

**CHANGES IN BLOOD CHEMISTRY OF ALBINO MICE DUE  
TO INDUCED INTOXICATION WITH THE  
ORGANOPHOSPHORUS PESTICIDE CYANOPHOS.**

By  
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**ABSTARCT**

The present study examines the toxic effects of single as well as repeated sublethal doses of the organophosphate, cyanophos (animals' were injected intraperitoneally with 1/4 LD<sub>50</sub> value once daily over 1, 2, or 4 days) on male adult albino mice (Mus musculus). Animals received the single dose were decapitated at 24, 48, 96 and 192 hrs post exposure, while those treated repeatedly were sacrificed at 24 hrs after the last injection.

Results indicate the occurrence of subtle decline in serum albumin in both cases of treatment whereas both globulins and total proteins exhibited escalation, particularly during the follow-up period for the single dose. Also, this effect vigorously potentiated by elongating duration of administration. The foregoing alterations resulted in a severe disturbance of the albumin/globulin (A/G) ratio where it underwent highly significant decrease in almost all treated groups as compared with their respective controls.

Elevation in activity of aminotransferase enzymes (GPT and GOT) has occurred especially after dosage, whereas alkaline phosphatase was inhibited markedly under the same conditions. This study suggests that the severity of the toxic effect of repeated exposure is duration dependent whereas the toxic action of the single dose is relatively reduced within 8 days post-treatment.

## INTRODUCTION

Pesticides used in plant protection should effectively kill the pests and cause little or no harmful effect to non-target individuals. Unfortunately, this is not the case, therefore, a number of WHO reports (1985) have voiced concern about the potential risk of increased production and use of pesticides at a rapid rate all over the world, especially developing countries.

In Egypt, as in many other countries, the continuous use of pesticides became an ever increasing problem that takes part in the environmental pollution since Egypt alone, as has recently reported by Amr (1990), consumes 1-2 % of the world wide production of pesticides.

Several pesticides at their recommended methods of application may cause great hazardous toxic side effects on living organisms (Sherif et al., 1990). Moreover, pesticides may have persistent occurrence in the environment and/or accumulation may take place in different tissues of animals (Eto et al., 1980) and human (Smally et al., 1968 and Robens, 1969), especially the liver which is particularly concerned with metabolism of toxic and foreign substances entering the body. As a matter of fact, pesticides induce serious side alterations in some key enzymes such as aminotransferases (GPT and GOT) as well as alkaline phosphatase which have prognostic and diagnostic significance (Zietsow 1971 and Coles, 1974) in evaluation of adverse health effects. According, this investigation was conducted to detect activities of the above mentioned enzymes in addition to some specific protein fractions in blood serum of adult male albino mice (Mus musculus) after exposure to single or repeated doses of the organophosphate, cyanophos.

## MATERIAL AND METHODS

Pesticide used :

The organophosphate; cyanophos (0-4 cyano-phenyl 0,0. dimethyl phosphorothioate) introduced by sumitomo chemical Co., as 50% EC ltd., (Japan) was used in this study. This compound has other names, S4048 and Cyanox.

#### Probit Mortality Line :

A series of concentrations were prepared in saline solution (0.9 %) and administered to the animals intraperitoneally (i.p). Eight concentrations of the pesticide were ranged from 180 to 1080 mg/kg body weight and each was replicated five times. Five animals were treated with saline solution only and used as a control. The probit line was drawn and the estimated  $LD_{50}$  value was 760 mg/kg (Fig. 1). The data were analyzed statistically according to Finney, (1971).

#### Animal :

Male albino mice (Mus musculus), 25-30 g., obtained from the breeding unit of the plant protection Dept., Faculty of Agriculture, Menoufia Univ., Egypt, were used for undertaking the present experiments. Animals were housed in groups in wired cages under uniform conditions of light and temperature. The animals were provided with balanced diet and water ad. libitum.

#### Experimental Design :

##### 1. Single Dosage :

The animals were randomly divided into 4 groups in addition to a control group of 10 mice each. The control animals received saline solution only while the experimental ones received one single dose equals to 1/4 of the  $LD_{50}$  of organophosphate, cyanophos, intraperitoneally (i.p). Treated animals were decapitated by crevical dislocation at 24, 48, 96 and 192 hrs post-treatment.

## 2. Repeated Dosage :

Animals of this experiment were divided into 3 groups which were treated once a day for 1,2 or 4 days. The pesticidal dose was i.p. injected into the test animal in a magnitude equals to 1/4 of the LD<sub>50</sub> and the animals were sacrificed at 24 hours after the respective last dose of each group. A control group whose animals received only saline solution in the same way was used for the comparison.

## Biochemical Analysis :

Total protein content in the serum was determined by biuret method according to Alexander et al., (1985) using bovine serum albumin as standard. Albumin content was determined using commercial kits of Boehringer Mannheim. Serum globulins were quantified by subtracting serum albumin from the total serum proteins. Serum aminotransferases (GPT and GOT) were determined according to the method of Reitman and Frankel, (1957) using Boehringer Mannheim kits. Serum Alkaline phosphatase activity was assayed by the method of Kind and King (1954).

## Statistical Analysis :

Data are presented as means  $\pm$  standard deviations. Student's t-test was used to evaluate the statistical significance of the results according to Hine and Wetherill, (1975).

## RESULTS

### Serum proteins :

#### A- Effect of a single dose :

The data obtained from the quantitative of some serum protein fractions in response to i.p. injection of a single dose (1/4 LD<sub>50</sub>) of the organophosphate, cyanophos during a follow-up period reached 192 hours are presented in Table 1

Table (1) Effect of a single dose (1/4 LD<sub>50</sub> value) of cyanophos on the blood serum proteins of male albino mico.

| Parameter      | Hours post-treatment |                           |                           |             |                           |
|----------------|----------------------|---------------------------|---------------------------|-------------|---------------------------|
|                | Control              | 24-hr                     | 48-hr                     | 96-hr       | 192-hr                    |
| Albumin        | 3.054±0.052          | 2.978±0.051 <sup>*</sup>  | 3.050±0.085               | 3.083±0.072 | 2.813±0.163 <sup>*</sup>  |
| Globulins      | 2.909±0.063          | 3.122±0.026               | 4.595±0.491 <sup>**</sup> | 2.738±0.394 | 3.366±0.092 <sup>**</sup> |
| A/G ratio      | 1.050±0.014          | 0.958±0.068 <sup>**</sup> | 0.644±0.052 <sup>**</sup> | 1.144±0.163 | 0.836±0.061 <sup>**</sup> |
| Total proteins | 5.963±0.108          | 6.101±0.313               | 7.625±0.556 <sup>**</sup> | 5.821±0.463 | 6.179±0.157 <sup>*</sup>  |

Values represent means ± S.D.

Albumin, Globulin and total proteins expressed as g/dl.

\* p < 0.05 ; \*\* p < 0.01

n = 5

Table (2) Effect of a single dose (1/4 LD<sub>50</sub> value) of cyanophos on the blood alkaline phosphatase and aminotransferase enzymes (GOT and GPT) in male albino mice.

| Parameter                                | Hours post-treatment |                           |                           |              |                           |
|------------------------------------------|----------------------|---------------------------|---------------------------|--------------|---------------------------|
|                                          | Control              | 24-hr                     | 48-hr                     | 96-hr        | 192-hr                    |
| Alkaline phos-<br>phatase <sup>(1)</sup> | 22.738±2.025         | 23.240±1.741              | 17.066±1.688 <sup>*</sup> | 21.738±2.532 | 12.400±2.773 <sup>*</sup> |
| <sup>(2)</sup><br>GOT                    | 39.413±2.625         | 49.033±2.449              | 41.310±2.357              | 38.984±0.659 | 43.411±6.673              |
| <sup>(2)</sup><br>GPT                    | 14.678±2.926         | 24.323±2.969 <sup>*</sup> | 21.226±1.562 <sup>*</sup> | 15.734±1.727 | 14.502±2.899              |

Values represent means ± S.D.

1) Activity is expressed as King and King Units/dl

2) Activity is expressed U/l.

n = 5

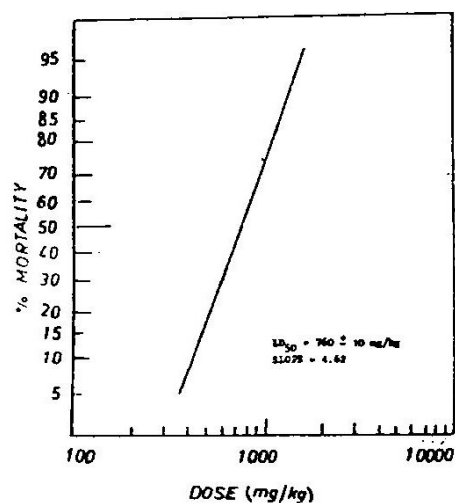


FIG. (1) : LOG PROBIT LINE OF MALE ALBINO MICE TREATED BY INTERAPERITONEALLY INJECTION WITH THE ORGANOPHOSPHATE, CYANOPHOS.

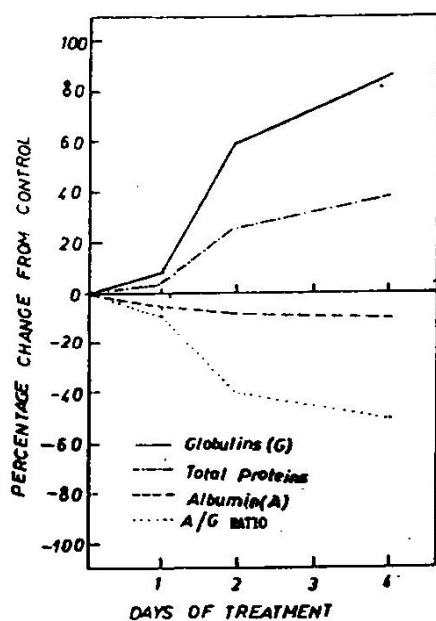


FIG. (2) : EFFECT OF DAILY REPEATED INJECTION WITH SUBLETHAL DOSE (1/4 LD<sub>50</sub> VALUE) OF THE ORGANOPHOSPHATE, CYANOPHOS ON BLOOD PROTEINS OF MALE ALBINO MICE.

