THE EMBRYOTÓXICITY AND TERATOGENICITY OF SOME CONVENTIONAL PESTICIDES ON WHITE LEGHORN CHICK-EMBRYO

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ABSTRACT

The embryotoxicity and teratogenicity of some conventional pesticides; cypermethrin, methamidophos (tamaron) and 2,4-D were studied. The effect of cypermethrin, tamaron and 2,4-D on egg-weight loss was found not to be dosage dependent, and subsequently this parameter could not be used as a reliable teratogenic sign. The data also emphasize that the phenomenon of embryotoxicity can not be used as a parameter for teratogenicity. The data also showed that cypermethrin was quite safe from the teratogenic point of view. Both tamaron and 2,4-D caused abnormalities including wing micromelia, leg hemimelia, deformed toe, asymmetrical development of the spine, lordosis of the spine, wry neck, twisted finger, sparse down and reduced body size. However, the severity of the abnormalities was dosage-dependent.

INTRODUCTION

According to the National Research Council Report (1977) on "Principles and Procedures for Evaluating Toxicity of Household Substances", the objective of teratogenicity testing is the identification of agents acting during embryonic development to produce or alter the incidence of congenital malformations and during the fetal period to produce functional changes in the off-spring. Teratogenicity from chemical agents and physical forces is a very real hazard to humans. No single atfinial test will provide assurances of safety. Because of the many factors involved in chemical-induced teratogenesis extrapolation of the dose-response relationship from animals to humans is difficult and/or arbitrary. Some believe that toxic ingredients of natural products might be much safe and suitable as a new generation of insecticides for controlling economic pests.

This study aimed to evaluate the embryo toxicity and teratogenicity of some conventional pesticides; cypermethrin, tamaron and 2,4-D.

MATERIALS AND METHODS

I- Chemicals:

All tested pesticides were in the formulated form:

1- Cypermethrin:

chemical name IUPAC : (R,S)-α-cyano-3-phenoxy benzyl (1RS,Cis-trans)-3-(2,2-dichlorovinyl)-2-2-dimethyl cyclopropane carboxylate

2- 2,4-D:

chemical name IUPAC : 2,4-dichlorophenoxy acetic acid

3- Methamidophos (Tamaron):

chemical name IUPAC : O.S-dimethyl phosphoramidothioate

II Organisms used for testing teratogenecity:

White leghorn egg: Fresh fertile white leghorn eggs of the average of 55.17± 3.75 g each were supplied from the Experimental Station Farm, Department of Poultry, Faculty of Agriculture Kafr El-Sheikh, Tanta University.

RESULTS AND DISCUSSION

Evaluation of embryotoxicity and teratogenicity of tested pesticides:

1- Effect on percent loss of egg-weight:

The effect of sublethal concentrations of cypermethrin, tamaron and 2,4-D on egg-weight and percent loss of egg weight during incubation were studied and the data are presented in Table (1). Selected eggs for chick-embryo inoculation 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 solution were injected directly into the yolk through a sterilized proper needle, then sealing the injection hole with paraffin wax. The treated eggs were incubated again in vertical position for examination on the 19th and 21st days. It was quite clear that all treatments including the control showed positive increases in percent losses of egg-weights throughout the incubation period. The percent increases of egg-weight losses ranged between 9.96 to 12.64, 10.33-11.51 and 11.45-12.84% for Tamaron, cypermethrin and 2,4-D respectively. These ranges of percent losses in egg-weight agreed fully with the previous finding

of Shanaway (1994) who stated that the loss of chick egg-weight resulted from the natural evaporation of egg-weight during incubation at an established rate of 11-13% of fresh weight.

The data also revealed that inspite of the existance of some significant variations in percent losses of egg-weights through the incubation period (Table 1), there is no positive correlation between the average percent loss of egg-weight and insecticidal treatments. In other words, the effects of Tamaron, cypermethrin and 2,4-D on egg-weight loss are not dosage dependent, and subsequently this parameter could not be used as a reliable teratogenic symptom. 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 used.

2- Effect on embryo-weight and emberyotoxicity:

The effect of cypermethrin, tamaron and 2,4-D on embryo-weight and embryotoxicity were also studied and the data are presented in Table (2). Precise investigation of the data revealed that, the effects of all tested compounds on chick embryos-weight are dosage-dependent. In term of figures, the emryos weights in case of cypermethrin-treatment were 33.91, 33.23, 30.24, 26.24, 23.84 and 21.02 gm for the control, 1, 3, 5, 10 and 20 mg cypermethrin per egg, respectively. In other words, the percent reductions in embryo-weight with respect to the control were 2.01, 10.83, 22.62, 29.70 and 38.01%, respectively. This trend of results agreed fully with the previous findings of Khera *et al.*, (1982), Voronina and Unii (1984) and Awadallh (1991).

Concerning the embryotoxicity of cypermethrin, the data presented in Tables (2) clearly showed that inspite of using sublethal concentrations of cypermethrin in the current experiments, there was a positive correlation between percent of embryonic mortality and insecticidal concentrations. However, the observed percent mortalities in case of cypermethrin were: 0.0, 0.0, 13.3, 28.7 and 36.9% for 1, 3, 5, 10 and 20 mg cypermethrin per egg respectively.

Table (1): Average egg-weight (gm) and percent loss of egg-weights after being injected on the sixth day of incubation with cypermethrin, tamaron and 2,4-D.

Pesticides	Treatment doses*		Average of egg-weights during incubation**					Average % loss of egg-weight relative to fresh weight**			
			Days of incubation					Days of incubation			
	mg/kg	mg/egg	0	7	11	14	18	7	11	14	18
Cyper-methrin	1	0.05	57.60	54.48	53.61	52.65	51.65	5.23	6.92	8.59	10.33
	3	0.15	58.00	55.01	53.27	52.80	51.34	5.15	8.15	8.97	11.51
	5	0.25	57.90	55.10	53.85	52.47	51.65	4.83	7.00	9.37	10.97
	10	050	54.20	51.51	50.79	49.69	48.08	4.97	6.29	8.32	11.30
	20	1.00	55.70	53.25	52.60	51.74	49.76	4.39	5.57	7.11	10.66
Tamaron	1	0.05	51.60	49.04	48.09	47.08	45.55	4.96	6.81	8.76	11.73
	3	0.15	52.10	49.98	49.38	48.38	46.91	4.07	5.23	7.14	9.96
	5	0.25	53.60	50.91	49.38	48.48	46.82	5.02	7.88	9.55	12.64
	10	050	52.80	50.06	49.24	48.48	47.09	5.19	6.74	8.19	10.82
	20	1.00	57.30	54.13	53.59	52.05	50.32	5.53	6.90	9.17	12.19
2,4-D	10	0.50	56.00	53.30	52.60	51.10	49.60	4.84	6.14	8.67	11.51
	20	1.00	50.60	48.20	47.30	46.10	44.70	4.81	6.48	8.85	11.65
	30	1.50	50.80	48.30	47.60	46.40	45.00	4.96	6.44	8.60	11.45
	35	1.75	53.50	50.60	49.60	48.60	46.90	5.45	7.25	9.14	12.27
	40	2.00	55.00	51.70	50.60	49.10	49.20	6.08	8.04	10.67	12.37
	50	2.50	56.20	52.60	51.30	50.00	49.40	6.33	8.65	10.96	12.02
	150	7.50	58.10	54.60	53.10	52.20	50.60	5.98	8.61	10.23	12.84
Control		1μ saline	60.40	57.50	56.50	55.50	54.30	4.82	6.44	8.13	10.16

* Each treatment includes 20 fertile eggs with an average weight of 55.17± 3.75 g.

** Average percent loss of egg-weights up to the sixth day of incubation.

It is noteworthy to mention that non of the dead embryos which resulted from eggs treated with cypermethrin showed any sign of teratogenicity as they were almost well developed (Figure 2) and the death might have occurred due to the hyper sensitivity of embryos to cypermethrin.

It is very important to emphasize that the phenomenon of embryotoxicity can not be used as a parameter for teratogenicity. Teratology concerns the functional, biochemical or structural deviations in development that are prenatally initiated. The term of embryotoxicity is widely used but not well defined (Vergieva, 1982 b). However, in the current research, embryotoxicity is the case in which the embryo was affected in a manner similar to that of fully developed mature organism.

