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Embryotoxic and teratogenic effects of certain pesticides
I. Weight changes of eggs during incubation

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ABSTRACT.

Embryotoxicity and teratogenicity of four tested compounds which use as fungicides benomyl carbendazim.Dimethoate as one of the organophosphorus compounds and cypermethrin as synthetic pyrethroids were carried out on fertile hen eggs of the Alexandria chicken strain injected into the yolk-sac on the 6th day to select the suitable vehicle carrier for testing the compounds and record their effects on weight changes of eggs during incubation. The data showed that all the tested solvent and pesticides caused decrease in the average weight loss of eggs during incubation as compared with the control Isopropanol tested eggs showed very close values to the control. The results of these experiments were in favor of the use of isopropanol as a carrier for tested chemicals has proved that the chick embryo injection technique is successful in detecting the adverse effects of embryotoxicity or teratogenicity in a dose dependent pattern.

INTRODUCTION

According to the report of the W.H.O. Expert Group (1967). The choice of doses with which th initiate teratoglogical studies.

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should be made against the carefully considered back ground ofthe pharmacological and toxicological activity of the chemical agent.There is a complex inter-relationship between letal action upon the embryo toxicity for the mother and teratogenic effect.The observer, therefore should be aware that the action of the drug on the mother may affect the results of teratological studies and thus make their interception more difficult. In most instances dosage should be based on body weight.

Henderson,et al. (1982),indicated that in ulteroethanol exposure in rat and mouse induced significant decrease in prenatal growth and in fetal survival.

Recently in the WHO.IARC monograph (1988). Effects of ethanol on reproduction and prenatal toxicity were reviewed oral doses of ethanol (1-2 g/kg b.w.) to rats throughout gestation caused decreases in litter size. litter weight,and mean pup weight. There was also confirming evidence of behavioral teratogenesis in treated rats. Ethanol increased the incidence of cleft palate in swiss-mice administered methyl mercuric chloride and retinyl acetate.

Ethanol also.in combination with lithium carbonate,had a synergistic effect on the induction of fetal abnormalities in albino rats. According to (shepard,1980) DMSO was injected on the 8th day to hamsters at the dose range (0.5-8.0 gm/kg)it produced exencephaly in a high proportion of fetuses. Rib fusions,micropholmia, limb abnormalities and cleft lip were also found .

Somlyay and varnagy(1983). reported that there are many good reasons for using the

avian embryo in first live screening of pesticides candidates. They added that the avien embryo has been used extensively in screening for toxic and teratogenic effects of pesticides.

It goes without saying that it was necessary to start by also screening some solvents to select a real inert solvent to be used as a vehicle carrier in the injection treatments of fertile eggs and to study the effect of these chemicals on average weight of eggs during incubation periods.

MATERIALS AND METHODS

A.Tested chemicals:-

The following pesticides were standard pure samples of active ingredient compounds provided by EPA (USA).

- Benomyl : LD50 (9920 mg/kg)
- Carbendazim : LD50 (6400 mg/kg)
- Dimethoate : LD50 (151 mg/kg)
- Cypermethrin : LD50 (251 mg/kg)

B.Selection of a suitable solvent:-

To select the suitable solvent for the tested chemicals. the following solvents were examined:-

a- Ethyl alcohol.chemical purity 98% CH3-CH2OH.

b- Isopropanol (Analar).100% CH3-CH(OH).CH3.

c- Dimethyl sulfoxide (DMSO),chemical purity (DMSO) 100%,(CH3)2-S=O.

d- Glycerol.chemical purity 100%. CH2 (OH).CH(OH).CH2OH.

The following protocol was applied to test the solvent:

- Thirty fertile eggs were selected for each solvent.and the same number served as a control group.

- Eggs were incubated for 21 days at 100 F _+ 20 (37.5 C _+ 1).
- After the sixth day of incubation 20 ul of the solvent was injected into the yolk sac of the embryo.
- Then. the eggs were returned to the incubator to compete their incubation period.
- On the 19th and 21st days of incubation 15 eggs were examined according to the following parameters.

a- Ability of naturally hatching on the 21st day of incubation and only the eggs unable to hatch were opened.

b- The percentage of living and dead embryos were calculated.

c- Weight of living chicks and the measurement of the legs,wings,body length and status of feathers and neck were recorded.

