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EFFECT OF PROFENOFOS ON HAEMOGRAM AND THYROID GLAND IN MALE ALBINO RATS

M.M. Farid[**], M.I. Kobeasy[*] and A.A. AbdAllah[**]

[*] *Agric. Biochem. Dept., Fac. Agric. Cairo Univ., Giza, Egypt.*

[**] *Mammalian and Aquatic Toxicology Dept. Central Agricultural Pesticide Laboratory, Agricultural Research Centre, Dokki, Giza, Egypt.*

ABSTRACT

The present study dealing with the effect of profenofos on subchronic toxicity test, including general toxicological (body weight changes and internal organ weights), and haematological indices (WBCs, RBCs, Hb, PCV, MCV, MCH and MCHC) and thyroid hormones analyses which supported by pathology of thyroid gland. Detectability of toxic effects of profenofos following adminstrate in drinking water at concentration of 22.25 ($1/20LD_{50}$) and 11.125 ($1/40LD_{50}$) ppm for 12 weeks in male albino rats. The results revealed a significant decrease in body weight gain, thyroxin (T4), Tri-iodothyronine (T3), white blood cells (WBCs), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), while significant increase in red blood cells (RBCs), haemoglobin concentration, packed cell volume (PCV) and mean corpuscular volume (MCV). On the other hand no significant changes in the weights of liver, kidney, spleen, lung, heart and brain of male albino rats was recorded. Histopathological examination showed that, atrophy and colloid free lumen were observed in few acini of thyroid gland in male albino rats.

Key words: Profenofos – Haemogram - Thyroid gland .

INTRODUCTION

Body weight is one of the most sensitive indicators of the condition of an animal if it is monitored frequently and carefully during a study. Marked body weight loss is usually a harbinger of ill health or death. Body weight loss can be due to decreased feed

consumption, water consumption, or disease or specific toxic effects (Hayes, 1994).

Blood is general turn used to describe a fluid that is circulated within the body to transport gases and other nutrient or waste products. It's often the source of fluid and products to be excreted. It is also a storage reservoir for a variety of metabolites. Blood often contains a respiratory pigment, present within blood cells. Respiratory pigments both increase the oxygen carrying capacity of the blood and confer a special relationship between the blood oxygen content and pressure of oxygen. The blood of most animals contains circulating cells, called blood cells or hemocytes. Some blood cells contain hemoglobin (erythrocytes or red blood cells), these are often present in high numbers to facilitate oxygen transport (Maile, 1972).

Thyroxin (T_4) is the major hormone secreted by the thyroid gland and is at least 25 times more concentrated than T_3. The T_4 levels are commonly used to measure thyroid hormone concentration and to determine thyroid function. Thyroxin stimulates oxygen consumption in most cells and helps regulate both lipids and carbohydrate metabolism. Moreover, thyroxin is the major regulator of food intake and the differentiation and maturation of numerous cell types. In addition, thyroxin (T_4) appears necessary for normal growth and development in the reproductive and nervous systems (Martin, 1985).

The present work was carried out to study the effect of O.P. compound profenofos on haemogram and thyroid gland in male albino rat.

MATERIALS AND METHODS

Insecticide used

Insecticide used was the profenofos (Cord) which belongs to organophosphorus family [O, (4–bromo–2– chloro phenyl) -O- ethyl – S– propyl phosphorothioate] empirical formula, $C_{11}H_{15}BrClO_3PS$ and molecular weight, 373.6 It is formulated in liquid form 72% EC as active ingredient.

Experimental Design

A total of 30 male albino rats weighting 140±10 g were raised in the animal house of Mammalian Toxicology Department, Central Agricultural Pesticide Laboratory (CAPL). The rats were kept under normal laboratory conditions for two weeks before the commencing of

the experiments. The animals were divided into three groups of the thirty animal each. Group (1) was served as control animals. The Group (2) and Group (3) were given the profenofos at concentrations of 75 and150 ppm (equivalent to 1/20 and 1/40 of oral LD_{50}, respectively) daily in drinking water for 90 successive days.

