EMBRYOTOXICITY AND TERATOGENIC POTENTIAL OF SOME CONVENTIONAL PESTICIDES ON JAPANESE QUAIL CHICK-EMBRYOS

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ABSTRACT

This study was designed to explore the toxic and teratogenic effects of chlorpyrifos (organophosphate pesticide), carbofuran and methomyl (carbamate pesticides) and a mixture between chlorpyrifos and carbofuran on the development of Japanese quail chick embryos. Treatment of chick embryos was done by injecting a single dose of tested compounds into the air chamber of eggs on the 6th day of incubation. The effect of sublethal doses of chlorpyrifos, carbofuran and methomyl on quail egg-weight and their percent loss during incubation was found not to be dose dependent, and subsequently this parameter could not be used as a reliable teratogenic sign. Data pertaining to impact of tested pesticides on embryo weight and embryotoxicity exhibited that, the effects of all tested insecticides on chick embryo-weight are dose-dependent. Moreover, the current data also emphasize that the phenomenon of embryotoxicity is not a reliable parameter for teratogenicity. Concerning the teratogenic effects of the tested insecticides, the results quite indicate that all tested compounds caused significant abnormalities in chick embryo, and these effects are concentration-dependant. However, the highest increase was observed in carbofuran- and methomyl-treated eggs at 1/20 LD_{50} (95, 100% of control, respectively) followed by that exhibited by chlorpyrifos at the same doses (85% of control). Notice that, the high concentration of both carbofuran and methomyl (1/10 LD_{50}) caused complete embryo-toxicity at the early stage of development. On the other hand, the teratogenic potential was less pronounced for the dose, 1/60 LD_{50}. Perusal of the early mentioned results clearly showed that, the teratogenic effects were found to be dose dependent. Combination between chlorpyrifos and carbofuran caused 100% malformation in chick embryo. Generally, the most pronounced malformations are wing micromelia, leg hemimelia, deformed toe, clubfoot, asymmetrical development of the spine, lordosis of the spine, wry neck, shortened tibio-fibulae and toes, twisted finger, sparse down, reduced body size and retardation of skeletal growth.

INTRODUCTION

There is an increasing concern over the possible toxicological hazards of wide spectrum of synthetic organic chemicals including industrial pollutants, food additives, drugs, mineral fertilizers and pesticides. Because of their ubiquity, persistence, presence and/or concentration in food chains and toxicological properties; pesticides constitute a major group of potential environmental hazards to mammalian species, which is attributed to their possible teratogenic, mutagenic, carcinogenic and endocrine disruption effects (**Hoppin *et al.*, 2000; Millikan *et al.*, 2000; Sarkar *et al.*, 2000 and Calle *et al.*, 2002**). Organophosphates such as chlorpyrifos and the carbamates carbofuran and methomyl have been demonstrated to have severe effects on experimental animals (**Kaplan and Sherman, 1977; Pawar and Katdare1984; Roy *et al.*, 2005 and Tian *et al.*, 2005**).

Chlorpyrifos is an extensively used insecticide in agricultural and non-agricultural settings (**Racke, 1993**). It considered one of the most heavily used organophorus insecticides in the U.S.A. (**EPA, 2000**). While there have been many studies and reviews of the toxicologic effects of chlorpyrifos, especially its neurotoxic effects (**ATSDR, 1997**), its teratogenicity potential had few studies.

Carbofuran was found to be the second highest among carbamate pesticides responsible for avian mortality in the United States, causing deaths in 45 species (**Fleishli *et al.*, 2004**). In 1978 all methomyl products were classified as restricted use pesticides (**EPA, 1998**).

Based on the increasing uses of pesticides which is characterized by their high toxic potential for non-target organisms, containing toxic impurities causing irreparable damage to human and environmental health and the little available information about their teratogenic effects, thus it becomes a must to investigate the possibility of their teratogenic potential.

The check embryo technique (**Bowman, 1967**) is a useful method to study the action of a proximate teratogen, one that acts directly, without the necessity of prior metabolic transformation.

The current study aimed to investigate the teratogenic effects of three commonly used insecticides namely chlorpyrifos, methomyl and carbofuran as well as one mixture between carbofuran and chlorpyrifos on Japanese quails chick embryos.

MATERIALS AND METHODS

I. Chemicals:

Selected pesticides, organophosphorus, chlorpyrifos (O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl)-phosphorothioate) and carbamate pesticides methomyl (S-methyl N-[(methylcarbamoyl)oxy]-thioacetimidate) and carbofuran (2,3-dihydro-2,2-dimethyl benzofuran-7-yl methylcarbamate) were obtained as analytical grade from the Environmental Protection Agency (EPA), USA. Alizarin red S, ethanol, glycerin and benzylalcohol as well as potassium hydroxide were obtained from Merck Co., Germany.