C.The Source of tested Chick embryo:

The "Alexandria" chicken strain from the university of Alexandria faculty of Agriculture experimental station was used as the source of eggs used in the experiments of the present investigation. The "Alexandria" strain was developed by Egyptian breeders from inter-crossing between fayoumi,plymouth:Red Rhode Island and white leghorn chicken strains.The "Alexandria" strain is now well developed as a homozygous strain.It is characterized by giving well grown hens and rosters or 1.5-2 kg each.They lay 150-180 eggs/year,weighing in the average of 50 gm each.The regular incubation period is 21 days.

D.Incubation Conditions:-

An electrical incubator with 500 eggs capacity and with five drowers was used

throughout the study. The apparatus is thermostatically controlled at 100 □+2 f (37.5_+ 1C). The relative humidity was maintained at 50-65%. Rotation of the eggs was performed 3-5 times every 24 hours. The incubation conditions were checked and recorded regularly.

E. Preparation of Eggs for Chick Embryo Inoculation

- Homogeneous eggs of the average weight 50.60 _+3.4 gm were selected from a fertile flock and were cleaned by gentle drying or rubbing with slight moisture.
- Incubation takes place while the air-sac is upward.
- Each egg was numbered and weighed before incubation and then at two days intervals till the 18th day.
- The selected eggs were incubated for six days before inoculation at (100 _+ F). This period was adopted to make sure that every egg had a living embryo. This was performed by candling each egg by lamp on the 6th day of incubation.
- The site of the air-sac and the site of the embryo were detected and marked before inoculation.
- The site of perforation (the site of inoculation) is disinfected by tincture of iodine (5%) in absolute alcohol.

F. Yolk Sac Route (Y.S.) Injection Technique:-

- The eggs were candled to define the site of the live embryo and the air-sac.
- The outer surface of the egg s shell over the air-sac was cleaned using tincture iodine 5% in absolute alcohol and then left to dry.
- Using an electrical teeth driller the egg shell was drilled at the pole point of the center of the air-sac.

- The needle was inserted vertically for a distance of 2-2.5 cm deepward. To make sure that the needle was in the yolk.0.1 ml of the liquid was withdrawn.and if the yolk material got out.Then the syringe was in the right position and the injection was completed. The volume of the inoculum was 20 ml in each egg.
- The needle was then withdrawn.and the perforation site was sealed with either paraffin wax or celluloid.
- The treated eggs were incubated in vertical position.

H.Number of replicates:-

for each treatment.ten eggs were used as replicate. including the negative control and the solvent-treated controls. The treatments were repeated in six batches at successive periods of time and the data were analysed statistically.

RESULTS AND DISCUSSION

The results of experiment which was designed to select a suitable solvent as a carrier for the tested pesticides are shown in (Table 1) untreated fertile eggs,with an average weight of 50.60_+ 3.40 gm before incubation.showed 1.32 _+ 0.18 gm weight loss at day 6 of incubation. Control eggs exerted 4.69_+ 0.20 gm decrease in weight at day 19 of incubation. The eggs treated with glycerol showed the least weight loss.while those treated with isopropanol showed very close values as the control.Weight loss of the eggs treated with ethanol and DMSO were also lower than that of the control ones. Statistical analysis of the data revealed a highly significant difference ($p<0.001$) between weight changes of control eggs and those treated with glycerol.and only a significant difference ($p<0.01$) between DMS or ethanol.

Table (1)

Weight Changes of the Eggs* During Incubation After Injection with Solvents.

Treatment Groups* (Solvents)***	**Average weight loss of eggs (gm) mean ± SD Days of incubation 8	10	12	15	19
Control	1.85 ±0.29	2.90 ±0.23	3.76 ±0.14	4.69 ±0.20	5.87 ±0.70
Isopropanol	1.86 ±0.20	2.93 ±0.13	3.58 ±0.55	4.73 ±0.17	5.90 ±0.80
Ethanol	1.63 ±0.09	2.54 ±0.30	2.77 ±0.63	3.50 ±0.60	3.85 ±0.50
DMSO	1.67 ±0.30	2.01 ±0.20	2.23 ±0.50	2.60 ±0.60	3.05 ±0.30
Glycerol	1.25 ±0.23	1.93 ±0.10	2.40 ±0.10	2.98 ±0.56	3.26 ±0.62

*Each group includes 30 fertile eggs with an average weight 50.60 ±3.4 gm.