Sampling

Blood samples were collected from orbital sinus vein at the end of each experimental period (30, 60 and 90 days) according to Schalm (1986). Whole blood samples were divided into two aliquots, the first aliquot was collect in tubes containing of EDTA as anticoagulant (1 mg/ml blood) for the hemogram analyses, and the second aliquot was collected in tubes containing the heparin to obtain the plasma which used in hormonal analysis and kept in a freezer at (-20C°) until all assays were carried out.

Hormonal Analysis

Thyroxin (T_4) and triiodothronine levels were determined in plasma according to Britton *et al.* (1975) by the coat –A- count technique, using kits purchased from Diagnostic Products Corporation (DPC).

Haematological analysis

Blood samples were taken for the following hematological studies.

1- Hemoglobin concentration was determined according to the method of Drabkin and Austin (1935).
2- Hematocrit (Packed cell volume) was estimated in percent by the microtechnique method. Whole blood was centrifuged at 12.000 r.p.m. for 10 min; as described by Dacie and Lewis (1984).
3- The erythrocyte and total leukocyte counts were performed according to Schalm (1986).
4- Erythrocyte indices:

Erythrocyte indices were calculated according to Wintrobe (1981) as follows:

A. Mean corpuscular volume (MCV):

$$MCV = \frac{PCV\ (Vol.\%)}{RBCs\ (X10^6/\mu l)} \times 10 = Ft\ (Femtoliters)$$

B. Mean corpuscular hemoglobin (MCH):

$$MCH = \frac{\text{Hb (g/100 ml)}}{\text{RBCs (X10}^6/\mu l)} \times 10 = \text{Pg (Picograms)}$$

C. Mean corpuscular hemoglobin concentration (MCHC)

$$MCHC = \frac{\text{Hb (g/100 ml)}}{\text{PCV (Vol. \%)}} \times 100 = (\%)$$

Histopathological Analysis

Histopathological procedures were done and sections were stained by hematoxylin and eosin Stain according to Harris (1898).

Statistical Analysis

The obtained data were statistically analyzed using the method of Snedecor and Cochran (1982); and t-test was performed to evaluate the difference between mean values of the treated group and those of control group. Values are expressed as mean ± S.E.

RESULTS AND DISCUSSION

1- Effect of profenofos insecticide on body weight and internal organs body weight ratio

Data presented in Table (1) showed a significant decrease in body weight gain after 30,60 and 90day of treatment showing 29%, 36.4% and 44.5% for 150 ppm respectively. While significant decrease in the body weight after 30 days for 75ppm 17.8% showed relative to control individuals.

Table (1): Effect of treatment with different concentrations of profenofos on body weight gain (g) in male albino rats.

Conc.	Treatment Period (days)		
	30	60	90
Control (0 ppm)	43.75 ± 6.25	30.20 ± 2.04	40.00 ± 6.88
%	100	100	100
75 ppm.	7.8 ± 3.08***	14.04 ± 6.30	20.00 ± 8.9
%	17.8	46.5	50
150 ppm.	12.72 ± 1.7**	11.00 ± 4.00**	17.8 ± 1.4***
%	29	36.4	44.5

Each value represents Mean ± SE; ** Significant differences versus control at p <0.01; * Significant differences versus control at p <0.001.**

It's of interest to indicate that the present results are generally similar to those reported by several workers i.e. Chaturvedi (1993) and Undeger *et al.* (2000), noticed that, the body weight gain decreased in the treated groups after 31 and 28 days from treatment. While, Antony *et al.* (1990) and Ahmed *et al.* (2000), reported that, the body weight was increased markedly according to the type of insecticide and the dose level, which used in treatment with different pesticides i.e, pyrethroids (fenpropathrin, alphacypermethrin and fenvalerate), organophosphate insecticide (methyle parathion) and two herbicides, proturon and O-endimethaline. On the other hand, Abd-Ellah (1987) and Shaker *et al.* (1988), they observed that, no significant change in the body weight as a result of synthetic pesticides administration to laboratory animals.