II.Teratogenecity test:

The teratogenic effects of tested insecticides were evaluated by using the chick embryo technique advised by **Bowman, (1967)**. Fresh fertile Japanese quail eggs of the average of 12.64± 2.83 g each were supplied from the Experimental Station Farm, Department of Poultry, Faculty of Agriculture, Kafr El-Sheikh University. Eggs were kept vertical in a thermostatically controlled incubator at a preset temperature of 38.5±0.2 °C with 60-65% relative humidity until pesticidal administration.

The LD_{50} values of chlorpyrifos, carbofuran and methomyl (102.0, 5.0 and 15.40 mg/kg body weight respectively) were obtained from The Pesticide Manual, 1998. The effects of sublethal dosages (1/10, 1/20, 1/40 and 1/60 LD_{50}) of tested pesticides as well as a mixture between 1/40 LD_{50} carbofuran and 1/40 LD_{50} chlorpyrifos on egg-weight and percent loss of egg weight during incubation were studied.

Twenty fresh fertile eggs were randomly selected for each sublethal concentration of tested pesticides, and the same number was served as a control group. Three groups were done for the control treatment, the first was injected with saline, the second was hole only and the third was left as non-treated eggs. Selected eggs were incubated for six days and regularly candled to make sure that every egg had a living embryo. The outer surface of the egg's shell over air-sac (site of perforation) was cleaned using tincture iodine 5% in absolute ethanol. The tested compounds in saline were injected (100 µl) directly into the yolk through a sterilized proper needle, then the hole was sealed with paraffin wax. The treated eggs were returned back to the incubator to complete their incubation period and were examined on the 16th days.

The treated eggs were weight at different intervals of incubation i.e. 7, 11, 14 and 16 days. Each quail embryo was weighed and examined for

external malformations. On the 17th day the rest of the treated eggs were expected to give rise to fully developed chicks. Those which are unable to open the eggs are considered dead. Alizarin red S staining was used to detect skeletal anomalies (**Dowson, 1926**). Quail embryo were processed for Alizarin stain according to the method of **Staples and Schnell (1964)**.

RESULTS AND DISCUSSION

1. Effect on loss percentages of egg-weight:

The effects of sublethal doses of chlorpyrifos, methomyl, carbofuran and a mixture of (carbofuran + chlorpyrifos) on quail egg-weight and their percentages loss during incubation were studied and the data are presented in Table (1). It is quite clear that all treatments including the control showed positive increases in percentages loss of egg-weights throughout the incubation period. The positive increases in percent losses of egg-weight ranged between, 6.03-14.62, 5.23-14.74, 5.37-15.21, 5.03-9.43 and 7.46-14.75% for chlorpyrifos, carbofuran, methomyl, chlorpyrifos-carbofuran-mixture and control, respectively. The data also demonstrated that in spite of the existence of some significant variations in loss percentages of egg-weights through the incubation period, there is no positive correlation between the average loss percentages of egg-weight and insecticidal concentration. In other words, the effects of tested pesticides on egg-weight loss are not dose dependent. These results are in coincidence with **Salama et al., (2006) and Hosney et al., (2006)** who stated that methamidophos (organophosphorus pesticide) caused elevation in egg-weight losses ranged from 9.96 to 12.64%. However, our results contradict with the previous finding of **El-Sebae et al., (1992)** who found that the average weight loss in chick egg was inversely proportional with the doses of cypermethrin. Loss of egg weight of chick embryo during incubation is a natural phenomenon resulted from the fact that the egg contents must evaporate at an established rate of 11- 13 % of fresh weight (**Shanaway, 1994**). Accordingly, it could be concluded that loss percentages of egg-weight is not a good parameter to rely upon for teratogenic evaluation.

2. Effect on embryo weight and embryotoxicity:

Data pertaining to the impact of tested insecticides on embryo weight and embryotoxicity are shown in Tables (1 and 2). Perusal of these results clearly exhibited that the effect of all tested pesticides on chick embryo-weight are dose-dependent. Chlorpyrifos, for instance, caused reductions in chick embryo weight representing 16.67, 53.95, 69.30 and 58.99% as

compared to the control after being injected by 0.023, 0.034, 0.067 and 0.133 mg/egg, respectively. The same trend of results occurred with both carbofuran and methomyl (Table 2), but with different percent reductions of embryo-weight (Table 2).