**Average weight loss up to day 6 of incubation was 1.32 ±0.15 gm before injection.

***20 µl of the solvent was injected in each egg.

Table (2)

Egg Weight Loss (gm) After Incubation with Sub-lethal Doses of Benomyl.

Treatment doses * (mg/kg)	Treatment doses (mg/egg)	Average weight loss of eggs (gm) Mean ±SD**				
		Days of incubation				
		8	10	12	15	15
Control (untreated)	-	1.85 ±0.29	2.90 ±0.23	3.76 ±0.14	4.69 ±0.20	5.90 ±0.70
Control (Isopropanol)	20µl/egg	1.86 ±0.20	2.93 ±0.13	3.54 ±0.55	4.73 ±0.17	5.90 ±0.80
$\frac{LD_{50}}{100}$ (99.00)	4.[illegible]	1.44 ±0.53	1.39 ±0.97	1.91 ±0.17	1.82 ±0.21	2.45 ±0.16
$\frac{LD_{50}}{200}$ (49.40)	2.47	1.50 ±0.36	1.40 ±0.39	2.65 ±0.16	2.77 ±0.13	3.60 ±0.21
$\frac{LD_{50}}{300}$ (33.60)	1.65	1.63 ±0.10	2.35 ±0.16	2.96 ±0.03	2.62 ±0.11	3.52 ±0.13
$\frac{LD_{50}}{500}$ (19.80)	0.99	1.61 ±0.09	2.43 ±0.03	2.89 ±0.07	3.10 ±0.03	3.37 ±0.08
$\frac{LD_{50}}{1000}$ (9.90)	0.495	1.61 ±0.05	2.15 ±0.09	2.63 ±0.51	3.14 ±0.03	4.41 ±0.01
$\frac{LD_{50}}{1500}$ (6.60)	0.330	1.73 ±0.01	2.76 ±0.22	2.89 ±0.25	3.41 ±0.28	4.91 ±0.11

*Each group includes 30 fertile eggs with an average weight of 50.69 ±3.4 gm.

**Average weight loss up to day 6 of incubation was 1.32 ±0.15 gm before injection.

Table (3)

Egg Weight Loss (gm) After Incubation with Sub-lethal Doses of Carbendazim.

Treatment Doses * (mg/kg)	Treatment doses (mg/egg)	Average weight loss of eggs (gm) Mean ±SD** Days of Incubation				
		8	10	12	15	19
Control (untreated)	-	1.85 ±0.29	2.90 ±0.23	3.76 ±0.14	4.69 ±0.20	5.90 ±0.70
Control (Isopropanol)	20µl/egg	1.86 ±0.20	2.93 ±0.13	3.58 ±0.55	4.73 ±0.17	5.90 ±0.80
$\frac{LD_{50}}{100}$ (64.00)	3.2	1.44 ±0.63	2.09 ±0.37	2.31 ±0.02	2.81 ±0.11	3.02 ±0.02
$\frac{LD_{50}}{200}$ (32.00)	1.6	1.55 ±0.11	2.29 ±0.01	2.40 ±0.03	2.63 ±0.03	3.06 ±0.30
$\frac{LD_{50}}{300}$ (21.33)	1.06	1.52 ±0.03	2.61 ±0.04	2.75 ±0.34	3.22 ±0.37	3.81 ±0.12
$\frac{LD_{50}}{400}$ (11.60)	0.64	1.63 ±0.2	2.41 ±0.05	2.56 ±0.14	3.54 ±0.36	4.21 ±0.07
$\frac{LD_{50}}{1000}$ (6.40)	0.32	1.76 ±0.21	2.46 ±0.5	2.83 ±0.12	3.70 ±0.14	4.56 ±0.14
$\frac{LD_{50}}{1500}$ (4.26)	0.213	1.84 ±0.13	2.41 ±0.31	2.85 ±2.65	3.65 ±0.11	4.58 ±0.31

*Each group includes 30 fertile eggs with an average weight of 50.60 ±3.6 gm.
**Average weight loss up to day 8 of incubation was 1.32 ±0.15 gm before injection.

