـم .