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Teratogenic and Biochemical Effects of Glyphosate on Chick Embryo

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ABSTRACT

The herbicide glyphosate was tested for its teratogenic effects by injection of sublethal doses of $LD_{50}/10$, $LD_{50}/50$, $LD_{50}/100$ and $LD_{50}/1000$ in fertile eggs on day 6 of incubation. Body weight of fertile chicken eggs were recorded on 0, 6, 9, 12, 15 and 19 days. The fetuses on day 21 were examined. The results showed that mortality of fetuses was 100% for $LD_{50}/10$ level, and decreased with the decrease of concentration. Similarly, the body weight of fetus and the abnormalities were dose dependent. Cases of opened abdomen, brain edema, eye enlargement, deficiency in feathers and appendages were recorded in eggs treated either with $LD_{50}/50$ or $LD_{50}/100$, while at $LD_{50}/1000$ level least abnormalities were observed. Acetylcholinesterase (AChE) and Alkaline phosphatase (ALP) activities were measured in brain and liver of chicks. The results indicated that AChE activity was inhibited, while ALP activity was stimulated in a dose dependent manner. Finally, it can be concluded that both AChE and ALP enzymes can be used as biomarkers for exposure to glyphosate which can be harmful to humans and nontarget organisms.

INTRODUCTION

Glyphosate is a post-emergent, systemic and non-selective herbicide used in both agricultural and non-agricultural areas worldwide.

The major formulation is Roundup in which glyphosate is formulate as the propylamine salt (WSSA, 1983). A rapid uptake of ^{14}C-glyphosate within few hours was indicated in sugar beets (Gougler and Geiger, 1981) and potatoes (Smid and Hiller, 1981). As the glyphosate concentration in foliage may increase up to high levels immediately after application, this implies the possibility of entry the food chain and exerting toxicological impact on humans either directly or indirectly. The mechanism of toxic action of glyphosate was studied in rats by Olorunsogo *et al.*, (1979) and they found a dose-dependent respiratory toxicity and increased phosphatase *in vivo* activity in rat liver mitochondria after doses ranging from 15-120 mg/kg (Babaunmi *et al.*, 1979 and Olorunsogo, 1982). They suggested that the mode of toxic action is through uncoupling of oxidative phosphorylation. Glyphosate even at very low concentrations cause hepatic changes through induction of aminotransferases and phosphatases enzymes (Osman, 1994). These changes also results in changing of normal levels of endogenous hormones and biogenic amines which regulate many vital biological process (Enan and Berberian, 1986). The few data available for the longterm and chronic toxicity effects of glyphosate encouraged the initiation of this work to study the teratogenic toxic potential of the compound on chick embryo and to record the *in vivo* biochemical effect of the compound on AChE and ALP enzymes in liver and brain of the developed exposed embryos. Besides, the morphological teratogenic and fetotoxic will be recorded and discussed.

Materials and Methods

Chemicals. A technical grade of glyphosate, N-(phosphonomethy glycine) was obtained from Kafr El-zayat Co., Egypt. The chemical reagents and enzymes substrates were obtained from BDH Chem. Co. as chemical pure agents.

Fertile Eggs. The Red Road Island fertile eggs were used in the present study. Eggs were obtained from the Poultry Improvement Center, Hoash Essa, Behera Governorate.

Incubation Technique. Selected homogenous eggs of average weight 54.55 ± 0.43 gm were assigned to seven groups, each of 10 eggs and incubated for six days at 37.5-37.8°C, and relative humidity of 60-65%. Automatic rotation of the eggs was performed 4 times every 24 hours. the living chick embryo in the incubated eggs was confirmed by candling using a light bulb on the *6th* day of incubation. The weight of the incubated eggs was recorded on days 6, 9, 12, 15, and 19. On day *6th*, the eggs were pierced by a piercing electrical machine and treated with the solvent or glyphosate. Treatment included untreated control, isopropanol-treated control, sodium chloride (0.9%)-treated control, 1/10, 1/50, 1/100 and 1/1000 of LD_{50} value of glyphosate. The holes were waxed to be closed under antiseptic conditions The changes in the treated incubated eggs were recorded. On day *19th*, the half number of the eggs were inspected by opening, weighting and examining the embryo and recording the observation regarding the mortality, the abnormal growth, size and morphological features. On day *21st* the untreated control eggs begin to hatch. Few of the treated eggs hatched normally especially at the lower concentration. The other eggs were forced to open and embryos were weighed. Chick embryos were sacrificed and brain and liver quickly dissected and frozen at -20 °C until used for enzyme assays.

Assay of AChE and ALP

Brains and livers were homogenized in 10 volumes (W/V) of ice cold 0.1M Phosphate buffer pH 8.0 using a polytron homogenizer for 15 second. Homogenates were centrifuged at 10000 xg for 20 min. at 4°C in HS-21 (MSE) Centrifuge. The supernatant obtained (microsomal plus soluble fractions) were used for determination of AChE and ALP.

AChE activity was determined according to Ellman *et al.*, (1961) using acetylthiocholine as a substrate. Activity was expressed as µmole of acetylthiocholine hydrolyzed per min per gram tissue.

ALP activity was measured according to the method of Bessy *et al.*, (1946) using sodium p-nitrophenyl phosphate as a substrate. Enzyme activity was calculated as µmole of liberated p-nitrophenol per min per gram tissue.

RESULTS AND DISCUSSION

Perhatching changes in glyphosate-treated fertile after incubation for 21 days

Data in Table 1 show the gradual changes in eggs weight during incubation periods. The normal embryo growth in the untreated controls exerting the highest percentage of egg weight reduction. The highest level glyphosate (LD_{50}) caused the slower egg weight reduction due to retarded growth. Lower concentrations showed relatively lower growth resulting in higher reduction in egg's weight after incubation.

Embryotoxicity and morphological malformations at day 21 after incubation

Data in Table 1 show the percentages of mortality, embryo weight, and body size measurements at day 21 after incubation. Natural mortality did not exceed 7% in the controls. The highest level of glyphosate ($LD_{50}/10$) showed 100% mortality of the embryos followed by 96, 60 and 20% mortality for 1/50, 1/100 and 1/1000 of LD_{50} value, respectively. The offspring embryos were of lower body weight in a dose dependent trend. The body size dimensions were all lower than the untreated control. Being dose dependent suggests that glyphosate is the causing agent.

Teratogenic effects of glyphosate

Figure 1 shows the normal growth in the chicks that normally hatched On day 21 from the untreated control. In contrary, fetus mortality and undeveloped embryos of eggs treated with the highest concentration, $LD_{50}/10$ is clearly shown in Fig. 2. At 1/50 LD_{50} treatment, fetus mortality occurred and cases of early toxicity after formation of early parts are shown in Fig. 3 where severe malformations of open abdomen, brain edema and enlargement swallowed eyes in the dead fetus are found. At $LD_{50}/100$ treatment, the fetus was developed but with lower body weight and malformed wings and legs (Fig. 4). The chick was helped to hatch and was unable to stand on legs, feathers incomplete and the yolk was not consumed. the least concentration ($LD_{50}/1000$) showed least teratological symptoms where the fetus mortality was slightly higher than the control. The fetus weight was also lower than the control, and the wings and legs were malformed not enabling the chick to stand on legs (Fig. 5).

Table 1: Change in egg weight during incubation

Treatment	Decrease in egg weight during incubation									
	W (gm)	D %	W (gm)	D %	W (gm)	D %	W (gm)	D %	W (gm)	D %
Control Untreated	52.26	4.19	51.16	6.21	50.22	7.93	49.70	8.89	48.90	10.35
Control Isoprop-anol	52.03	4.61	50.90	6.69	50.06	8.23	49.52	9.22	48.83	10.48
Control (0.9%)	52.11	4.47	51.07	6.37	50.08	8.19	49.63	9.01	48.86	10.43
$LD_{50}/10$	52.80	3.20	52.59	3.59	52.32	4.08	52.12	4.45	51.70	5.22
$LD_{50}/50$	51.90	4.85	51.33	5.9	50.50	7.42	50.04	8.26	49.70	8.89
$LD_{50}/100$	52.23	4.25	51.80	5.04	51.30	5.95	50.89	6.7	50.50	7.42
$LD_{50}/1000$	52.26	4.19	51.50	5.59	50.84	6.8	50.44	7.53	49.74	8.81

*W=(weight in grams& D = decrease of egg weight as percentage of control)

The average egg mass at zero day of incubation is 54.55 ± 0.43 gram.

Table 2: Effect of glyphosate on mortality, body weight and size of chick embryo after 21 days of incubation

Treatment	% Mor-tality	Body weight (gm)	Measurements (Cm)				
			Body Leg	Wing	Foot	Thigh	
Control (Untreated)	6.0	38.04	8.50	3.50	2.74	3.30	1.72
Control Isopropanol	6.5	37.99	8.20	3.41	2.64	3.24	1.69
Control NaCl, 0.9%	7.0	37.64	8.00	3.35	2.62	3.22	1.70
$LD_{50}/10$	100	-	-	-	-	-	-
$LD_{50}/50$	96	5.60	4.04	1.32	1.12	1.56	1.08
$LD_{50}/100$	60	34.50	7.32	2.60	2.22	2.54	1.25
$LD_{50}/1000$	20	36.00	7.86	3.20	2.24	3.00	1.64

#The tabulated values are averages of all the fetuses after 21 days.