Asignificant decrease in body weights after treatment with profenofos which may be attributed to interfere with metabolic process and or this may be due to pathological lesions in the gastrointestinal tract, which reflected in mal-absorption of nutrients in treated groups with profenofos (Kaneko, 1989).

Data in Table (2) revealed that, rats treated with both concentrations of profenofos had no changes in the weights of different internal organs (liver, kidney, spleen, lung, heart and brain) in male albino rats.

Table (2): Effect of profenofos on internal organs weight (g/ 100 g b.wt) in male albino rats after 90 days from treatment.

Organ	Organ weight at different concentration (ppm.)		
	Control (0 ppm)	75 ppm	150 ppm
Liver	10.29 ± 0.316	9.51 ± 0.466	9.71 ± 0.167
%	100	92.3	94.3
Kidneys	4.76 ± 0.18	4.595 ± 0.179	4.38 ± 0.213
%	100	96.6	92.4
Spleen	3.49 ± 0.16	3.36 ± 0.0186	3.98 ± 0.548
%	100	96.3	114
Lungs	15.78 ± 0.78	16.56 ± 0.56	16.89 ± 0.333
%	100	104.9	107
Heart	3.39 ± 0.04	3.15 ± 0.129	3.36 ± 0.09
%	100	92.9	99.1
Brain	4.24 ± 0.2	4.03 ± 0.093	4.4 ± 0.092
%	100	95	103.7

Each value represents Mean ± SE.

Such results are agreement with those obtained by Cannon and Kimbrough (1979) who mentioned that treatment rats with chlordane (kepone) showed no differences in organs weight, except the liver and kidney. Also the findings of Renner *et al.* (1981), showed, no changes in the internal organ weights following treatment with pentachloro-nitrosobenzene (PCNO) into rats.

2- Effect of profenofos on haemogram in male albino rats

The haematological changes in male albino rats following treatment with profenofos are shown in Table (3). A remarkable elevation in erythrocyte counts (RBCs) associated with increasing in haemoglobin concentration (Hb) and packed cell volume (PCV) was observed in rats treated with profenefos at higher concentration (150 ppm) after 60 and 90 days of treatment. Also, a significant incr2ease in mean corpuscular volume (MCV) and (PCV) values was recorded in rats treated with profenofos at 150 ppm for 30 days and at 75 ppm for 90 days of treatment, respectively. Meanwhile, a significant reduction in Hb concentration and mean corpuscular haemoglobin concentration (MCHC) was noticed in rats treated with lower concentration (at 75 ppm) of tested insecticide after 60 days of treatment Moreovar, a marked decrease in mean corpuscular haemoglobin (MCH)was observed in rats treated with profenofos, at 75 ppm for 90 days. With respect of total leukocyte counts, (WBCs), there was a significant reduction WBCs counts in rats treated with profenofos at both concentrations for 30 days of treatment.

Sub-chronic toxicity of profenofos to male albino rats produced secondary erythrocytosis, which referred to absolute polycythemia, that may be arising from hypoxic hypoxemea. This could be attributed to erythropoietin (EP) stimulation, which in turn, activated the erythroid cell line in bone marrow of treated rats. A significant reduction in Hb concentration and MCHC values was noticed in rats treated with profenofos at 75 ppm for 60 days, this may be due to increase numbers of reticulocytes, incirculating blood in response to anaemia.

A significant increase in PCV values, without increasing of erythrocyte count, may be due to the dehydration in profenofos treated rats (Jain, 1993).

Table (3):Effect of treatment with different concentration of profenofos on haemogram parameters in male albino rats.