As for the effects of tamaron on embryo-weight and embryotoxicity was studied and the data are presented in Table (2). Statistical analysis showed that the body weights of the embryos on the twentieth day of incubation were significantly lower than those of the control group. In term of figures, the percent reductions of chick embryo-weight with respect to the untreated control are: 3.67, 6.52, 22.15, 34.83 and 39.84%, for 0.0125, 0.025, 0.05, 0.1 and 0.2 mg tamaron/egg, respectively.

Concerning the embryotoxic effect of tamaron, the data presented in Table (2) clearly indicate that no case of embryomortality was observed. This result was expected since all tested concentrations are sublethals. However, the data clearly showed that, there was no correlation between embryotoxicity and teratogenicity since tamaron which was not embryotoxic at all tested concentrations, it caused severe teratogenic signs in embryos that resulted from treated eggs (Table 2 and figure 3). On the other hand, cypermethrin which was relatively toxic to chick embryo (Table 1) had no teratogenic effect on chick embryos development (Figure 2).

This trend of results agreed fully with the previous findings of many investigators; Kimbrough and Gaines (1968), Kavlock *et al.*, (1979) and Budreau and Singh (1979).Accordingly, it is fair to state that, there is no correlation between pesticidal potency as chick embryotoxic agent and the teratogenic property of such pesticide.

With regards to the effect of 2,4-D on embryo-weight and embryotoxicity, the statistical analysis of data in Table (2) clearly show, that the body weights of the chick embryos on the twentieth day of incubation were significantly lower than their corresponding control values particularly

at the higher doses of 2,4-D. In other words, the percent reductions of the chick embryo-weights with respect to the untreated control group were: 6.1, 6.75, 16.28, 21.76, 28.18, 33.0 and 45.36%, for 0.5, 1.0, 1.5, 1.75, 2.0, 2.5 and 7.5 mg 2,4-D per egg, respectively.

Table (2): Teratogenic signs of chick embryos from eggs injected on the sixth day of incubation with sublethal concentrations of cypermethrin, Tamaron and 2,4-D (Examination was done one the twentieth day of incubation).

Treatment Concentrations mg/egg	% Morta- lity	Chick embryo weight		Teratogenic signs (cm) Lengths of:					
		(g)	%R	Body	Leg	Foot	Thigh	Wing	Neck
Cypermethrin									
1	0.0	33.23	2.01	8.58	7.20	2.29	3.50	3.20	3.40
3	0.0	30.24	10.83	8.23	6.88	2.31	3.10	3.40	3.10
5	13.3	26.24	22.62	8.29	6.98	2.09	2.80	2.90	2.98
10	28.7	23.84	29.70	8.53	6.93	2.33	3.00	3.00	3.19
20	36.9	21.02	38.01	8.49	7.05	2.42	2.70	3.20	3.02
Tamaron									
0.0125	0.0	32.70	3.67	7.80	6.19	2.16	3.20	3.40	3.48
0.0250	0.0	31.70	6.52	8.00	5.90	1.93	2.00	3.15	2.55
0.0500	0.0	26.40	22.15	8.20	6.28	1.98	3.06	2.80	3.61
0.1000	0.0	22.10	34.83	6.80	3.35	2.70	1.68	3.65	2.53
0.2000	0.0	20.40	39.84	6.20	3.13	1.46	1.24	1.98	1.36
2,4-D									
0.50	0.0	31.84	6.10	7.92	7.10	2.46	3.10	3.58	3.65
1.00	0.0	31.62	6.75	7.53	6.89	2.13	2.98	3.00	3.13
1.50	0.0	28.39	16.28	7.01	6.64	1.51	2.54	2.90	3.00
1.75	10.0	26.53	21.76	6.53	6.25	1.31	2.10	2.00	3.00
2.00	10.0	24.35	28.18	6.01	4.54	1.12	2.00	1.50	2.50
2.50	20.0	22.72	33.00	5.03	3.87	1.00	1.98	1.40	2.10
7.50	40.0	18.53	45.36	3.25	0.25	0.13	1.36	1.10	1.50
Control	0.0	33.91	0.00	8.61	7.10	2.38	3.00	3.60	3.55

%R=% Reduction in emberyo weight with respect to control.