Table (1): Average egg-weight (gm) and loss percentage of egg-weights after being injected on the sixth day of incubation with chlorpyrifos, methomyl, carbofuran and.a mixture of (chlorpyrifos+carbofuran).

Pesticides	Doses mg/kg	Average of egg-weights during incubation					Average % loss of egg-weight relative to fresh weight			
		Days of incubation					Days of incubation			
		0	7	11	14	16	7	11	14	16
Chlorpyrifos	1.70	13.36	12.43	12.36	11.68	11.40	6.93	7.47	12.57	14.62
	2.55	13.28	12.29	11.99	11.72	11.53	7.42	9.73	11.75	13.21
	5.10	13.17	12.38	12.03	11.93	11.76	6.03	8.68	9.47	10.72
	10.20	13.07	12.22	12.00	11.77	11.55	6.50	8.15	9.91	11.64
Carbofuran	0.083	12.95	12.06	11.84	11.81	11.64	6.90	8.60	8.78	10.15
	0.125	12.85	12.06	11.80	11.47	11.39	6.15	8.14	10.72	11.35
	0.250	12.68	11.49	11.24	10.99	10.82	9.38	11.42	13.39	14.74
	0.500	12.51	11.85	11.65	11.53	11.36	5.23	6.87	7.86	9.18
Methomyl	0.257	12.34	11.67	11.42	11.18	10.88	5.37	7.45	9.35	11.82
	0.385	12.13	11.30	10.65	10.42	10.28	6.84	12.15	14.07	15.21
	0.770	11.90	10.64	10.60	10.44	10.24	10.65	10.96	12.31	13.96
	1.540	11.68	10.93	10.72	10.57	10.11	6.40	8.26	9.49	13.47
Ca+Ch*	0.125+2.55	14.32	13.60	13.41	13.24	12.97	5.03	6.34	7.56	9.43
Control	Control	11.06	10.24	10.12	9.70	9.43	7.46	8.56	12.30	14.75

*Ca = Carbofuran , Ch = Chlorpyrifos

Generally, the highest increase in the percent reduction of chick embryo weight was observed in methomyl-treated eggs at 1/10 LD_{50} (74.78% of control) followed by chlorpyrifos and carbofuran at the same dose (69.30 and 57.02% of control, respectively). The effects were less pronounced for the dose, 1/60 LD_{50}. However, the highest increase in the mortality percentages was observed in methomyl -treated eggs at 1/10 LD_{50} and 1/20 LD_{50} (100 and 100 % of control, respectively) followed by that exhibited by carbofuran and chlorpyrifos at the same doses (100, 100 and 90, 85% of control, respectively). The effect was less powerful at a concentration of 1/60 LD_{50}. With regards to the effect of combination between chlorpyrifos and carbofuran on embryo weight the data presented in Table (2), showed that embryo weight decreased significantly (52.85% of control).

The obtained results are in accordance with those found by **(Pawar and Katdare, 1984)** who stated that carbofuran retarded growth in frog embryos. Furthermore, **Cummings *et al.*, (1992)** administered rats by methyl benzimidazolecarbamate (MBC) at 0, 100, 200, 400, or 600 mg/kg/day during days 1-8 of pregnancy and killed on day 11 or day 20 of gestation.

Evidence of developmental delay was apparent on day 11 at all doses, and fetal weight was reduced by day 20. MBC exposure during the first week of pregnancy was shown to be embryotoxic, resulting in embryonic death, growth retardation. Carbadox (growth-promoting and antibacterial agent that has been used in veterinary practice) was administered by gavage once daily to pregnant rats at doses of 0 (control), 10, 25, 50 or 100 mg/kg on days 8 through 15 of pregnancy. There was a dose-related decrease in fetal body weights which was statistically significant at 25 mg/kg and above (**Yoshimura, 2002**).

Table (2): Effects of tested pesticides on weight, toxicity and malformation of Japanese quail chick embryo

Pesticides	Doses*		Chick embryo weight		Normal embryo		Dead embryo		Malformed embryo	
	mg/kg	mg/egg	(g)	%R	No.	%	No.	%	No.	%
Chlorpyrifos	1.70	0.023	3.80	16.67	12	60	0	0	8	40
	2.55	0.034	2.10	53.95	7	35	0	0	13	65
	5.10	0.067	1.87	58.99	3	15	0	0	17	85
	10.20	0.133	1.40	69.30	2	10	0	0	18	90
Carbofuran	0.083	0.0011	4.07	10.75	8	40	1	5	11	55
	0.125	0.0016	3.66	19.74	5	25	0	0	15	75
	0.250	0.0032	3.58	21.49	0	0	1	5	19	95
	0.500	0.0063	1.96	57.02	0	0	20	100	0	0
Methomyl	0.257	0.0032	3.49	23.46	17	85	1	5	2	10
	0.385	0.0047	2.11	24.78	9	45	0	0	11	55
	0.770	0.009	3.43	53.73	0	0	0	0	20	100
	1.540	0.018	1.15	74.78	0	0	20	100	0	0
Ca+Ch	0.125 +2.55	0.0016 +0.0.034	2.15	52.85	0	0	0	0	20	100
Control	Control		4.56	0.00	20	100	0	0	0	0