Parameters		Control (0.0 ppm)	75 ppm	150 ppm
After 30 days	RBCs ($\times 10^6$ µl)	7.29 ± 0.13	7.025±0.27	6.775±0.23
	%	100	96.3	92.8
	WBCs ($\times 10^3$ µl)	7.3±0.71	4.98±0.36*	5.35±0.33*
	%	100	68.2	73.3
	Hb (g/dl)	16.75±	17.4±1.06	15.65±1.15
	%	100	103.8	93.4
	PCV (%)	54.5±2.63	54.5±1.32	54.33±0.48
	%	100	100	99.6
	MCV (Fl)	72.4±2.8	77.96±2.4	92.62±0.94***
	%	100	107.6	128
	MCH (Pg)	20.95±1.04	19.32±0.33	23.61±3.19
	%	100	92	112.7
	MCHC (%)	33.4±3.33	28.02±2.00	29.31±1.94
	%	100	83.9	87.8
After 60 days	RBCs ($\times 10^6$ µl)	7.18±0.12	7.68±0.25	8.37±0.09***
	%	100	107	116.6
	WBCs ($\times 10^3$ µl)	7.4±0.73	6.62±0.17	8.81±0.85
	%	100	89.5	119
	Hb (g/dl)	16.68±0.34	15.26±0.32*	18.32±0.16**
	%	100	91.5	109.8
	PCV (%)	52.00±0.83	52.00±0.63	60.66±1.42***
	%	100	100	116.7
	MCV (Fl)	68.49±0.23	69.05±3.1	72.46±2.5
	%	100	100.8	105.8
	MCH (Pg)	22.37±0.79	19.53±1.01	2.88±0.34
	%	100	87.3	102.3
	MCHC (%)	33.7±0.7	29.55±0.6**	32.7±2.05
	%	100	87.7	97
After 90 days	RBCs ($\times 10^6$ µl)	7.61±0.21	7.62±0.28	8.42±0.16*
	%	100	100	110.6
	WBCs ($\times 10^3$ µl)	8.61±0.98	7.46±0.25	7.4±0.42
	%	100	86.6	86
	Hb (g/dl)	16.65±0.54	16.33±0.33	18.98±0.21**
	%	100	98	114
	PCV (%)	52.66±1.01	58.33±1.11**	59.00±2.51*
	%	100	110.7	112
	MCV (Fl)	78.73±3.016	74.64±1.26*	73.04±2.88
	%	100	94.8	92.7
	MCH (Pg)	20.91±0.39	15.75±0.29**	21.85±0.63
	%	100	75.3	104.5
	MCHC (%)	31.66±1.73	27.32±0.77	31.8±0.59
	%	100	86.3	100.5

Each value represent Mean ± SE; * Significant differences versus control at $p < 0.05$; ** Significant differences versus control at $p < 0.01$; *** Significant differences versus control at $p < 0.001$

A leukopenia (a decrease in total leuckocyte count) was observed in rats treated with profenofos for 30 days of treatment, this may be due to the lymphopenia, which occurred as a results of lympholysis, consequently to release of corticosteroids as a stress hormone, during exposure to profenofos (Marx, 1996) Meanwhile, a significant increase in MCV values in rats, post – treatment with profenofos at 150 ppm for 30 days, may be arising from swelling of erythrocytes as consequence of releasing of catecholamines(stress hormone) in profenofos treated rats (Soivio and Nikinnmaa, 1981).

3- Effect of profenofos insecticide on thyroid hormones

Data in Table (4) illustrated that treatment rats with the profenofos at 75 ppm produced a significant decrease in the concentration of thyroxin (T_4) and Tri-iodothyronine (T_3) (hypothyrodism) after 90 days of treatment (50.8% and 74.4%) respectively. While, the low concentration induced a marked elevation in the level of T_3 after 60 days of treatment (The increased values 151% for 75 ppm relative to control). Our results confirmed with the histopathological findings in thyroid gland of profenofos treated rats.