Table 3: *In vivo* effect of glyphosate on ALP activity of brain and liver of chick embryos

Treatment	Brain Mean ± SD[1]	Liver Mean ± SD[1]
Control	194.24±14.59	244.60±7.19
LD50/10	238.21±12.31*	283.53±12.31*
LD50/50	239.01±7.33*	244.60±11.99
LD50/100	221.42±10.81*	237.41±2.40
LD50/1000	205.43±7.71	239.81±8.58

1. Activity is expressed as µmole of p-nitrophenol produced/min. g tissue.
*. Significantly different from control value ($p \leq 0.05$).

Table 4: *In vivo* effect of glyphosate on AChE activity of brain and liver of chick embryos

Treatment	Brain Mean ± SD[1]	Liver Mean ± SD[1]
Control	372.55±37.07	600.49±34.75
LD50/10	237.77±18.52*	348.04±15.31*
LD50/50	254.90±30.61*	372.55±37.73*
LD50/100	299.02±33.96*	539.21±48.96*
LD50/1000	328.44±37.71	555.15±37.42

1. Activity is expressed as µmole of acetylthiocholine hydrolyzed /min. g tissue.
*. Significantly different from control value ($p \leq 0.05$).

The fetotoxicity and teratolgical effects being dose dependent suggest that glyphosate is responsible for these toxic effects. The present data co'ncides with the data by Yousef (1995) who reported that glyphosate has no adverse effect on reproduction in mammals. Long term laboratory trials have shown decreased body weight gain, increased incidence of cataract and lens abnormalities, increased liver weight and degeneration of liver and kidney at high doses (USEPA, 1993).

Biochemical Effects of Glyphosate

***In vivo* effect of glyphosate on ALP activity of brain and liver chick embryo**

Table 3 represents the activity of ALP in both untreated and treated fertile eggs with glyphosate at different concentration. The alkaline phosphatase activity in either brain or liver was stimulated at the higher level of glyphosate ($LD_{50}/10$). This induction effect was reduced at lower concentrations. The activity of ALP enzyme was relatively higher in liver tissues than brain. This is expected as the liver is the active organ for dephosphorylation of xenobiotics by phosphatases in the alkaline medium (Osman, 1994). Thus ALP which act as a detoxifying enzyme particularly in the liver will be highly released in both liver and brain. However, this inductive effect when extended is harmful to the balance between metabolic and catabolic enzymes incorporating phosphate groups. The long term effect is undesirable and can be toxic.

***In vivo* effect of glyphosate on AChE activity of brain and liver chick embryo**

The activity of AChE in both brain and liver of chick embryos is illustrated in Table 4. Maximum inhibition of AChE was exerted at the higher glyphosate level ($LD_{50}/!0$). The enzyme activity was almost recovered at the lowest concentration of glyphosate. Thus The AChE inhibition goes parallel to the long term teratological effects. The high ability of glyphosate to inhibit liver AChE is probably due to high residues in liver (Osman, 1994). The ability of glyphosate to inhibit AChE explains its poisoning incidents to farmers and field workers of weed control as reported in New Zealand by Waltts (1994). Finally, it can be concluded that the herbicide glyphosate can be hazardous to farmers during application and its residues can be of chronic adverse effect to mass and the nontarget organisms.

Fig. 2. Undeveloped embryo in eggs treated with 1/10 LD_{50} of glyphosate.

Fig. 3. . Retaded and malformed growth Of the embryo of eggs treated with 1/50 LD_{50} of glyphosate.

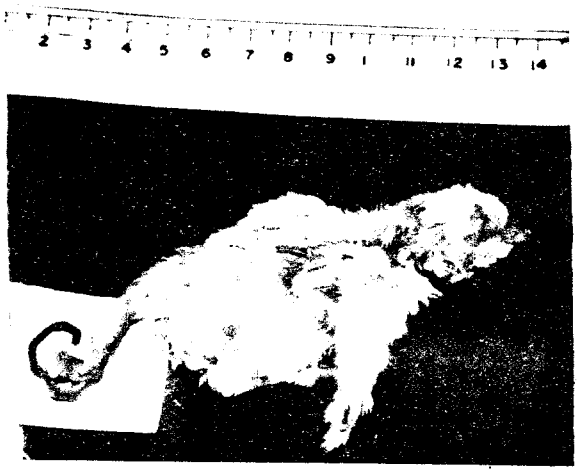

Fig. 4. Malformed chick of egg treated with 1/100 LD_{50} of glyphosate.