Ca = Carbofuran, Ch = Chlorpyrifos,* Each treatment includes 20 fertile eggs.

Concerning the embryo-toxic effect of tested insecticides, it is very important to distinguish between embryo-mortality resulted from the toxic action of tested compounds and those resulted from the teratogenic action which occurred in a vital target(s).

Since, the injection of tested insecticides was done on the sixth day of incubation; thus, we removed gently the shell of untreated eggs after 6 days of incubation to get the 6-day normal embryo free from the yolk to figure out the normal external developmental stage of the Japanese quail embryo. The embryo is inside a fluid filled sac called the amnion (fig. 1-A). This embryo was taken as a standard for comparison between the embryos, which died from the toxic action of the insecticides, and those died from teratogenic action. Notice the wing is beginning to form, while the legs are only very small buds (Fig. 1-B). Accordingly a comparative qualitative study of the emberyotoxic and teratogenic activities of the tested insecticides was done. Embryos died at a very early stage of developments similar to those embryos in Fig. (2a) which are considered dead and the death resulted from the toxic action of the tested compounds (embryotoxicity), while those died after developing some deformed organ(s) are considered dead but the death resulted from the teratogenic action, which occurred at vital target(s). Accordingly, the data presented in Table (2) revealed that, apart from the highest concentrations of both carbamates, no single case of embryo-toxicity was observed. However, these results were expected since all tested concentrations are sublethal and ranged between 1/60 to 1/10 of the LD_{50}. On the other hand, the highest concentration of both carbofuran and methomyl resulted in complete mortality of the embryos at early stage of developments. This might be due to the high sensitivity of Japanese quail embryos at their early stage of development and/or the lack of biodegradation mechanisms towards both insecticides leading to long persistence of the toxic parent compound for each insecticide.

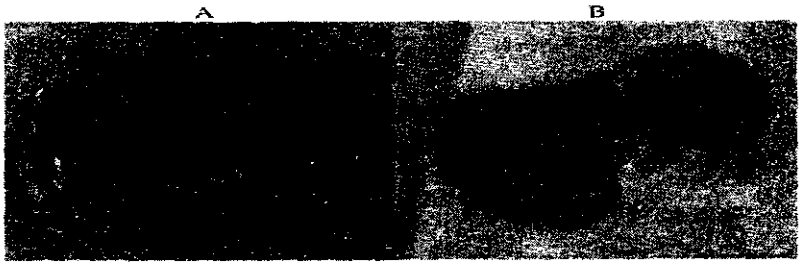

Fig (1) Normal Japanese quail chick embryo after six bays of incubation:
(A) The embryo is inside a fluid filled sac called the amnion
(B) Normal Japanese quail chick embryo after 6 day of incubation remove from the yolk. Notice the wing is beginning to form, while the legs are only very small buds.

The data also showed that, there is no correlation between embryotoxicity and teratogenecity. Based on LD_{50} values, carbofuran is considered the highest toxic compound (5 mg/kg b.w), followed by methomyl (15.4 mg/kg b.w), while chloropyrifos as considered the least toxic compound in this respect (10.2 mg/kg b.w). The corresponding malformathion percentages for the highest concentration of each compound are 90, 0.0 and 0.0 for chlorpyrifos, carbofuran and methomyl respectively. In other words, the least toxic compound caused 90% malformation while both carbamate insecticides, which are the highest toxic compound, did not show any sign of malformation. Accordingly, it is quite fair to emphasize that the phenomenon of embryotoxicity is not good parameter for teratogenicity. Teratology concerns the functional, biochemical or structural deviations in development that are parentally initiated; the term of embryotoxicity is widely used but not well-defined **(Vergieva, 1982)**. These findings are somewhat in agreement with those of many authors. **Sahu and Ghatak (2002)** found that dimecron, an organophosphorus insecticide, caused developmental alterations in the developing chick embryo when administered at two different doses (25 μg and 35 μg) into the egg yolk through a pore into the equatorial region at day 0 of incubation. Significant overall retardation in growth were noted in the insecticide-treated embryos, revealing that dimecron played a role in producing embryotoxicity at different stages of embryogenesis.