Table (4): Effect of Treatment with different concentration of profenofos on thyroxin (T_4) and tri-iodothyronine (T_3) levels (n mol/l) in male albino rats.

Treatments		Thyroxin T_4		Tri-iodo hyronine T3	
		(n mol/L)	%	(n mol/L)	%
After 30 days	Control (0.00 ppm)	58.00 ± 2.73	100	1.133 ± 0.197	100
	75 ppm	62.55 ± 1.206	107.8	1.32 ± 0.03	116.5
	150 ppm	71.38 ± 5.55	123	1.523 ± 0.1	132.7
After 60 days	Control (0.00 ppm)	54.5 ± 3.74	100	0.825 ± 0.123	100
	75 ppm	70.32 ± 6.31	129	1.24 ± 0.07*	151
	150 ppm	55.49 ± 3.35	102	1.095 ± 0. 17	134
After 90 days	Control (0.00 ppm)	51.83 ± 2.48	100	0.842 ± 0.05	100
	75 ppm	26.36 ± 1.46***	50.8	0.625 ± 0.05*	74.4
	150 ppm	51.99 ± 4.03	100	0.934 ± 0.2	110.7

Each value represent Mean ± SE; * Significant differences versus control at $p < 0.05$; * Significant differences versus control at $p < 0.001$**

The results of the present study were in contrary with those found by Abd-Ellah (1987), Hotz *et al.* (1997), Salem *et al.* (1999), Kandil *et al.* (1991) and Akhtar *et al.* (1996), they found that, a significant

decrease of T_4 and T_3 were occurred after administration of different pesticides to experimental animals.

On the contrary, Abd-Allah (1998) on carbosulfan in rats, Porter *et al.* (1993) on aldicarb and methomyl in rats, El-Kashoury (1999) on three pesticides in rats and Farid (1997) on insect growth regulators (IGR) (i.e., diafenthiuron) in rats proved opposite finding. Proved positive findings, they mentioned that, the thyroxine (T_4) and Tri-iodothyronine (T_3) levels were significantly increased after administration with different agro-chemicals by rats.

In this respect; Salem *et al.* (1989) and Ozmen & Okay (1993) they mentioned that, the T_4 and T_3 levels did not alter after treatment with methamidophos and malathion in male albino rats.

The thyroid gland of untreated male albino rats showed the normal histological structure of the acini and the colloid material in their lumenae as shown in Fig. (1). Thyroid gland of male rats treated with 75 ppm of profenofos for 90 days, showed degeneration and desquamation of the lining epithelium of some acini and the acinar lumenae were completely free from the colloid (Figs. 2, 3). While, histopathological examination of thyroid gland of high concentration (150 ppm), showed a few atrophy and colloid free lumen in few acini (Fig., 4).

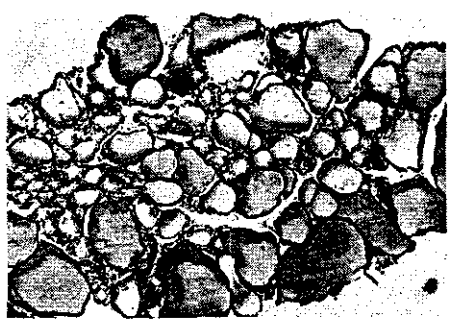

Fig. (1): Section in thyroid gland of untreated male albino rat showing the normal histological structure of the acini and the colloid material in their

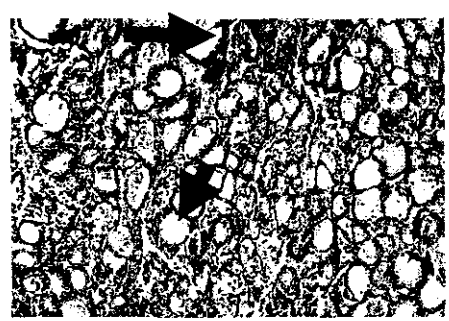

Fig. (2): Section in thyroid gland of male albino rat given 75ppm of profenofos for 90 days showing the acini completely free from the colloid (H&EX40).