On the other hand,the eggs treated with isopropanol showed an insignificant difference ($p>0.1$) from the control ones. The results thus favor the adaption of isopropanol as a successful carrier for the pesticides used in the present investigation.

The screening test of the solvents thus proved that ethyl alcohol,glycerol and DMSO were teratogenic,and hence cannot be used as a carrier for injection of the tested pesticides Shepard (1980) reported positive teratogenic effects of both ethyl alcohol and DMSO.The alcoholic characteristic of the trihydroxy glycerol might suggest similar adverse effect similar to ethyl alcohol to the treated chick embryo.On the other hand,isopropanol did not show any adverse teratogenic effect to the treated chick embryos.No embryo toxicity or malformation effects were recorded.Therefore, isopropanol was selected as the suitable inert solvent carrier for the tested pesticides during injection experiments.

The average weight loss of incubated eggs of the control-untreated,control isopropanol treated,and benomyl treated groups are illustrated in(Table 2).The high dose groups. namely LD50/100. Ld50/200 and LD50/300 showed marked effect of average egg-weight loss during incubation and the most marked effect was observed in LD 50/100 group.Close values were showed by groups LD50/200.LD50/300 and LD50/500,which were slightly higher than those of LD50/100 group.The average egg-weight loss showed by groups LD50/1000 and LD50/1500 were the highest recorded values in benomyl treated groups.However,they were still lower than the controls average values (Table2)statistical analysis of the data revealed that egg-weight loss in benomyl-treated egg groups LD50/100, LD50/ 200.LD50/300 and LD50/500 were

Table (4)

Egg Weight Loss (gm) After Incubation with Sub-lethal Doses of Dimethoate.

Treatment doses * (mg/kg)	Treatment doses (mg/egg)	Average weight loss of eggs (gm) Mean ±SD** Days of incubation 8	10	12	15	19
Control (untreated)	-	1.85 ±0.29	2.90 ±0.23	3.76 ±0.14	4.69 ±0.20	5.87 ±0.70
Control (Isopropanol)	20µl/egg	1.86 ±0.20	2.93 ±0.13	3.58 ±0.55	4.73 ±0.17	5.90 ±0.80
$\frac{LD_{50}}{10}$	(15.20) 0.76	0.54 ±0.58	0.73 ±0.38	1.07 ±0.30	1.35 ±0.11	1.70 ±0.76
$\frac{LD_{50}}{50}$	(3.04) 0.152	1.23 ±0.64	1.69 ±0.19	2.31 ±0.19	2.97 ±0.63	2.99 ±0.93
$\frac{LD_{50}}{100}$	(1.52) 0.076	1.13 ±0.12	1.79 ±0.73	2.58 ±0.31	2.89 ±0.01	3.89 ±0.21
$\frac{LD_{50}}{250}$	(0.608) 0.0304	1.10 ±0.03	1.87 ±0.31	2.69 ±0.21	3.00 ±0.12	3.07 ±0.78
$\frac{LD_{50}}{500}$	(0.304) 0.0152	1.03 ±0.01	1.83 ±0.24	2.23 ±0.13	2.91 ±0.30	3.12 ±0.41
$\frac{LD_{50}}{1000}$	(0.152) 0.0076	1.65 ±0.04	2.11 ±0.21	2.75 ±0.28	3.95 ±0.27	4.87 ±0.60

* Each group includes 50 fertile eggs with an average weight of 50.50 ±3.4 gm.
**Average weight loss up to day 6 of incubation was 1.32 ±0.15 gm before injection.

Table (5)