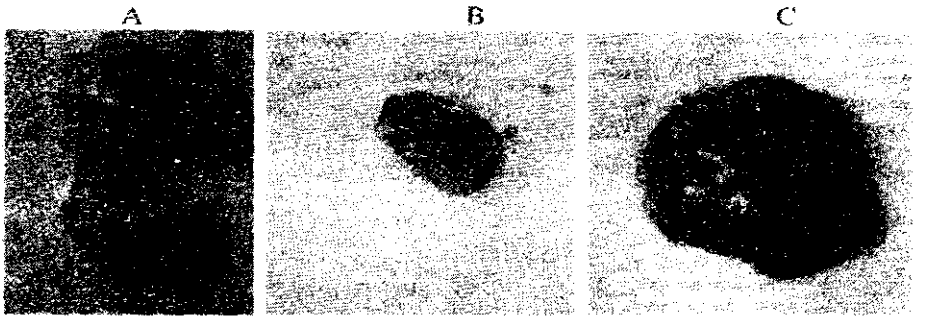

Fig (2) Japanese quail chick embryo died at early stage of development from the toxic action of insecticides at 1/10 LD_{50}:
(A) Control embryo after 6 days of incubation
(B) Methomyl-treated group after 16th day of incubation
(C) charbofuran-treated group after 16th day of incubation

Breslin *et al.*, (1996) evaluated chlorpyrifos for its potential to produce developmental toxicity in rats following oral exposure of pregnant rats to doses of zero (corn oil vehicle), 0.1, 3.0, or 15 mg chlorpyrifos/kg/day,

by gavage, on gestation days 6 through 15. the data showed that, parental toxicity at the high dose was accompanied by decreased pup body weight and increased pup mortality in the F1 litters. **Anonymous (2000)** reported that chlorpyrifos caused foetal toxicity in rats at 25 mg/kg/day resulting in reduced pup weight and crown-rump length. **Farag et al., (2003)** found that chlorpyrifos caused fetotoxic effects at a maternal dose of 25 mg/kg per day. Fetal weight and viability were decreased, and fetal death was increased at the 25 mg/kg/day maternal dose. Moreover, decreased birth weight and length of new-borns have been associated with high levels of chlorpyrifos in plasma samples of urban minorin women (**Perera et al., 2003**). **Tian et al., (2005)** indicated that chlorpyrifos treatment resulted in a significant reduction in numbers of live fetuses, versus control litters when the pregnant females were given a single intraperitoneal injection (40 or 80 mg/kg) on day 10 of gestation and fetuses were evaluated on gestation day 17, at 80 mg/kg.

3. Teratogenic actions of tested insecticides on Japanese quail chick embryos:

A. Normal chick embryo:

Figure (3) shows a completely well development Japanese quail chick embryo on day 17after few hours from hatching. Notice that, the quail chick will be up and walking, eating and chirping in just a few hours after hatching.

Fig (3) Normal Japanese quail chick embryo on 17 day of incubation after few hours from hatching

B. Teratogenic action

Teratogenic signs of Japanese quail chick embryos as affected by the tested insecticides are recorded in Table (2) and illustrated in figures (4,

5, 6 and 7). The results quite indicate that, all tested insecticides caused significant increase of severe abnormalities in quail chick embryo. The highest increase was observed in the case of methomyl-treated eggs at 1/20 LD_{50} (100%) followed by carbofuran and chlorpyrifos (at the same concentration) with malformed values of 95 and 85% of control, respectively. As for the effect of chlorpyrifos-carbofuran mixture on teratogenic signs in chick embryo, the data showed that, the percentage of malformed embryos increased significantly (100% of control). The data also revealed that, the teratogenic effects were less pronounced for the lowest concentration 1/60 LD_{50} (Table 2). It is of great interest to mention that all tested insecticides caused almost similar teratogenic signs but differed in their magnitude. This might be due to the fact that all tested insecticides have the same mode toxic action as anticholine esterase-inhibitors.

Generally, the abnormalities included wing micromelia, leg hemimelia, deformed toe, clubfoot, asymmetrical development of the spine, lordosis of the spine, wry neck, shortened tibio-fibulae, and toes, twisted finger, sparse down and reduced body size.

