

## EFFECT OF CERTAIN PESTICIDES ON SOME ENZYMES OF CHICK EMBRYOS

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### ABSTRACT

Effects of Chlorpyrifos 48 % EC ( Dursban & Pestban ) and Profenofos 72 % EC ( Selecron & Ictacron ) at two sublethal concentrations ( 1/100<sup>th</sup> and 1/10<sup>th</sup> of the field recommended rate ) on some serum , brain and liver enzymes as well as total protein from 18 and 21 days old - chick embryos (hatching) were determined. In general, Dursban and Pestban at the two concentrations significantly decreased brain acetylcholinesterase (AChE) either at 18 or 21 days old chick embryos, except Dursban in 18 days at the low concentration. On the other hand, Selecron and Ictacron at the two concentrations caused insignificant inhibition in brain AChE activity after 18 and 21 days compared with the control groups.

Serum alanine amino transferase (ALT) specific activity was significantly increased with Chlorpyrifos and Profenofos at the used concentrations. On the contrary, Chlorpyrifos from the two formulations at the high concentration significantly inhibited the serum aspartate amino transferase (AST). There were no differences between the two formulations of Profenofos on the serum amino transferases ( ALT & AST ).

The results also revealed that the activity of liver acid phosphatase (AcP) in 18 days old chick embryos was insignificantly inhibited at the two concentrations of Chlorpyrifos ( Dursban & Pestban ). There were no significant differences between Selecron and Ictacron on the liver enzyme activity at 18 days. Chlorpyrifos and Profenofos were caused significant inhibition of 21 days old chick embryo liver (AcP) activities from the two formulations, except Selecron at the high concentration.

The activity of liver alkaline phosphatase (AIP) in the 18 days old - chick embryos was decreased insignificantly at the two concentrations of Dursban, in contrast, the activity was increased by increasing the concentration of Pestban, Selecron and Ictacron. On 21 days old - chick embryos, Chlorpyrifos (Dursban & Pestban) caused significant decrease of liver AIP activity at the two concentrations. Selecron at the two concentrations had insignificant effect on serum AIP activity, but Ictacron caused significant activation of serum AIP activity at the high concentration.

There were insignificant changes in the serum total protein between the treated groups with Dursban, Pestban, Selecron, Ictacron and control groups.

### INTRODUCTION

The intensive use of organic pesticides on a wide variety of crops, fruit orchards and vegetable crops for controlling pests leads to environmental contamination and killing wild life (Flint and Bosch, 1981).

The earliest observations on the effects of external applications of pesticides to bird eggs by spraying DDT revealed few apparent toxic effects (Mitchell, 1946 and Somers *et al.*, 1974). Paraquat proved to be highly toxic following its application to eggs of chickens and Japanese quail (Lutz-Ostertag and Henou 1975) or mallards (Hoffman and Eastin 1982). Some organophosphorus pesticides are known to cause delayed neurotoxicity in chickens (Abo-Donia and Graham 1978; EL-Sebae *et al.*, 1980). Mallard

(*Anas platyrhynchos*) eggs are intensive to external exposure of organophosphorus pesticides including parathion, which results in mortality, stunted growth and teratogenicity. These effects are accompanied by inhibition of brain acetylcholinesterase (Hoffman and Eastin 1981; Hoffman and Albers, 1984). Chickens (*Gallus gallus*) and Japanese quail (*Coturnix japonica*) are also sensitive to external application of parathion (Meiniel, 1973).

Activity of brain acetylcholinesterase (AChE), plasma cholinesterase, alkaline phosphatase were inhibited, while, plasma aspartate amino transferase was increased at one or more stages of mallard development treated with the organophosphorus insecticide; phenyl phosphonothioic acid – O - ethyl - O ( - 4 - nitrophenyl) ester (EPN) (Hoffman and Sileo, 1984). The *in vivo* results of brain ChE activities revealed that Profenofos was not a potent ChE inhibitor, while sulprofos was a relatively stronger ChE inhibitor insecticide at the 1/4 LD<sup>50</sup> levels (Enan, 1979). Dimethoate and Chlorpyrifos exposed mallard (*Anas platyrhynchos*) ducklings exhibited activity depression of brain acetylcholinesterase (AChE) activity (Martin and Forsyth 1998). Also, Chlorpyrifos, parathion, acephate and trichlorfon inhibited brain AChE of chick embryos (Lesser *et al.*, 2000).