Accordingly, it is quite fair to conclude that the effect of the tested herbicide 2,4 D) on chick embryo-weights was dosage-dependent and re-cconfirmed our previous results that the effects of cypermethrin, and tamaron on chick embryo-weight positively correlated with insecticidal concentrations.

Concerning the embryotoxic effect of 2,4-D, the data presented in Table (2) revealed that, although sublethal concentrations of 2,4-D were used in the current experiments (Table 2), but few cases of embryogenic

mortality were observed particularly at the higher Concentrations. However, the observed percent mortalities are: 0.0, 0.0, 0.01, 10, 10, 20, and 40% for 0.5, 1.0, 1.5, 1.75, 2.0, 2.5 and 7,5 mg/egg, respectively. The observed percent mortalities positively correlated with pesticidal concentrations.

The data also revealed that, 2,4-D was relatively low toxic to chick embryo. Based on the toxicological point of view, the mode of 2,4-D as a herbicide differed greatly from the conventional tested insecticide, and that might be behind its low toxic effect against the chick embryos. It is very important to re-emphasize that embryotoxicity is not a good parameter for teratogenicity.

3- Teratogenic effects of tested pesticides on chick embryo:

Teralogenic assessments in studies up to date have been limited to external, gross visceral, and skeletal examination. Therefore, all external features of chick embryos such as lengths of body, leg, foot, thigh, wing and neck as well as straight legs, shot spine, wry neck, parrot beak, abnormal feathering, visceral hernia, …etc., were taken in considerations.

Table (2) showed the various growth parameters of the chick embryos on the twentieth day of incubation. The data clearly show that were no significant difference between the various growth parameters and their corresponding values of the untreated control. In other words, cypermethrin did not affect either the embryological growth parameters nor caused any case of embryonic retardation. Moreover, all other external morphological features are quite normal and similar to the control ones (figure 2). This means that no morphological abnormalities were observed even at the high concentration of cypermethrin.

Concerning the skeletal examination, Figure (2) clearly illustrated that no skeletal defects were observed in embryos hatched from eggs previously treated with cypermethrin. These data confirmed the previous findings that cypermethrin had no teratogenic potentials. Accordingly, it could be fairly concluded that the tested pyrethroid is quite safe from the teratogenic point of view.
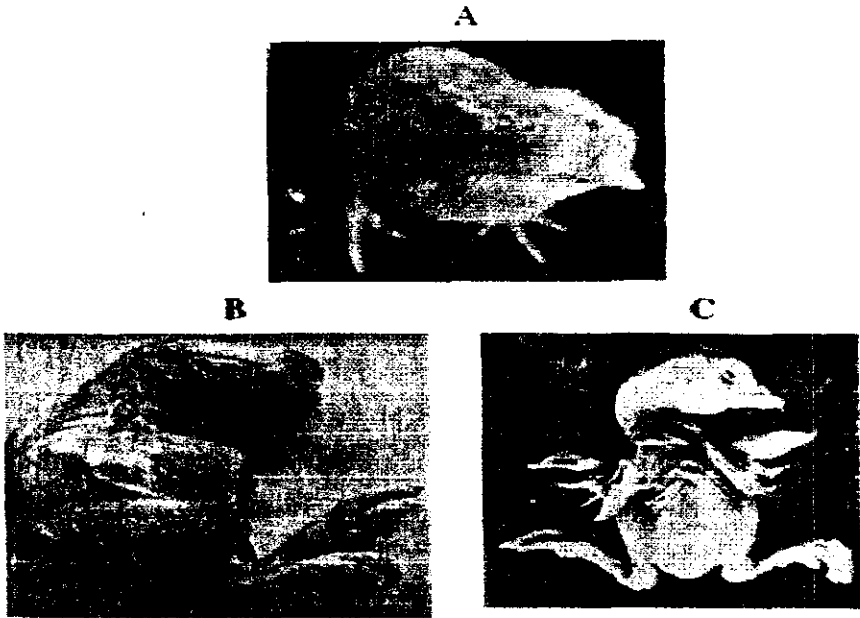
Fig.(1): **(A)** 21-day chick, saline-treated group, **(B)** 20-day chick untreated control (hole only) and **(C)** 20-day chick, saline-treated group, showing complete and fully developed embryo.

Fig (2): Cypermethrin-treated group **(A)** 20-day chick, showing complete and fully developed embryo and **(B)** alizarin preparation of 20-day chick embryo, showing no skeletal defects.