Concerning the teratogenic effects of chlorpyrifos on Japanese quail chick embryos, figure (4) showed that, all tested concentrations caused remarkable reductions in body size and weight. In addition at the highest concentration (1/20 LD_{50}), the treated group are completely naked without feathers, the right leg is short, the feet are deformed and the eyelid is absent (fig. 4-A). As for 1/40 LD_{50} treated-group, the embryos are characterized by abnormal feathering, short neck, twisted leg and wing micromelia (fig. 4-B). In case of the lowest concentration (1/60 LD_{50}), the embryos showed micromelia of right wing, abnormal toes, twisted right leg and abnormal feathering (fig. 4-C).

It is more likely that, the malformed effects might be due to the action of the anticholinestrase inhibitors (tested insecticides) which were introduced into the fertile eggs during the course of embryonic development had lead to the development of behavioral and morphological disturbances in the process of embryonic organogenesis.

Acetylcholinesterase (AChE) is an enzyme playing a key role in the modulation of neuromuscular impulse transmission. However, in the past decades, there has been increasing interest concerning its role in regulating non-neuromuscular cell-to-cell interactions mediated by electrical events, such as intracellular ion concentration changes, as the ones occurring during gamete interaction and embryonic development. An understanding of the mechanisms of the cholinergic regulation of these events can help us foresee

the possible impact on environmental and human health, including possible teratogenic effects on different models, and help elucidate the extent to which OP exposure may affect human health **(Aluigi *et al.*, 2005)**. Moreover, **Roy *et al.*, (1998)** concluded that chlorpyrifos specifically targets brain development at low concentrations.

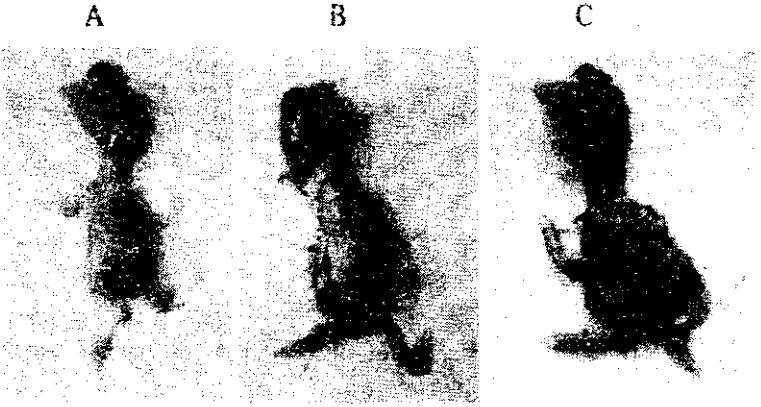

Fig (4): Chlorpyrifos treated group of Japanese quail chick embryo after 16-day chick of incubation:

(A) Treated with 1/20 LD_{50} showing reduced body size and weight, without feathers, notice that the right leg is short, the feet is deformed and the eyelid is absent.

(B) Treated with 1/40 LD_{50} showing reduced body size and weight, abnormal feathering, short neck, twisted leg and wing micromelia

(C) Treated with 1/60 LD_{50} showing reduced body size and weight, micromlia of right wing, abnormal feathering, abnormal toes and twisted right leg

The obtained results of chlorpyrifos are in coincidence with those previously reported by many investigators **(Stecher, 1960; Smith ,1981; EPA, 1998; Gibson, 1996; Sherman 1996; Roy *et al.*, 1998; Sarneckis and Kumar, 2001). Farag *et al.*, (2003) and Tian *et al.*, (2005)** who confirmed the role of chlorpyrifos in increasing the teratogenic effects in mammals.

Regarding, the effect of methomyl and carbofuran on teratogenic signs in chick emberyos, the obtained results are show in figures 5 and 6, respectively. In case of methomyl, the group treated with 1/20 LD_{50} showed reduced body size and weight, abnormal feathering, deformed right leg and abdominal hernia (fig. 5-A). the corresponding teratogenic sign in case of

1/60 LD_{50}-treated-group are reduction in body size and weight and the right leg is deformed (fig. 5-B).

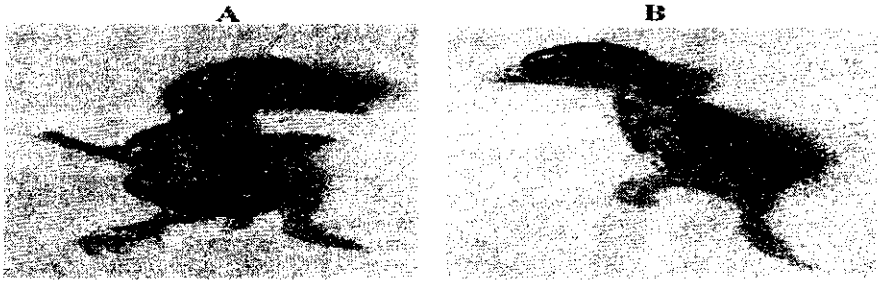

Fig (5): Methomyl treated group of Japanese quail chick embryo after 16-day chick of incubation:

(A) Treated with 1/20 LD_{50} showing reduced body size and weight, abnormal feathering, notice that the toes of the right leg is deformed, abdominal hernia, the feet is deformed and the eyelid is absent.