The purpose of the present study is to investigate the potential toxic effects of Chlorpyrifos and Profenofos on some serum, brain and liver enzymes as well as the serum total protein of chick embryos by applying different formulations (exported or locally) at different concentrations to the incubating eggs.

## MATERIALS AND METHODS

### Chemicals

The organophosphorus, insecticides used were;

- I. Two formulations of Chlorpyrifos 48 % EC { ( O, O diethyl - O - ( 3,5,6 - trichloro - 2- pyridyl ) phosphorothioate } from two chemical companies:
  - 1) Dursban was obtained from Dow Agro Sciences (England).
  - 2) Pestban was obtained from Agrochem Company (Egypt).
  
- II. Two formulations of Profenofos 72 % EC { O - ( 4 - bromo - 2 - chlorophenyl) O - ethyl S - propyl phosphorothioate ) } from two chemical companies:
  - 1) Selecron was obtained from Sengenta
  - 2) Ictacron was obtained from International Company of Chemicals and Trade Agencies (ICCTA), (Egypt).

All other chemicals were of highest purity grade commercially available.

### Tested chicken eggs:

Fertile eggs of Alexandria Strain chicken ( *Gallus gallus* ) obtained from the Experimental Station of Faculty of Agriculture , Alexandria University, Alexandria, Egypt. Eggs, weighting 52.2 ± 1.6 gm were placed in the incubator maintained at 37.5 ° C and 65 - 75 % relative humidity and 3-5 times rotation every day.

**Eggs treatment:**

On the fourth day of incubation all eggs were candled before treatment, and infertile ones and those with dead embryos were discarded. Eggs were randomly divided into ten groups ( 50 eggs per each group ) and treated as follows:

1-Untreated control

1) Treated control with water

2- Two groups treated with Dursban 48 % EC (1/100<sup>th</sup> and 1/10<sup>th</sup> of the field recommended rate)

3- Two groups treated with Pestban 48 % EC (1/100<sup>th</sup> and 1/10<sup>th</sup> of the field recommended rate)

4- Two groups treated with Selecron 72 % EC (1/100<sup>th</sup> and 1/10<sup>th</sup> of the field recommended rate)

5- Two groups treated with Ictacron 72 % EC (1/100<sup>th</sup> and 1/10<sup>th</sup> of the field recommended rate)

Eggs were treated by immersing the eggs in aqueous emulsion of pesticides for 15 sec according to the method of Meiniel, 1973. Eggs were allowed to dry for 5 min on racks and were then returned to the incubator.

**Biochemical studies:**

Blood samples were obtained from hatching embryos of each group by decapitation. Brain and liver of 18 and 21 days old embryos (hatching) were removed for biochemical parameter measurements. Brain acetylcholinesterase (AChE) activities was determined spectrophotometrically using the method of Ellman *et al.*, (1961), serum alanine amino transferase (ALT) and aspartate amino transferase (AST) were determined according to the method of Reitman and Frankel (1957). Also, acid phosphatase activities (AcP) in liver were determined according to Bessey *et al.*, (1946). Serum and liver alkaline phosphatase activities (AIP) were determined by the method of Hausman *et al.*, (1967).

**Total protein:**

Total serum protein was determined by the method of Weichsebaum, (1946).

**Statistical analysis**

The data were expressed as mean  $\pm$  SD. Data were statistically analyzed using one – way analysis of variance ( $p < 0.05$ ) according to Dixon and Massay (1957).

## RESULTS AND DISCUSSION

**Acetylcholinesterase activity (AChE):**

Specific activities of brain (AChE) of 18 and 21 days old embryos treated with Chlorpyrifos; Dursban and Pestban and Profenofos; Selecron and Ictacron with the two concentrations were summarized in table (1). Chlorpyrifos from the two formulations (Dursban or Pestban) was caused significantly inhibition of brain AChE activities in 18 and 21 days embryo, except Dursban at 18 days with the low concentration. There were

insignificant differences between Dursban and Pestban effects on brain AChE activities of 18 and 21 days old chick embryos. Profenofos (Selecron and Ictacron) at the two used concentrations; 1/100<sup>th</sup> and 1/10<sup>th</sup> of the field recommended rate were caused insignificant inhibition of brain AChE activity either in 18 days or 21 days old embryos.

