J.Pest Control & Environ. Sci. Vol: No:1 pp 1-15 (1993)

EFFECT OF THE ORGANOPHOSPHATE, CYANOPHOS ON SOME BIOCHEMICAL PARAMETERS IN THE LIVER AND BRAIN OF THE MALE ALBINO MOUSE MUS MUSCULES

By

Mohamed F.F. Bayomy* and Amal A. Eisa**
*Zoology Dept. Fac. of Science and **Plant Protection Dept., Faculty of Agric. Menoufia Univ.,
Recived 27/10/1992 Accepted 24/12/1992

ABSTARCT

The effect of the sublethal dose (1/4 LD_{50} value) of the organophosphate, cyanophos was studied in the tissues of liver and brain of male albino mouse, Mus muscules. The results cleared tnat :

1- In the treated mice with either a single dose or repeated one, of tested sublethal dose, there is an exhibited significant decrease in the total hepatic protein compared with the control. The decline was observed in all time points of the experiment.

2- The activity of the liver enzymes Glutamate Oxaloacetate Transaminase and Glutamate Pyruvate Transaminase (GOT, GPT) and alkaline phosphatase was noticed to be decreased.

3- Little decrease in the amount of brain protein content was observed with no significant difference compared with the control at all time intervals in case of both the single and repeated dose.

4- The treatment has led to a significant decrease in the activity of brain enzymes (GOT, GPT and acetyl choline esterase) at 24 hr after intoxication and there after till 192 hrs.

INTRODUCTION

The organophosphorus (OP) compounds are still the most abundant class of chemicals in large scale use as pesticides. But, the unwise use of synthetic toxicants, created problems more serious than those for which they were originally applied. Adverse impact on nontarget organism is one of those problems.

Persons frequently exposed to agricultural poisons either those who work with agricultural pesticides during manufacturing, preparation for use, storage or application in addition to those who come in contact with these chemicals accidentally,the cases which can be faced either through improper storage, by entering sprayed areas, or by eating sprayed foods from which spray residues have not been removed. Subsequently, prevention of intoxication requires adequate knowledge of the hazardous properties of substances and hence basic research on physiological, biochemical, pharmacological and toxicological effects of these compounds are prerequisite.

Fortunately, there is a growing international concern about the potential hazards to human and animal health caused by toxic residue after single exposure to pesticides and after the repeated exposure to such compounds as we have done in this investigation. However, information concerning currently used pesticidal effects on human is limited and therefore, these studies are urgently needed to provide data in order to give a chance for using relatively safe pesticides in a large scale application.

Some biochemical targets were found to be linked by some insecticides (Satoh and Moral, 1973), as for example :
acetylcholinesterase which is regarded as a target of organophosphorus pesticides and hence called anticholinesterases (Burgen and Mitchell, 1978). Also, determination of proteins has been shown to be sensitive indicator for the disturbances in liver (Bruin and Zielhuis, 1967; Eisa and Bayomy, 1992). Meanwhile, measurements of certain enzymes may be great value, although their activities may depend upon different factors including rate of synthesis, degradation and liberation of enzymes from cells due to increased enzyme synthesis which perhaps associated with regeneration (Baron, 1984).

The present study was intended to investigate the biochemical targets in male albino

mice that can be affected by the organophosphate, cyanophos. The study includes determination of total proteins and aminotransferase enzymes, GOT and GPT, in both the liver and brain and also activity of alkaline phosphatase in the liver as well as brain acetylcholinesterase activity after animals exposure to single or repeated doses of the pesticides.

MATERIALS AND METHODS

Experimental animal :

Adult male albino mice (Mus musculus) were used in this study. The animals were of the same age (2 months) and of 20-25 gm body weight. The animals were obtained from a strain reared in the laboratory of plant protection Department, Faculty of Agriculture, Shebin El-Kom, under normal laboratory conditions. Balanced diet and tap water were provided ad. libitum.

Insecticide and route of administration :

The organophosphate, cyanophos, (O-4 cyanophenyl O,O, dimethyl phosphorothioate) introduced as 50 % E.C by Sumitomo chemical Co., Japan, was used in this study. The compound was administered via intraperitoneal injection.

Experimental design :

1- Single dosage :

The animals were randomly divided into 4 groups in addition to a control one of 10 mice each. The control animals received physiological saline solution only while experimental ones received a single dose equals to 0.25 of the LD_{50} (190 mg/kg body weight) value which was obtained from a mortality curve drawn previously by the authors elsewhere (Eisa and Bayomy, 1992). Treated animals were decapitated by cervical dislocation at 24, 48, 96 and 192 hrs post exposure.

2- Repeated dosages :

Animals of this experiment were divided into 3 groups which were treated with 0.25 of the LD_{50} (190 mg/kg) for 1, 2 or 4 days respectively. The animals were sacrificed at 24 hrs after the respective last dose of each group. A control group were injected with physiological saline solution in order to balance the effect of injection.

Tissue sampling and preparation :

At the time of sacrifice, animals were dissected where liver and brain were excised, blotted with filter papers, placed in petri dishes and kept at - 20 °C until analyzed within a week.

Tissues were homogenized in 9 volumes of distilled water using a motor derived glass homogenizer in an ice bath. The homogenates were centerifuged (under cool 5 °c) at 3000 xg for 15 minutes and the supernatant was used for biochemical determination after suitable dilution with the same buffer.

Statistical analysis :

Data were presented as means ± standard deviation (S.D.) either in tables or figures. Significance of the changes from control values was estimated using student's t-test according to Hine and Wetherill, (1975).

Biochemical analyses :

Total protein content was determined by Lowry method (Lowry et al., 1951) using bovine serum albumin as standard. Aminotransferases (GOT and GPT) were determined according to Reitman and Frankel, (1957) using Boehringer Mannheim kits. Alkaline phosphatase activity was assyed by the method of Kind and King (1954). Cholinesterase activity was assayed in brain of all animals according to the method of Elman et al. (1961).

RESULTS AND DISCUSSION

Liver

The results presented in table (1) clearly indicate that treated mice with a single dose of cyanophos exhibited significant decrease in their hepatic total proteins compared with their respective controls especially 24 and 48 hrs after treatments whereas animals resumed their normal hepatic protein contents at 96 and 192 hrs post exposure which indicate their ability to recover by time upon cessation of the treatment.

Generally, protein inhibition is considered undesirable adverse effect or one of the important symptoms of cytotoxicity because of the diversion that induces from the normal rate or equilibrium state. Meanwhile, Sharma et al. (1976) have reported similar trend of effects, observed in our study, when they introduced dieldrine to mallard ducks. The decreased protein content has been interpreted, by Baron (1984), as a result of chronic liver disease when large number of parenchymal cells have been destroyed. In this context it is worthy to mention that repeated dosages, in this work, has also led to a significant decline in total hepatic proteins in all time points of the experiment (Fig. 1).

The results compiled in table (1) also showed that assayed enzymes activities were generally affected in livers of the treated animals as compared to their controls.

As for the enzyme, GOT its activity decreased significantly at 48 hr post treatment only when the activity was expressed as u/g wet tissue while GPT activity affected earleir, i.e after 24 after intoxication.

These transaminases, GOT and GPT, are regarded as key enzymes known for their role in utilization of protein and carbohydrates since they are concerned with the biosynthesis and degradative processes of the glutamic acid and

Table (1) Effect of a single dose of cyanophos administrated by intraperitoneal injection on liver enzymes (GOT, GPT and alkaline phosphatase) and total protein.

Parameter	Control	24-hr	48-hr	96-hr	192-hr
Total proteins[1]	214.83±5.42	185.76±17.69 **	198.57± 8.84 *	220.83±14.22	220.98±18.97
GOT[2]	7.46±0.21	7.15± 0.36	6.87± 0.37 *	7.29± 0.39	7.36± 0.15
GOT[3]	34.46±0.97	38.86± 4.75	34.52± 1.43	33.05± 1.47	33.49± 2.51
GPT[2]	22.13±1.64	17.64± 1.09 **	19.24± 2.37	19.25± 2.30	21.87± 1.18
GPT[3]	103.64±8.70	95.49± 9.91	97.06±12.76	87.70±13.64	99.23± 7.36
Alk.Ph[2]	1.32±0.08	0.73± 0.09 **	1.10± 0.13 *	0.79± 0.11 **	1.11± 0.18 *
Alk.Ph[3]	6.17±0.37	3.96± 0.77 **	5.51± 0.46 *	3.57± 0.35 **	5.07± 1.17

1- Total protein (mg/g wet tissue)
2- Concentration calculated as U/g wet weight of the liver tissue.
3- Concentration calculated as U/g protein
Values represent means ± S.D. * P < 0.05 ** P < 0.01 n = 5
Alk.Ph (Kind & King Units), one Kind & King Unit is that amount of enzyme which in the given conditions liberates 1 mg of phenol in 15 minutes at 37 °C.

Table (2) Effect of a single dose of cyanophos administrated by intraperitoneal injection on brain total protein and enzymes (GOT, GPT and acetylcholinesterase)

Parameter	Control	24-hr	48-hr	96-hr	192-hr
Total proteins[1]	82.56±9.81	77.21±7.08	78.62±0.36	74.57±12.39	74.90±11.55
GOT[2]	6.43±0.32	5.80±0.30 *	6.04±0.14 *	5.98± 0.17 *	5.81± 0.35 *
GOT[3]	78.50±6.23	75.49±5.85	77.64±9.75	82.22±15.44	79.10±13.39
GPT[2]	0.84±0.17	0.33±0.05 **	0.56±0.04 *	0.62± 0.05 *	0.77± 0.11
GPT[3]	10.07±1.11	4.67±0.88 **	7.13±1.45 **	8.50± 1.38	10.19± 1.55 **
AChE[2]	2.78±0.24	1.88±0.17	2.11±0.19	2.41± 0.22	1.76± 0.14
AChE[3]	29.02±5.03	24.07±1.83	26.64±4.18	33.06± 4.33	23.97± 3.16

1- Total protein (mg/g wet tissue)
2- Concentration calculated as U/g wet weight of the brain tissue.
3- Concentration calculated as U/g protein
Values represent means ± S.D. * P < 0.05 ** P < 0.01 n = 5
The units represent enzyme activities of GOT, GPT and AchE are calculated according to Boehringer Mannheim company.

other keto acids and ammonia (Meister, 1965) and therefore, changes in their activity especially in the liver, may have a role in the disorder occurred in the metabolism of amino acids and hence proteins.

Similar results in transaminases were also demonstrated by Abston and Yarbrough (1974 and 1976) as they reported transaminases depressed significantly in rats fed merix. Likewise, results documented by Kondela et al. (1972) following oral administration of endrin to pheasants and those recorded by Kadota et al. (1976) using permethrin in rats were also compatible with results reported herein.

Regarding the effect of repeated administration of the pesticide, it was clearly evident that both transaminases assyed were significantly less active when their activities were expressed per gram wet tissue while GPT only exhibited this behaviour when the activities were expressed per gram protein and this is likely due to decreased protein content documented in this work. Similarly, the activities of both aminotransferase enzymes were also found to be significantly inhibited in the liver after acute and such chronic oral and dermal application of the organophosphorus insecticide, Rogor in the rat (El-Elaimy et al., 1988a).

Activity of liver alkaline phosphatase was noticed to be profoundly decreased following either single or repeated dosage (table 1 and Fig. 1) which may be due to the direct binding of the test compound with the enzyme protein or due to toxic effects produced in tissue leading to decreased synthesis of enzyme protein as has been declared by Sastry and Sharma (1979).