Egg Weight Loss (gm) After Incubation with Sub-lethal Doses of Cypermethrin

Treatment doses * (mg/kg)	Treatment doses (mg/egg)	**Average weight loss of eggs (gm) Mean ±SD				
		Days of incubation				
		8	10	12	15	19
Control (untreated)	-	1.85 ±0.29	2.90 ±0.23	3.76 ±0.14	4.69 ±0.20	5.87 ±0.70
Control (Isopropanol)	20µl/egg	1.86 ±0.20	2.93 ±0..3	3.58 ±0.55	4.73 ±0.17	5.90 ±0.80
$\frac{LD_{50}}{10}$ (25.00)	1.25	1.43 ±0.08	2.13 ±0.52	2.20 ±0.06	2.85 ±0.15	4.06 ±0.03
$\frac{LD_{50}}{50}$ (5.00)	0.25	1.45 ±0.07	2.17 ±0.06	2.24 ±0.05	2.90 ±0.2	4.09 ±0.12
$\frac{LD_{50}}{100}$ (2.50)	0.125	1.51 ±0.21	2.23 ±0.11	2.32 ±0.01	2.98 ±0.03	4.13 ±0.06
$\frac{LD_{50}}{250}$ (1.0)	0.0500	1.53 ±0.16	2.32 ±0.10	2.51 ±0.01	3.07 ±0.12	4.19 ±0.15
$\frac{LD_{50}}{500}$ (0.50)	0.0250	1.62 ±0.10	2.38 ±0.13	2.55 ±0.07	3.16 ±0.11	4.23 ±0.29
$\frac{LD_{50}}{1000}$ (0.25)	0.0125	1.64 ±0.15	2.45 ±0.17	2.63 ±0.011	3.28 ±0.18	4.43 ±0.21

*Each group includes 30 fertile eggs with an average weight of 50.60 ±3.4 gm.
**Average weight loss up to day 8 of incubation was 1.32 ±0.15 gm before injection.

significantly lower($p<0.001$) than those of the control groups.Meanwhile egg-weight-loss in groups LD50/1000 and LD50/1500 showed a significant decrease ($p<0.01$) compared with the control groups carbendzim in doses LD50/100,LD50/200 and LD50/300 caused a highly significant decrease ($p<0.001$) in egg-weight loss during incubation as compared with the controls.Lower doses caused a significant decrease ($p<0.01$) in egg weight loss as compared with the controls(Table 3).The average weight loss of eggs of dimethoate treated group LD50/10 and LD50/50 was significantly lower ($p<0.001$) than the control group.However,while groups LD50/100,LD50/250 and LD50/500 showed a significant decrease ($p<0.01$)than the control.group LD50/1000 showed significant difference ($p<0.1$) to the control group (Table 4).

The average weight loss is inversely proportional with the dose of cypermethrin used.Statistical analysis shows that the egg weight loss during incubation was significantly lower ($p<0.01$) in the treatments than that of the control (Table 5).

The present work conclusion goes parallel to the data reported by voronina and unii (1984), who found that administration to rats of 40 mg/kg of cypermethrin during active organogenesis (6-15 day of pregnancy) showed an increased tendency of the post-implantation deaths and thus a decreasing in the number and body weight of litters.However,no teratogenic action was seen.

David (1982) studied the action of deltamethrinn on quail biotic potential and its effect on the embryonic genital system. He found that pur deltamethrin reduced the hatching rate and the embryonic viability.

In addition.pure deltamethrin and its commercial solution in xylene had limited teratogenic effect in the quail.They were also found to affect the embryonic genital system more in the males than in the females.There findings regarding decamethrim partially support the embryotoxic potential trend shown by cypermethrin and also indicate that synthetic pyrethroids might not be potent teratogenic agents.However,the group has the tendency to induce certain cytotoxic effects.

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١- تاثير بعض المبيدات على الاجنه و البيض
الحشرات فى وزن البيضه خلال فترة التحضين

تم خلق مبيدى البيوتوميل و الفارباندازيم مبيدات قطريه "الدايميثويت" مبيد حشرى فوسفورى "السيرميثرين" مبيد حشرى بيريثرويد مخفر صناعى فى بيض الدجاج نوع الاسكندرانى المخصب فى اليوم السادس فى منطقة الصفار. ولذلك لاختيار افضل المذيبات التى لاتحدث اضرار للبيض و التى لاتنتج عنها تشوهات واضحه اثناء تطور الجنين عن طريق تسجيل الانخفاض فى الوزن خلال فترة التحضين. ومن الواضح ان جميع المركبات و المذيبات المختبره احدثت نقصا فى متوسط وزن البيض خلال فترة التحضين مقارنته بالكنترول. ومن هذه النتائج يمكن استخلاص ان الايزوبروبانول يمكن استخدامه كمذيب لاختبار المبيدات حيث ان هذه الطريقه تعتبر طريقه سريعه وحساسه للكشف عن التاثير الضار للمركبات سواء على الاجنه او تسبب تشوهات فى مراحل تطور الجنين و الذى يعتمد على الجرعه المستخدمه.