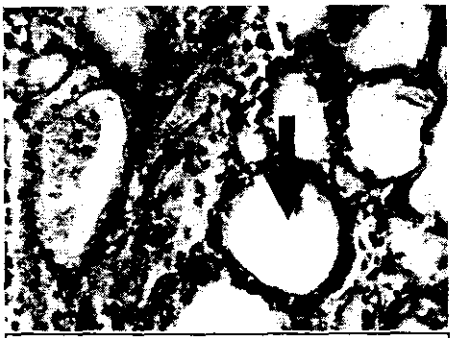

Fig. (3): Section in thyroid gland of male albino rat given 75ppm of profenofos for 90 days showing degeneration and des quamation of the lining epithelium of the acini which completely free from colloid in

Fig. (4): Section in thyroid gland of male albino rat given 150 ppm of profenofos for 90 days showing few atrophy and colloid free acini (H&E X40).

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تأثير المعاملة بالبروفينوفوس على الدم والغدة الدرقية في ذكور الفئران البيضاء

محمد محمود فريد** محمد إبراهيم محمد قبيصى* عمرو عبد الرازق عبدالله**

* قسم الكيمياء الحيوية – كلية الزراعة – جامعة القاهرة.

** قسم سمية المبيدات للتدييات والأحياء المائية المعمل المركزي للمبيدات – الدقي – مركز البحوث الزراعية.

في هذا البحث تم دراسة تأثير الجرعة المنخفضة والعالية (١/٢٠ ، ١/٤٠ من LD_{50} على التوالى) من المبيد الحشرى (البروفينوفوس) على فئران التجارب البيضاء ولمدة ٩٠ يوما فى ماء الشرب يوميا. وتم دراسة كل من تأثير المبيد على وزن الجسم بالإضافة إلى وزن الأعضاء المختلفة (الكبد والكلية والطحال والرئة والقلب والمخ) منسوبا لوزن الجسم. كما تم دراسة تأثير المبيد على مكونات الدم (RBCs و WBCs و Hb و PCV و MCV و MCH و MCHC) بالإضافة إلى تأثيره على هرمونات الغدة الدرقية (T_3 و T_4). أوضحت النتائج الخاصة بالدراسة الفسيولوجية حدوث انخفاض معنوي في الزيادة في وزن الجسم في المراحل المختلفة من المعاملة كما أنه لم يحدث أى تأثير بعد المعاملة بالمبيد على نسبة وزن الأعضاء بالنسبة لوزن الجسم (الكبد والكلية والطحال والرئة والقلب والمخ). كما أوضحت النتائج الخاصة بدراسة مكونات الدم (الدراسات الهيماتولوجية) زيادة معنوية ملحوظة فى كل من عدد كريات الدم الحمراء والهيموجلوبين والهيماتوكريت بينما حدث نقص معنوي ملحوظ فى كريات الدم البيضاء ودلائل كريات الدم الحمراء (MCV و MCH وMCHC). أما بالنسبة للدراسة النسيجية (هستولوجية) الخاصة بالغدة الدرقية أوضحت النتائج إنه مع التركيز المنخفض حدث تدمير شامل فى الخلايا أو الأنسجة المبطنة للحويصلات والتي لها دور كبير فى تخليق هرمونات T_3 و T_4 ومن ثم أدى ذلك إلى خلو هذه الحويصلات من مادة الـ Colloid والتى تعتبر المصدر الأولى التى يخلق منها الهرمونات الخاصة بالغدة الدرقية (T_3 و T_4) كما حدث نفس التأثير بالنسبة للتركيز العالى من المبيد ولكن لوحظ أن التركيز المنخفض من المبيد كان تأثيره أكثر ضرراً من التركيز العالى.