On the other hand, alkaline phosphatase was reported to have a significant role in cellular proliferation and differentiation (Karasaki 1975) and as a regulator in gene transcription (Hwang and Cough, 1976) in addition to the role of phosphatase as detoxifying enzyme for

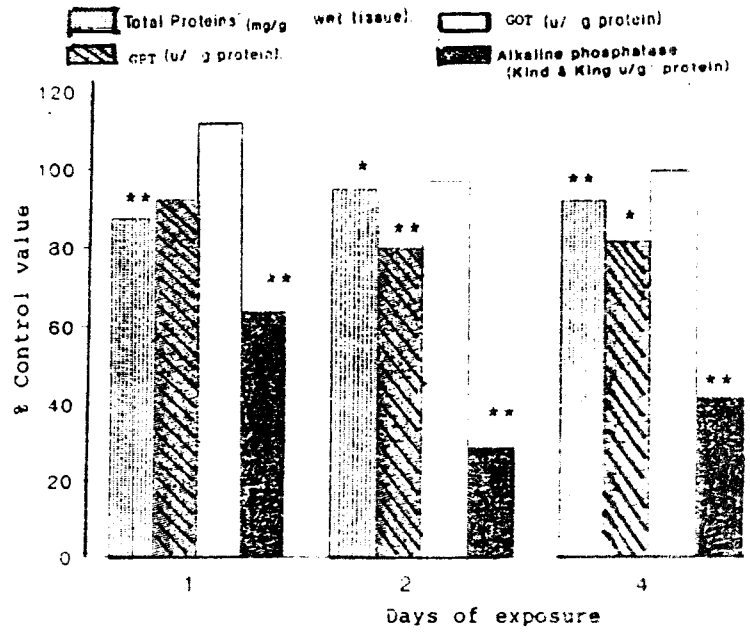

Fig. (1) Percentage of the control values of protein content enzymes activities (transaminases and alkaline phosphatase) in hepatic tissue after daily injection of cyanophos with 1/4 LD_{50} value (190 mg/kg).

* P < 0.05 ** P < 0.01 n = 5

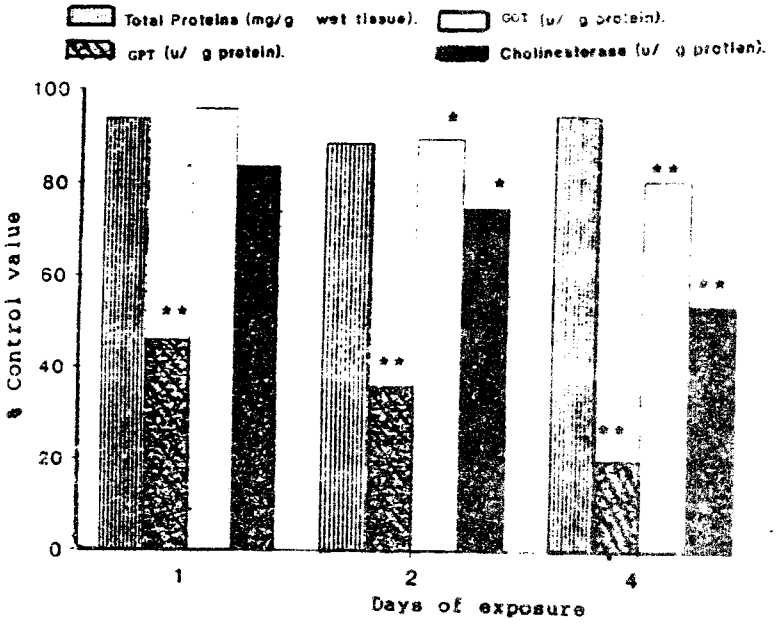

Fig. (2) Percentage of the control values of protein content enzymes (transaminases and acetylcholinesterase) in brain tissue after daily injection of cyanophos with 1/4 LD_{50} value (190 mg/kg).