(B) Treated with 1/60 LD_{50} showing reduced body size and weight and the toes of the right leg is deformed.

Charbofuran-treated group showed sever reduction in body size and weight and abnormal feathering at all tested concentrations. Moreover, the highest concentration caused remarkable reduction in the development of doun plumules, syndactylous polydactyly in fore and hind limbs, absence of eyelid (fig. 6-A). At a concentration of 1/40 LD_{50} of carbofuran, the treated group showed deformed toes (fig. 6-B), while the lowest concentration caused wry neck (fig. 6-C).

The obtained results are in accordance with those found by **Pawar and Katdare (1984), Cummings *et al.*, (1992), Extoxnet (1993) and Yoshimura (2002)** who confirmed the role of carbamate insecticides in increasing the teratogenic effects in mammals. On the contrary, there was no evidence of teratogenicity resulted neither from carbofuran-treated mice or rats **(Okeefe and Pierse, 1980 and Baron 1991)** nor from methomyl-treated rats **(EPA, 1998, Baron 1991)** or methomyl-treated rabbits **(Anonymous, 1995)**. The contradiction may be simplified as follows; the absorption, metabolism and excretion of methomyl after oral administration to rats are very rapid, the processes being completed within a few days. When rats were given radiolabelled methomyl (5 mg/kg body weight), 54% of the dose was excreted in urine and 2-3% in faeces within 7 days, and 34% in expired air within 5 days. After 7 days, 8-9% of the ^{14}C dose remained in the tissues and carcass, which was incorporated into endogenous constituents. The highest

concentration of radioactivity was in the blood (representing 2% of the dose), **Harvey *et al*, 1973.**

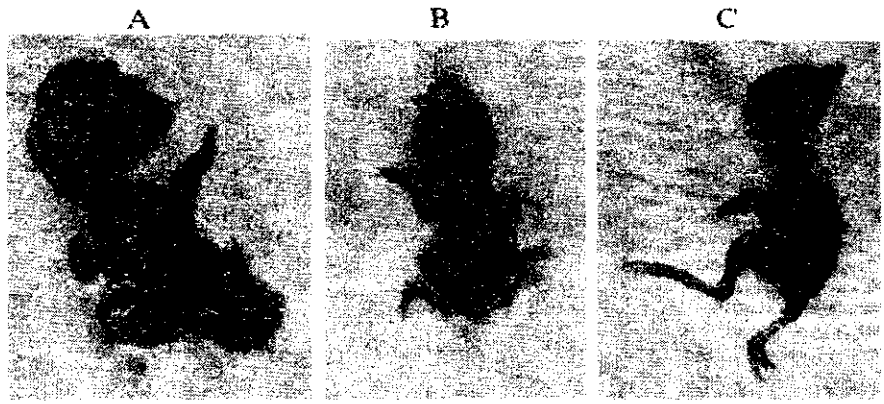

Fig (6): Charbofuran treated group of Japanese quail chick embryo after 16-day chick of incubation:

(A) Treated with 1/20 LD_{50} showing reduced body size and weight, growth retardation, remarkable reduction in the development of doun plumules and syndactylous polydactyly in fore and hind limbs, absence of eyelid and feathers. absence of eyelid

(B) Treated with 1/40 LD_{50} showing reduced body size and weight, abnormal feathers, deformed toes.

(C) Treated with 1/60 LD_{50} showing reduced body size and weight and wry neck

In case of using a mixture between 1/40 LD_{50} carbofuran and 1/40 LD_{50} chlorpyrifos, the resulted teratogenic effects were more effective and pronounced than using the same concentration of each insecticide alone. The data are illustrated in figure (7). However, the most pronounced signs are reduced body size and weight, deformed leg and toes, wry neck and abnormal feathering.

It is very important to mention that, the same treatment showed different malformations. In other words, figure (7-2) showed wry neck and abnormal feathering while figure (7-1), the embryo was completely necked with deformed toes. Figure (7-3) showed abnormal feathering and deformed legs and toes. The logic interpretation for such teratogenic variance might be due to the teratogenic activates include several vital targets and/or due to the variance between the individuals (eggs) within the same treatment.