        The present results agreed fully and are compatible with the previous findings of many investigators i.e. Swentzal *et al.*, (1978), Kavlock *et al.*, (1979) David (1982), Polakova and Vergieva (1982 a) and Voronina and Unii (1984) indicated that synthetic pyrethroids might not be potent teratogenic agents. However, the pyrethroid group has the tendency to induce certain cytotoxic effects. El-Sebae (1986) reported that permethrin and cypermethrin of the synthetic pyrethroids were considered carcinogenic to rats. Moreover, Abdel-Khalik *et al.*, (1993) found that no skeletal changes were observed in rat-foetuses recovered from deitamethrin treated females.

        Concerning the teratogenic signs of tamaron and its effect on the growth status of chick-embryo, the data presented in Table (2) reveated that there is a positive decrease in the proportion of embryos with increasing insecticidal concentrations showing arrested growth at an early stage of development, particularly at higher concentrations (Figure 3). The percent reductions of the body dimensions of the chick embryo at a dose level 0.2 mg tamaron/egg with respect to the untreated group are: 28, 55.9, 38.7, 58.7, 45.0 and 61.7% for total body, leg, foot, thigh, wing and neck, respectively. However, most of the reductions of the body dimensions are dosage-dependent, as the embryos which resulted from the eggs treated with the high doses, i.e. $LD_{50}/5$, $LD_{50}/10$ and $LD_{50}/20$ showed highly significant decrease (P< 0.001) in their body weights and sizes as compared with the control.

        The data also revealed that the response of the different body dimensions differed significantly from one treatment to another. The logic interpretation for such results could be explained by the fact that in each species, there is a relative short critical period of sensitivity to teratogens, when early organogenesis is in progress. This period extends from the time of incubation to the end of embryogenic period. The sensitive periods correlate well with the known sequence of organogenesis, in this species. If the time of incubation when a particular organ complete its differentiation is known, then a congenital defect of that organ could only be caused by a teratogen that, acted prior to that time (Harbison and Beeker,1969).

        The foregoing results of tamaron agreed fully with the trend of results of Hanafy *et al.*, (1986), Sherif (1991) and Awadallah (1991). In general, it could be fairly that the external morphology of the embryos and the changes in their body dimensions are clear and good teratogenic parameters that could be relied upon.

The teratogenic effect of tamaron on chick embryo was also studied. The data presented in Table (2) and illustrated in Figure (3) indicated that injection of tamaron into chicken eggs on the sixth day of incubation caused anomalous development. The abnormalities included wing micromelia, leg hemimelia, deformed toe, asymmetrical development of the spine, lordosis of the spine, wry neck, shortened tibiofibulae and toes, twisted finger, sparse down and reduced body size. However, the severity of the abnormalities was dosage dependent, as embryos given very small amounts of tamaron (0.0125 mg/ega) showed almost no damage and appeared similar to control ones except reduce body size (Figure 3). At the dose level of 0.025 mg tamaron /egg, the incidence of growth retardation accompanied with twisted limb and reduced body size were higher than in the previous dose level of tamaron. 0.05 mg tamaron /egg caused moderate reduction in body size, short neck, short limbs and wing micromelia (Figure 3).

Embryos given 0.1 mg tamaron /egg were very severly deformed, with small bodies, wry neck, wing micronelia, severity deformed legs and feet (shortened tibio-fibulae and toes, twisted finger) and sparse down. Administration of 0.2 mg tamaron/egg resulted in very severe malformation. However, the most pronounced abnormalities are: wry neck, edema, dysmelia, reduced body size, wing micromelia, deformed toe, reduction of upper beak, sparse down and lordosis of the spine (Figure 3).

Fig (3): Tamaron-treated group, 20-day chick embryo, (A) 0.0125 mg/egg, showing almost no damage. Embryos appeared similar to control except reduced body size. (B) 0.05 mg/egg showing short neck, short limbs, short spine and wing micromelia. (C) 0.2 mg/egg showing wry neck, edema, dysmelia, reduced body size, wing micromelia, deformed toe, reduction of upper beak, sparse down and lordosis of the spine.