This data was supported by several reports for the effect of organophosphorus pesticides on the AChE activity in birds. Chlorpyrifos caused significant inhibition in the brain ChE from 15 days old chick embryos (Lesser *et al.*, 2000). Helal, 2000 reported that Chlorpyrifos significantly decreased AChE of Japanese quail at 1 / 50 or 1 / 100 of LD<sub>50</sub> for 30 days, when compared with the control. The *in vivo* results of brain ChE activities revealed that Profenofos was not a potent ChE inhibitor, while sulprofos was a relatively stronger ChE inhibitor insecticide at the 1/4 LD<sub>50</sub> levels ( Enan, 1979). Esterases in some avian species were also, found to be affected by sublethal doses of organophosphate compounds (El – Hamady *et al.*, 1996). Also, brain and serum AChE activities were strongly inhibited after the treatment with azamethiphos and methomoyl (Fossi *et al.*, 1992).

In general, Chlorpyrifos is neurotoxic in nature by acting as inhibitor of neuronal cholinesterase activity (Altuntas *et al.*, 2002). In contrast, Profenofos is known as non-ChE inhibitor insecticide. Classes of compounds known to have the common side chain of S-n-propyl OP's are characteristically very low in their potency as ChE inhibitors particularly *in vivo*. The producer of Profenofos state that acute toxicity of the compound to human or mammals cannot be treated or pretreated by known antidotes atropine sulfate or PAM (Enan, 1979).

Table (1): Effect of Chlorpyrifos and Profenofos on brain acetylcholinesterase (AChE) activity in 18 and 21 days chick embryos .

Treatments	OD / mg protein / min. Mean ± SD	
	18 day	21 day
Untreated control	2.56 ± 0.14 <sup>c</sup>	4.05 ± 0.65 <sup>c</sup>
Control treated with water	2.59 ± 0.16 <sup>c</sup>	4.03 ± 0.11 <sup>c</sup>
<b>Chlorpyrifos</b>		
1-Dursban at low conc.	2.165 ± 0.14 <sup>abc</sup>	1.66 ± 0.03 <sup>a</sup>
2-Dursban at high conc.	1.94 ± 0.13 <sup>ab</sup>	1.12 ± 0.12 <sup>a</sup>
3-Pestban at low conc.	1.98 ± 0.20 <sup>ab</sup>	1.72 ± 0.15 <sup>a</sup>
4-Pestban at high conc.	1.74 ± 0.14 <sup>a</sup>	1.51 ± 0.25 <sup>a</sup>
<b>Profenofos</b>		
1-Selecron at low conc.	2.29 ± 0.36 <sup>bc</sup>	3.82 ± 0.36 <sup>bc</sup>
2-Selecron at high conc.	2.18 ± 0.37 <sup>bc</sup>	3.77 ± 0.47 <sup>bc</sup>
3-Ictacron at low conc.	2.33 ± 0.14 <sup>bc</sup>	3.76 ± 0.101 <sup>bc</sup>
4-Ictacron at high conc.	2.10 ± 0.38 <sup>bc</sup>	3.58 ± 0.24 <sup>bc</sup>

Significantly different from the control groups by one- way analysis of variance of ( p < 0.05 ) .

Each group includes nine replicates.

**Transaminases activities:**

Table (2) showed the effect of Chlorpyrifos and Profenofos from the two formulations at the two concentrations on the transaminases (ALT & AST) in serum of 21 days old chick embryos. ALT activities were significantly increased with Chlorpyrifos in a concentration dependent manner. Dursban increased ALT activity about 75.6 % and 153.7 % of control at low and high concentration respectively, while Pestban increased the activity about 118.5 % and 226.63 % of control, respectively. Profenofos ( Selecron & Ictacron ) caused significantly activation in the (ALT) activity , but there were no significant differences between them. In contrast, Chlorpyrifos from the two formulations was significantly decreased the activities of AST at the high concentration. The activity of AST decreased to 48.6 % and 45.75 % of the control at high concentration of Selecron and Ictacron respectively. The present data are also in agreement with Dieter and Wiemeyer (1978) who reported elevation of AST and ALT values in adult birds following an acute dosage of dieldrin, a known hepatotoxin. Also, Chlorpyrifos significantly elevated AST and ALT of Japanese quail when daily treated with sublethal doses (1/50 and 1/100 of LD<sub>50</sub>) for 30 days (Helal, 2000).