* P < 0.05 ** P < 0.01 n = 5

organophosphorus compounds (Moustafa et al., 1990).In this context, the present data confirmed cyanophos teratogenic effect in albino mice, as it has been reported before by Murphy, (1965). Recently, El-Elaimy et al. (1988b) have reported similar inhibiting effect on hepatic alkaline phosphatase using another organophosphorus member, Rogor (dimethoate).

Brain

Data presented in table (2) show the effect of a single dose of cyanophos on the brain total proteins, aminotransferases enzyme, as well as acetylcholinesterase activity in male adult mice. Little decrease in the amount of protein content was observed but with no significant difference compared with controls at all time interval monitored. Similarly, repeated dosage has induced negligeble decrease in brain total proteins which suggests that protein synthesis in the brain escapes the effect of cyanophos due to blood-brain barrier which protect or decrease effect of many toxicants including pesticides on the brain. On the other hand, the relative resistence of the brain may indicate that this pesticides is relatively safe in contrast to dieldrin which induced severe decline in brain protein content in the rat (Ahmed et al., 1986) due to single and repeated injection of the pesticide.

On the other hand, the treatment has led to a significant decrease in GOT activity even after 192 hr post exposure when the activity is expressed per gram wet tissue, however, when protein content of the brain was taken into consideration the decrease in GOT activity at that time was insignificant (table, 2).

Similar result was also observed concerning GPT activity, although, the magnitude of inhibition was larger following 24 hr of insecticide injection when enzyme activity was expressed either per gram wet tissue or per gram protein, but recovery has been obtained by the end of the follow up period in contrast to GOT.

Concerning the effect of repeated administration of cyanophos on both aminotransferase enzymes, activities were noticed to be depressed markedly at all time intervals tested when enzyme activities were expressed either per gram tissue or per gram proteins (Fig. 2). These results coincided with results of some authors using different insecticides (Enan et al. 1982; El-Elaimy et al., 1988b).

The O.P. compounds are known to exert substantial alterations in certain parameters indicative of neurotoxic and hepatotoxic actions which are dose-dependent (Abdel-Massih, 1977; Stevens and Michael, 1982; Ahmed et al., 1986 and Eisa and Bayomy, 1992). One of these important parameters is the acetylcholinesterase enzyme which is responsible for the degradation of the most general neurotransmitter, acetylcholine that can be either excitatory or inhibitory according to the receptor (Prosser, 1991) and normal level of the enzyme activity granteed the hydrolysis of the neurotransmitter one it performed its action otherwise had side effects would have been occurred.

The present data (Table 2 and Fig. 2) have clearly indicated marked significant decrease in acetylcholinesterase activity at 24 hr after intoxication and there after till 192 hr following a single dose when enzyme activity was expressed per gram wet tissue whereas repeated dosage has induced highly significant decrease in the 2 levels of enzymatic expression.

The decrease in enzyme activity reported herein may result from the inhibiting action of the pesticide or due to partial decreased de novo synthesis as evidenced by the relative decrease in brain protein content observed in this study, although it is difficult to have a direct practical support of such a claim since the enzyme comprises only a minor fraction of the total protein content (Lai et al. 1981).

Other studies have demonstrated sever inhibition of ACHE following intoxication with different insecticides including organophosphates in various animals (Abdel-Massih, 1977; Ahmed et al., 1986; El-Elaimy et al., 1988a and Ahmed et al., 1990).

In conclusion, the general inhibition of different enzymes assyed in this paper could be due to damage of liver as well as extrahepatic tissues which is much evident in repeatedly dosed-animals as a result of relatively cumulative property O.P. compounds (Amr, 1990) leading finally to functional impairment as has been indicated by a preceding work reported by Eisa and Bayomy, (1992).

REFERENCES

Abdel-Massih, V.F. (1977) Thesis submitted for M.Sc. degree, Cairo University.