As for the teratogenic effects of 2,4-D on chick embryo were also studied and the data are presented in table (2) and were illustrated in figures (4). Reviewing the previous results, the following points could be fairly concluded in general, growth retardation was common feature in all embryos of 2,4-D treated groups. The effect of 2,4-D on chick embryos body-dimensions is dosage-dependent, as the embryos which resulted from eggs treated with the high doses of 2,4-D, i.e. 2.0, 2.5, and 7.5 mg/egg showed highly significant decrease (P< 0.001) in their body weights and sizes as compared with the control.

The lengths of the foot and wing give the best graded response to increasing dosages of 2,4-D. The reduction of embryos body weights as well as the changes in their body dimensions are good teratogenic signs that could be relied upon.

The results reconfirmed our previous findings concerning the effect of cypermethrin and tamaron on chick embryos.
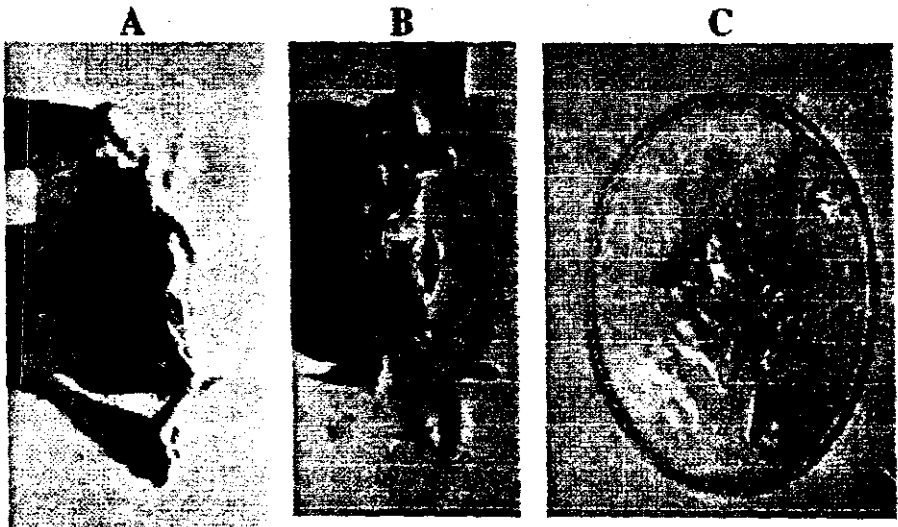
Fig (4): 2,4-D-treated group, 20-day chick embryo (A) 2.0 mg/egg, showing reduced body size, straight legs and growth retardation. (B) 7.5 mg/egg, showing growth retardation, sever reduction in body dimensions and weights, reduction of the beak, dysmelia and syndactyl. (C) 7.5 mg/egg, complete cessation of chick embryo at early stage of development.

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الملخص العربى

التاثيرات السامة والتشويهية لبعض المبيدات التقليدية على اجنة بيض الدجاج

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قسم المبيدات ــ كلية الزراعة ــ بكفر الشيخ ــ جامعة طنطا
*معهد بحوث وقاية النباتات ــ مركز البحوث الزراعية ــ الدقى

تـم دراسـة التـاثير الـعام والتـشويهى لـبعض المبيدات التقليديـة مثـل السيبرمثرين والتمارون والـ٤،٢- دى على أجنة بيض الدجاج. وقد اوضحت النتائج ان الفقد فى وزن البيض اثناء التحضين لا يعتمد على الجرعة ولا يمكن اعتباره كمعيار يعتمد عليه فى دراسة التشوهات الجنينية.

ولقد اوضحت النتائج ايضا ان السمية على الاجنة لا يمكن ايضا اتخاذها كمعيار للتاثيرات التشويهية وكذلك اوضحت ان مبيد السيبرمثرين يعتبر من المبيدات الامنة وليس له اى تاثير تشويهى على بيض الدجاج بينما تسبب كل من التمارون والـ٤،٢- دى فى احداث تشوهات واضحة اشتملت على فقد احد الاجنحة او الساق او التواء الاصابع او التواء فى العمود الفقرى او التواء فى الرقبة او الاصابع او فقد فى وزن الجسم وجميعها تعتبر ظواهر قوية لحدوث التاثيرات التشويهية . هذا وقد وجد ان جميع هذه الظواهر تعتمد على الجرعة.