The observed changes in serum AST and ALT activities are consistent with the possibility of cellular damage in liver. Elevated serum activities on intracellular enzymes characteristic of certain organs have indicated cellular leakage associated with probable cellular injury following exposure to pesticides during avian embryogenesis ( Baker *et al.*, 1972 ).

**Table (2) : Effect of Chlorpyrifos and Profenofos on serum transaminases ( ALT & AST ) of 21 days chick embryos .**

Treatments	Units / L Mean ± SD	
	ALT	AST
Untreated control	127.15 ± 3.04 <sup>a</sup>	134.95 ± .3 <sup>e</sup>
Control treated with water	140.3 ± 6.08 <sup>a</sup>	133.6 ± 13.2 <sup>e</sup>
<b>Chlorpyrifos</b>		
1. Dursban at low conc.	215.5 ± 3.54 <sup>b</sup>	126.85 ± 0.21 <sup>de</sup>
2. Dursban at high conc.	312.05 ± 15.1 <sup>c</sup>	104.25 ± 2.2 <sup>c</sup>
3. Pestban at low conc.	268.7 ± 12.3 <sup>bc</sup>	119.99 ± 0.7 <sup>de</sup>
4. Pestban at high conc.	401.75 ± 50.4 <sup>d</sup>	116.3 ± 5.6 <sup>d</sup>
<b>Profenofos</b>		
1. Selecron at low conc.	372.8 ± 6.3 <sup>d</sup>	75.67 ± 7.1 <sup>ab</sup>
2. Selecron at high conc.	232.25 ± 28.4 <sup>b</sup>	68.96 ± 1.4 <sup>a</sup>
3. Ictacron at low conc.	376.1 ± 11.0 <sup>d</sup>	76.65 ± 3.2 <sup>ab</sup>
4. Ictacron at high conc.	244.3 ± 7.64 <sup>b</sup>	72.85 ± 1.91 <sup>ab</sup>

Significantly different from the control groups by one-way analysis of variance of ( p < 0.05 ).

Each group includes nine replicates.

**Acid phosphatase activity ( AcP):**

Effects of Chlorpyrifos and Profenofos in the two formulations on liver AcP in 18 and 21-day chick embryos treated at 4 day of development are summarized in table (3). The results revealed that the activity of 18 days old chick embryos liver AcP was insignificantly inhibited with the two concentrations of Chlorpyrifos from the two formulations. While with 21-days old chick embryos the activity was significantly decreased by Chlorpyrifos (Dursban, Pestban). There were no differences between selecron and Ictacron on the enzyme activity in the liver of 18 and 21- days chick embryos, except for Selecron at the high concentration after 21 days.

The present results are in agreement with the results of Sati, (1996) who reported some changes in the liver AcP activity of hen after single or multiple treatments with some organophosphorus esters. The increment of AcP activity seems to result from enhanced enzyme turnover under pesticide stress, whereas the reduction of its activity may be related to leakage of the enzyme into the extracellular compartment. Barzu *et al.*, (1973) demonstrated that Op's may cause a release of some hydrolytic enzymes from lysosomes