Abston, P.A., and J.D. Yarbrough (1974) In vivo effects of dietary mirex on hepatic lache dehydrogenase and glutamic oxaloacetic transaminase levels of rats. J. Agr. food chem., 22(1) : 66 - 8.

Abston, P.A., and J.D. Yarbrough (1976) The in vivo effects of mirex on soluble hepatic enzymes in the rat. Pestic. Biochem. Physiol., 6(1) : 192-99.

Ahmed, N.A.; S.M. Rawi, and M.H. El-Behary, (1986) Effect of dieldrin injection on the level of certain amino acids and some enzymes in rat brain. Comp. Biochem. Physiol., 85 c; 437 - 442.

Ahmed, N.A.; N.M.M. Radwan and S.M. Rawi, (1990) The sensitivity of cholinesterase to four insecticides in different organs of frog. Proc. Zool. Soc., A.R. Egypt, 18:71-85.

Amr, M.M. (1990) Project of pesticide Intoxication - Egypt. Kasr El - Aini Faculty of Medicine, Cairo Univ., Egypt.

Baron, D.N. (1984) "A short textbook of chemical pathology" Fourth Edition, E.L. B.S., Great Britain, 292 p.

Bruin, A.D., and R.L., Zielhuis, (1967) "Vroege Diagnostick in De Bedriifsgeneeskunde Met Behulp Van Biochemische Methoden". T. Soc. Geneesk, 45:128.

Burgen, A.S.V. and J.F. Mitchell, (1978) Cholinergic system In : Gaddum's Pharmacology. P.79:, Oxford Univ. Press, Oxford.

Eisa, A.A. and M.F.F. Bayomy, (1992) : Changes in blood chemistry of albino mice due to induced intoxication with the organophosphorous pesticide cyanophos. J. Pest Control Environ. Sci. 4 (2):135 - 150.

El-Elaimy, I.A.; I.M. Al-Sharkawi and M.F. Bayomy, (1988a): Intoxication potentialities of oral and dermal applications of some pesticides. I-Effect on cholinesterase and transaminases enzymes. 13th Int. Cong. Stat., Comput. Sci. Soc. and Demog. Res., pp. 109-128.

El-Elaimy, I.A.; I.M. Al-Sharkawi and M.F. Bayomy, (1988b) Intoxication potentialities of oral and dermal applications of some pesticides. II- Alterations in Gloucose-6- phosphatase, alkaline phosphatase and 5'- nucleotidase activites. 13th Int. Cong. Stat. Comput. Sci. Soc. and Demog. Res., pp. 149-178.

Elman, G.L.; K.D. Courtney; V.J.R. Andress and R.M. Eeatherstone (1961): A new and rapid colourimetric determination of ACHE activity Biochem. Pharmacol., 7 : 88 - 95.

Enan, E.E.; A.H. El-Sebae, O.H. Enan, and S. El-Fiki, (1982). In vivo interaction of organophosphorus insecticides with different biochemical targets in white rats. J. Environ. Sci. Health., 17 : 549 -570.

Hine, J. and G.B. Wetherill, (1975). A programmed Text Statistics. Book Three. The t-test and X^2 Goodness of Fit. Shapman and Hill, London.

Hwang, K.P. and T.A. Cough, (1976) IARC. Sci, Publ., 20 : 309.

Kadota, T., Y. Okumo; H. Kohdo; and J. Miyamoto (1976) : Mammalian toxicological study of permethrin, 3. phenoxy-benzyl(I) cis, trans-2,2 dimethyl-3- (2,2-dichlorovinyl) - cyclo-propane-1-carboxylate. Botyukagoku, 41 : 143-51.

Karasaki, S. (1975) Cell proliferative and subcellular localization of alkaline phos-phatase activity in rat liver parenchyma during azo-dye carcinogenesis. Cancer Res., 35 - 482.

Kind, P.R.N. and E.J. King, (1954). Estimation of plasma phosphatase by determination of hydrolysed with amino-antipyrine. J. Clin. Pathol., 7 : 322.