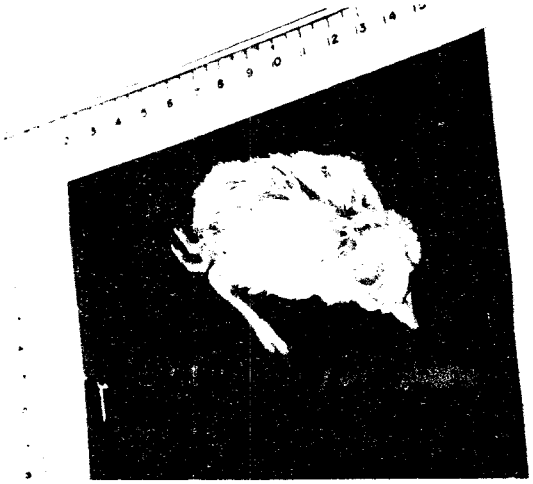

Fig 5, Retarded and malformed growth of embryo of eggs treated with 1/ 1000 LD_{50} of glyphosate.

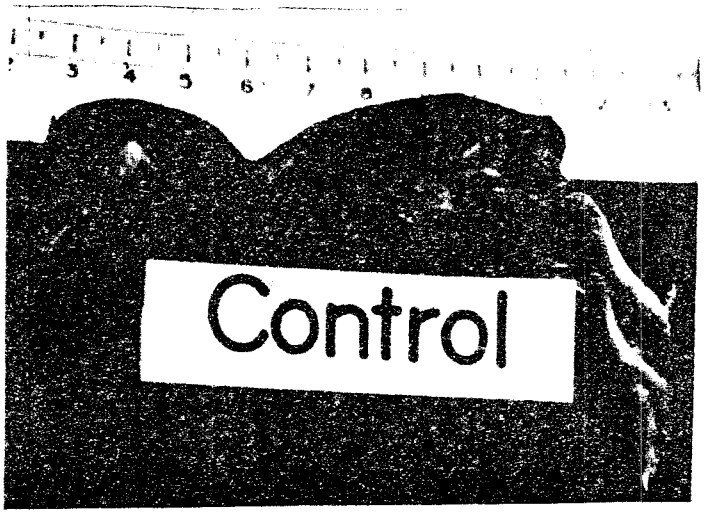

Fig. 1. Control of untreated eggs (normal growth).

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الملخص العربى

التأثيرات البيوكيميائية وتشوه الأجنة التى يحدثها مبيد الجليفوسات على جنين بيض الدجاج

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تم اختبار تأثيرات تشوه الأجنة لمبيد الحشائش الجليفوسات عن طريق حقن تركيزات أقل من المميتة من المبيد مذابة فى كحول الايزوبروبايل بجرعات ١/١٠ ، ١/٥٠ ، ١/١٠٠ و ١/١٠٠٠ من (LD_{50}) الجرعة المتوسطة للموت فى البيض المخصب عند اليوم السادس من بدأ التحضين مع استخدام عشرة بيضات لكل معاملة. وتم تسجيل وزن البيض عند الأيام صفر (بدأ التحضين) ، ٦ يوم ، ٩ يوم ، ١٢ يوم ، ١٥ يوم ثم ١٩ يوم . وفى اليوم ٢١ تم فحص الأجنة فى البيض الذى تم فقسه طبيعيا أو بعد فتح البيضة لخروجه . كما تم تسجيل وفيات الأجنة. وقد تبين موت ١٠٠% من أجنة البيض المعامل بجرعة ١/١٠ من LD_{50} وتناقصت النسبة المئوية للموت بانخفاض التركيز المستخدم. كما تم تسجيل وزن الأجنة وفحصها لرصد اية تشوهات التى وجد انها تزيد مع التركيز . وقد تم تسجيل أعراض البطن المفتوح - تورم المخ وتضخم وجحوظ العين - ونقص الريش وتشوه الأطراف وذلك فى المعاملتين عند التركيزين ١/٥٠ ، ١/١٠٠ LD_{50}. وكانت التشوهات أقل ما يمكن عند أقل التركيزات وهو ١/١٠٠٠ كما تم تقدير نشاط كل من انزيم الاستيل كولين

استريز والفوسفاتيز القلوى فى مخ و كبد عينات من الأفراد المعاملـــة مقارنـــة بالكونترول غير المعامل. وقد أتضح تثبيط نشاط انزيم الأسيتيل كولين اســـتريز بدرجة تتناسب طرديا مع قيمة التركيز المختبر ومن ذلك يمكن أقتراح اســـتخدام نشاط كل من انزيمى الاسيتايل كولين استريز والفوسفاتيز القلوى فى الأنســـجة كمؤشر حيوى biomarker عن درجة تعرض الكائن لمثل هذا المبيد الفوســفورى العضوى الذى قد يضر بالانسان والكائنات البرية وغير المستهدفة خاصة عنـــد التركيزات العالية .