**Table ( 3 ) : Effect of Chlorpyrifos and Profenofos on liver acid phosphatase in 18 and 21 days chick embryos .**

Treatments	µ mole P- nitrophenol / mg protein / min. Mean ± SD	
	18 day	21 day
Untreated control	0.64 ± 0.04 <sup>abc</sup>	1.35 ± 0.098 <sup>e</sup>
Control treated with water	0.72 ± 0.08 <sup>abcd</sup>	1.22 ± 0.075 <sup>de</sup>
<b>Chlorpyrifos</b>		
1. Dursban at low conc.	0.61 ± 0.086 <sup>abc</sup>	0.923 ± 0.02 <sup>ab</sup>
2. Dursban at high conc.	0.51 ± 0.03 <sup>ab</sup>	0.915 ± 0.035 <sup>ab</sup>
3. Pestban at low conc.	0.597 ± 0.08 <sup>abc</sup>	0.796 ± 0.03 <sup>a</sup>
4. Pestban at high conc.	0.43 ± 0.07 <sup>a</sup>	0.98 ± 0.09 <sup>abc</sup>
<b>Profenofos</b>		
1. Selecron at low conc.	0.75 ± 0.14 <sup>bcd</sup>	1.16 ± 0.13 <sup>cd</sup>
2. Selecron at high conc.	0.93 ± 0.13 <sup>d</sup>	1.4 ± 0.05 <sup>e</sup>
3. Ictacron at low conc.	0.67 ± 0.08 <sup>abcd</sup>	1.061 ± 0.04 <sup>bcd</sup>
4. Ictacron at high conc.	0.842 ± 0.23 <sup>cd</sup>	1.1 ± 0.18 <sup>bcd</sup>

Significantly different from the control groups by one-way analysis of variance of ( p < 0.05 ).

Each group includes nine replicates.

**Alkaline phosphatase activity (AIP) :**

The activity of liver alkaline phosphatase (AIP) of 18 - days old chick embryos was decreased insignificantly with the two concentrations of Dursban, while, the activity was increased by increasing the concentration of Pestban, Selecron and Ictacron (table 4). On 21- days, Chlorpyrifos caused significant decrease of liver AIP activity, but Profenofos inhibited the activity at

low concentration and changed to activation with the high concentration. The data indicated that the activities of serum AIP treated with Dursban and Pestban were decreased in a concentration dependent manner. Selecron had insignificant inhibition on serum AIP activity, but Ictacron caused significant activation of serum AIP activity. The present results are in agreement with some authors (Tag El-Din *et al.*, 1996 and Helal, 2000). Liver is often the primary target for the toxicity of various toxicants. The assessment of liver enzymes in blood is generally a more sensitive measure of hepatotoxicity and can be assessed within a shorter time ( Korsrud *et al.*, 1972). The reduction in AIP activity might be due to tissue damage, while the enhanced activity could be related to the influence of glucocorticoides (Murphy, 1966), or could be attributed to its release from ruptured cells due to the effect of pesticide (Shaffi, 1980).

**Table ( 4 ) : Effect of Chlorpyrifos and Profenofos on serum and liver alkaline phosphatase in 18 and 21 days chick embryos .**

Treatments	Mean $\pm$ S <sub>w</sub>		
	*Liver ( S.A X 10 <sup>-3</sup> )		**Serum of
	18 day	21 day	Hatched
Untreated control	216.6 $\pm$ 9.5 <sup>a</sup>	205.9 $\pm$ 2.3 <sup>bc</sup>	416.7 $\pm$ 29.3 <sup>cd</sup>
Control treated with water	215.5 $\pm$ 3.6 <sup>a</sup>	213.7 $\pm$ 12.8 <sup>bc</sup>	410.0 $\pm$ 10.0 <sup>cd</sup>
<b>Chlorpyrifos</b>			
1. Dursban at low conc.	213.6 $\pm$ 7.7 <sup>a</sup>	142.1 $\pm$ 34.4 <sup>a</sup>	328.6 $\pm$ 14.8 <sup>bc</sup>
2. Dursban at high conc.	194.5 $\pm$ 6.4 <sup>a</sup>	116.9 $\pm$ 14.1 <sup>a</sup>	261.2 $\pm$ 19.4 <sup>b</sup>
3. Pestban at low conc.	321.7 $\pm$ 49.1 <sup>ab</sup>	155.6 $\pm$ 1.4 <sup>a</sup>	287.5 $\pm$ 17.5 <sup>b</sup>
4. Pestban at high conc.	588.4 $\pm$ 162.7 <sup>d</sup>	110.9 $\pm$ 4.1 <sup>a</sup>	171.0 $\pm$ 22.0 <sup>a</sup>
<b>Profenofos</b>			
1. Selecron at low conc.	314.1 $\pm$ 27.3 <sup>ab</sup>	198.9 $\pm$ 38.2 <sup>b</sup>	387.7 $\pm$ 16.0 <sup>cd</sup>
2. Selecron at high conc.	429.3 $\pm$ 61.7 <sup>bc</sup>	274.2 $\pm$ 29.9 <sup>d</sup>	398.8 $\pm$ 45.2 <sup>cd</sup>
3. Ictacron at low conc.	465.4 $\pm$ 58.1 <sup>c</sup>	151.2 $\pm$ 7.3 <sup>a</sup>	442.2 $\pm$ 49.9 <sup>d</sup>
4. Ictacron at high conc.	746.7 $\pm$ 20.2 <sup>e</sup>	247.9 $\pm$ 13.9 <sup>cd</sup>	524.0 $\pm$ 84.5 <sup>e</sup>

\* Specific activity expressed as (Units / g wt.)