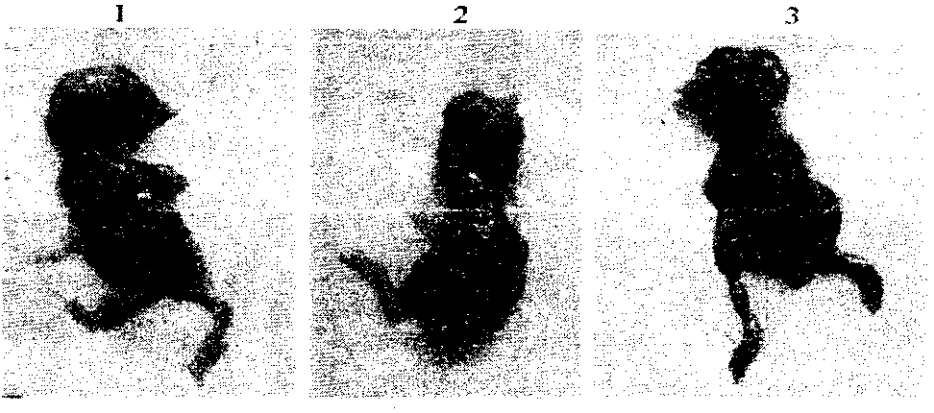

Fig (7): Chlorpyrifos-Carbofuran mixture treated group of Japanese quail chick embryo (1/40 LD_{50}) after 16-day of incubation, showing reduced body size and weight, deformed leg and toes, notice that although the above three pictures resulted from the same treatment but showing deferent malformations i.e picture 2 showed wry neck, abnormal feathering, while picture 1 and 3 showing deforming legs and toes and without feathers.

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الملخص العربى

التأثيرات السامه الجنينيه والتشويهيه المحتمله لبعض المبيدات التقليديه على أجنة السمان اليابانى

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إستهدفت هذه الدراسه إكتشاف تأثير الكلوربيرفوس (مبيد فوسفورى) ، والكربوفيوران والميثوميل (مبيدات كارباماتيه) و مخلوط من مركبى الكلوربيرفوس والكربوفيوران على أجنة السمان اليابانى أثناء المراحل المبكره من تطورها، حيث تم معاملة الأجنه بحقن جرعه واحده من المركبات المختبره فى الغرفه الهوائيه للبيضه وذلك فى اليوم السادس من التحضين. وكذلك شملت الدراسة تاثير الجرعات تحت المميتة من المبيدات المختبرة على الفقد فى وزن البيض أثناء التحضين، واظهرت النتائج النقاط التالية:

١- وجد ان تاثير الجرعات تحت المميتة من المبيدات المختبرة على الفقد فى وزن البيض لا يعتمد على التركيز، كذلك لا يمكن اعتباره كمعيار يعتمد عليه فى دراسة التشوهات الجنينية.

٢- كذلك اوضحت النتائج ان ظاهرة سمية الاجنة لا يمكن اعتبارها كدالة للتاثيرات التشويهية.

٣- فيما يتعلق بتاثير المبيدات المختبرة على وزن الاجنة، فقد اكدت النتائج ان جميع المبيدات احدثت خفض معنوى فى وزن الاجنة، كما ان هذا التاثير يعتمد على الجرعة ولذلك يمكن اعتباره كدالة لحدوث التاثيرات التشويهية فى الاجنة

٤- احدثت جميع المبيدات المختبرة زيادة معنوية فى التشوهات الخلقية حيث لوحظ ان اقصى زيادة فى هذه التشوهات نتجت من معاملة البيض بجرعة قدرها ١/٢٠ من قيمة LD_{50} من كل من الكربوفيوران و الميثوميل ، كذلك اوضحت النتائج ان هذا التاثير ينخفض جدا عند استخدام الجرعات المنخفضة (١/٦٠ من قيمة LD_{50}).

٥- ادت معاملة البيض بمخلوط من الكلوربيرفوس والكربوفيوران الى حدوث تشوهات جوهرية فى جميع البيض المعامل حيث بلغت نسبة التشوهات الجنينية ١٠٠%.

٦- اوضحت النتائج ايضا عدم وجود اختلافات جوهرية فى نوعية التشوهات الناتجة من المعاملات المختلفة من المبيدات المختبرة.

وبصفه عامه فقد أشتملت هذه التشوهات على فقد أحد الأجنحه أو الساق ،التواء الأصابع ،قدم مخلبى، التواء فى العمود الفقرى، التواء فى الرقبه، فقد فى وزن الجسم، تأخر فى نمو فقرات الهيكل العظمى، ووجود أوديما.