Kondela, K., E.Q. Sova; A. Vebenska; J. Houska; A. Hrdinova; Z. Nemec; J.J. Janda; and V. Pujman (1972) : The effect of small and large dose of endrin on biochemical indexes in pheasants, Biol. Chem. VYZ. Zvivat, 8(1) : 47 - 57.

Lai, J.C.K.; T.K.C. Leung, and L. Lim, (1981) J. Neurochem 36 : 1443; Cited from : I. Silman (1984) : Molecular properties and biosyn-thesis of acetylcholinesterase. In:Molecular Biology Approach to the Neurochemistry. [Ed: H. Soreq] pp. 53-57. John Wileyq Sons Ltd. Chichester.

Lowry, O.H.; N.J. Rosenbrough; A.L. Farr, and R.J. Randall, (1951) : Protein measurement with the folin phenol reagent. J. Biol. Chem., 193 : 265 - 275.

Meister, A. (1965) : Biochemistry of amino acids. Vol. I; Academic Press Inc., New York.

Moustafa, F.I.; M.S. Shawir, and A.A. Nomeir, (1990) : Enzymes involved in organophosphorus resistence. Proc. Int. Conf. Env. Haz. Agrochem. 2:968-980.

Murphy, S.D. (1965). : Enzymes and glycogen in livers of rats subjected to two stress. Toxicol. Appl. Pharmacol., 7 : 942.

Prosser, C.L. (1991) : Excitable membranes; Synaptic transmission and modulation. In: Neural and Integrative Animal Physiology. [Ed : C. Ladd Prosser] pp. 1-66. Wiley-Liss, Inc., New York.

Reitman, S. and S. Frankel, (1957). :A colorimetric method for the determination of serum glutamic oxaloacetic pyruvic transaminases : Am. J. Clin. Pathol., 28 : 56 - 63.

Satoh, T. and K. Moral, (1973) : Comparative studies on the inhibition of liver amidases, aminopeptidases and serum cholinesterases by EPN. Toxicol. Appl. Pharmacol., 25:553-559.

Sastry, K.V. and S.K. Sharma (1979) : In vivo effect of endrin on three phosphatases in kidney and liver of the fish (Ophiocephalus punctatus). Bull. Environm. Contam. Toxicol 21 (1/2) : 185 - 89.

Sharma, R.p., D.S. Winn, and J.B. Low (1976) Toxic, Neurochemical and behavioral effect of Dieldrin exposure in mallard ducks. Bull Environm. contam. Toxicol., 5(1): 43-53.

Stevens, K.R. and A.G. Michael, (1982): Practical considerations in the conduct of chronic toxicity studies. In:Principles and methods of toxicology. [Ed : A.W. Hayes] p. Raven Press, New York.

تأثير المبيد الفوسفوري سيانوفوس على بعض القياسات البيوكيميائية في كبد ومخ الفأر الأبيض

تم حقن ذكور الفار الأبيض في الفراغ البريتوني بجرعة من السيانوفوس مقدارها ١٩٠ مجم / كجم من وزن الجسم (ربع الجرعة النصفية) وتم دراسة التأثير على بعض القياسات البيوكيميائية في المخ والكبد واتضح من الدراسة مايلي:

في الكبد :-

١- عند المعاملة سواء بجرعة واحدة أو عند تكرار الجرعة حدث انخفاض معنوي في كمية البروتين الكلي عند كل الفترات التي أخذت عندها العينات بعد المعاملة.

٢- حدث انخفاض في نشاط أنزيمات GOT , GPT والفوسفاتيز القلوي.

في المخ :-

١- حدث انخفاض ولكنه غير معنوي في كمية البروتين عند الفترات التي أخذت عندها العينات.

٢- حدث انخفاض في نشاط أنزيمات GOT , GPT والاستيل كولين أستريس في المخ بعد ٢٤ ساعة وحتى ١٩٢ من المعاملة.