\*\* Specific activity expressed as (Units / L)

Significantly different from the control groups by one-way analysis of variance of ( p < 0.05).

Each group includes nine replicates

#### **Total protein:**

Table (5) illustrates the effects of the tested pesticides on serum total protein of chick embryos and the control groups. The data showed that there were insignificant differences in the serum total protein of the treated groups with Dursban; Pestban; Selecron and Ictacron at both concentrations and the control groups. This data is similar to those of Mandal *et al.*, (1992) who reported that fenvalerate at 5 mg / kg did not change the level of sheep serum total protein.

Table ( 5 ) : Effect of Chlorpyrifos and Profenofos on the serum total protein of 21- days chick embryos treated on the 4<sup>th</sup> day.

Total Protein ( g / d )	Untreated Control	Control Treated with water	Treatment							
			Chlorpyrifos			Profenofos				
			Dursban		Pestban		Selecron		Ictacron	
			Low conc.	High conc.	Low conc.	High conc.	Low conc.	High conc.	Low conc.	High conc.
Mean ± SD	2.32 ±0.31 <sup>a</sup>	2.36±0.29 <sup>a</sup>	2.26±0.65 <sup>a</sup>	2.18±0.69 <sup>a</sup>	2.34±0.65 <sup>a</sup>	2.23±0.25 <sup>a</sup>	2.27±0.19 <sup>a</sup>	2.16±0.1 <sup>a</sup>	2.54±0.19 <sup>a</sup>	2.48±0.37 <sup>a</sup>

Significantly different from the control groups by one-way analysis of variance of (p< 0.05). Each group includes nine replicates.

Also, Fayez and Kilgore (1992) reported that the serum total protein of male rat was not affected by the single acute oral dose of 1, 2.1 and 3.5 mg /kg of oxamyl. The insignificant effect of pesticides on the lipids and protein implies the possibility of the absence of any tendency of these chemicals to exert cytotoxic effects, which are highly dependent on interference with lipoprotein levels and rate of biosynthesis (Radwan *et al.*, 1993).

In conclusion, the external exposure of chicken eggs to the organophosphorus insecticide; Chlorpyrifos at the sublethal concentrations was found to be ChE inhibitor, while Profenofos is low in their potency as ChE inhibitor. The low potency in Profenofos can be attributed to less persistence *in vivo* and / or less affinity to the biochemical targets. We can be considering that the limiting factors for anticholinesterase effects are expected to be lipid solubility, stability and affinity to the ChE (Enan *et al.*, 1981). Also, Chlorpyrifos and Profenofos were found to be exerting hepatic action as showed by affecting phosphatases and transaminases. There were no differences between the two formulations of Profenofos in their effects on the studied biochemical targets. Certain Egyptian carriers are available and suitable to formulating pesticides with high good physics and chemical properties and without any apparent phytotoxicity that can serve the national pesticide industry (El-Sebae *et al.*, 1980). While the differences between the two formulations of Chlorpyrifos on the AChE activity and the other enzymes may be due to the differences of the additives. Also, toxic impurities in the pesticide products might be formed during the manufacturing process, during storage, or after opening the sealed pesticide container.

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**Aly, Nagat M. and S. M. Abd – El Rahman**

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## تأثير بعض المبيدات على بعض النظم الإنزيمية في أجنة الدواجن نجاهة محمد على و صفاء مصطفى عبد الرحمن المعمل المركزي للمبيدات - مركز البحوث الزراعية - الصباحية - الإسكندرية

يهدف البحث دراسة تأثير تجهيزات مختلفة لبعض المبيدات الفسفورية ؛ الكلوربيريفوس ٤٨ % مركز قابل للاستحلاب ( دورسيان - بستبان ) والبروفينوفوس ٧٢ % مركز قابل للاستحلاب ( سيليكرون- إكتاكرون ) بتركيزين ( ١٠٠/١ ، ١٠/١ من المعدل الموصى به حقلياً ) على بعض إنزيمات السيرم والمخ والكبد وكذلك البروتين الكلى في السيرم لأجنة الدجاج عند عمر ١٨ ، ٢١ يوم ( الفقس ) مقارنة بالمجموعات الضابطة ( الغير معاملة والمعامل بالماء ) . ويمكن توضيح النتائج في الآتى :

لقد أحدثت التجهيزات المختبرة لمبيد الكلوربيريفوس ( الدورسيان - البستبان ) انخفاضاً معنوياً لنشاط إنزيم الأستاييل كولين إستيريز ( AChE ) في المخ بكلما التركيزين المستخدمين في الأجنة بعد ١٨ ، ٢١ يوم ، فيما عدا المعاملة بالتركيز المنخفض من الدورسيان بعد ١٨ يوم حيث كان الانخفاض غير معنوي مقارنة بالمجموعات الضابطة. وعلى العكس من ذلك فإن مبيد البروفينوفوس بتجهيزتيه ( سيليكرون- إكتاكرون ) لم يحدثا تأثيراً معنوياً على نشاط إنزيم الأستاييل كولين إستيريز في المخ سواء عند ١٨ أو ٢١ يوم من المعاملة.

حدثت زيادة لنشاط إنزيم الألاتين أمينو ترانسفيريز ( ALT ) في سيرم الأجنة المعاملة بكل من الكلوربيريفوس ( دورسيان- بستبان ) والبروفينوفوس ( سيليكرون- إكتاكرون ) وبكلا التركيزين المستخدمين زيادة معنوية . وعلى العكس من ذلك فإنها أحدثت تثبيطاً معنوياً لنشاط إنزيم الإيسباريت أمينو ترانسفيريز ( AST ) في السيرم للأجنة المعاملة فيما عدا التركيز المنخفض من كل من الدورسيان و البستبان فكان الانخفاض غير معنوي مقارنة بالمجموعات الضابطة .

كما أوضحت النتائج أيضاً حدوث تثبيط غير معنوي لنشاط إنزيم الفوسفاتيز الحامضى ( ACP ) في الكبد لأجنة الدجاج المعامل بكل من الدورسيان و البستبان بالتركيزات المختبرة و بعد ١٨ يوم ، بينما السيليكرون والإكتاكرون فقد أحدثا تثبيطاً غير معنوياً لنشاط إنزيم عند التركيز العالى ( ١٠٠/١ من المعدل الحقلى ) . بينما أحدث كل من الدورسيان و البستبان و الإكتاكرون بالتركيزين المختبرين انخفاضاً معنوياً لنشاط الإنزيم . وعلى العكس فإن مبيد السيليكرون بالتركيز العالى أحدث زيادة غير معنوية في نشاط الإنزيم في الكبد للأجنة بعد ٢١ يوم من المعاملة.

ولقد حدثت زيادة في نشاط إنزيم الفوسفاتيز القاعدى ( AIP ) عند ١٨ يوم في الكبد المعامل بكل من البستبان ، السيليكرون و الإكتاكرون بعلاقة طردية مع التركيز . بينما انخفض نشاط الإنزيم إنخفاضاً معنوياً في كبد الأجنة عمر ٢١ يوماً من المعاملة بمبيد الكلوربيريفوس سواء عند ١٠٠/١ أو ١٠/١ من المعدل الحقلى . سبب كل من الدورسيان و البستبان تثبيطاً لنشاط الإنزيم في السيرم يزيد بزيادة التركيز . وقد أحدث مبيد السيليكرون انخفاضاً غير معنوي لنشاط إنزيم الفوسفاتيز القاعدى في السيرم بينما أحدث مبيد الإكتاكرون زيادة لنشاط هذا الإنزيم في السيرم وبالعلاقة طردية مع التركيز .

ولم تظهر أية تأثيرات على مستوى البروتين الكلى في السيرم للأجنة المعاملة بكل المبيدات المختبرة وبكلا التركيزين وذلك بالمقارنة بالمجموعات الضابطة.