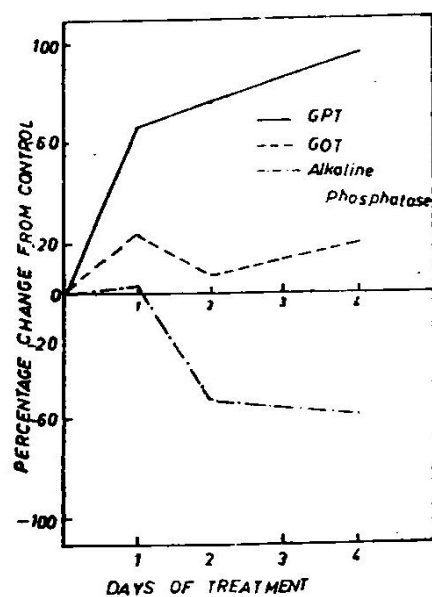


FIG. (3) : EFFECT OF DAILY REPEATED INJECTION WITH SUBLETHAL DOSE (1/4 LD<sub>50</sub> VALUE) OF THE ORGANOPHOSPHATE, CYANOPHOS ON BLOOD ALKALINE PHOSPHATASE AND AMINOTRANSFERASE ENZYMES (GOT AND GPT) OF ALBINO MICE.

The analysis of serum albumin indicated a small but significant decrease in this parameter only at 24 and 192 hrs after injection ( $P < 0.05$ ), while globuline showed nearly the reverse pattern where highly significant increases amounted to 58 and 16 % were recorded at 48 and 192 hrs post-treatment respectively.

Concerning A/G ratio, with the exception of the measurement at 96 hr post-treatment this parameter showed highly significant decreases amounted to 9, 37 and 20 % at 24, 48 and 192 hrs post-treatment respectively ( $P < 0.01$ ).

Total proteins exhibited moderate to small increases at 48 and 192 hrs after treatment, respectively, which were significantly higher than the control value.

#### B- Effect of Repeated Doses

Figure (2) represents the results of the effect of daily exposure of animals to  $1/4 LD_{50}$  for 1, 2 and 4 consecutive days. Serum albumin content displayed a gradual significant decrease reached its maximum decline after 4 days of exposure to the toxicant. On the other hand, globulins underwent progressive elevations which were dependent on duration of exposure amounted 7.3, 59.6 and 86.7 % after 1, 2 and 4 days of pesticide administration respectively.

With respect to A/G ratio, it showed gradual decrease amounted to 9, 40 and 51 % after 1, 2 and 4 days of intoxication respectively ( $P < 0.01$ ) whereas, serum total protein content exhibited progressive elevation reached its maximum magnitude (38 %) after 4 days of pesticide administration.

#### Serum Enzymes :

##### A. Effect of a Single Dose :

With respect to GPT, it showed sharp elevations in response to a single i.p. injection

of the test compound amounted to 66 % and 45 % at 24 and 48 hrs post exposure respectively. This elevation in sGPT was attenuated during the rest of the follow-up period where complete restoration of the normal control level was attained at 192 hrs after exposure.

Similar pattern was also observed concerning sGOT, although the magnitude of alterations was lower and the restoration of the normal value occurred earlier.

Regarding alkaline phosphatase, it showed a statistically significant decreased level compared to the control and only at 48 and 192 hrs post-treatment (Table 2).

#### B- Effect of Repeated Dosages :

In response to repeated dosage, sGPT displayed an abrupt increase in its activity which did further elevate with increasing dosing duration.

Serum GOT exhibited a moderate significant increase amounted to 24 and 20 % only after 1 and 4 days of treatment with the test article respectively.

The effect on serum alkaline phosphatase appeared only after 2 and 4 days of cyanophos injection where the percentage declines from the control level were 52 and 85 % respectively (Fig. 3).

### DISCUSSION

Organophosphorus compounds which are extensively used in agriculture as pesticides leaves a strong biological activity. This effect exceeded the target pests for warm blooded animals, and man and finally present a great risk of acute poisoning. However, the toxic action of these compounds depends largely on their routs or entry into the body (Amr., 1990).



It is evident from the present findings that intraperitoneal administration of cyanophos induced a subtle decrease in serum albumin content which was relatively more pronounced in case of repeated than in single dosage. The reduction of this parameter, under the present experimental conditions might be due to mild damage of hepatic cells that are responsible for the synthesis of albumin with urea (Baucht et al., 1980).

On the other hand, serum globulins showed the quite opposite picture where they increase with both single as well as repeated dosage, the observation which holds true for total proteins in both cases of treatment which might be linked with the animal defence action to counteract toxic effects of cyanophos. The enhancement of globulins as specific protein compounds is not due to increased hepatic protein synthesis since these protein fractions are also produced by extrahepatic tissues although total hepatic protein content was shown to increase in response to intoxication with an organochlorine insecticide, dieldrine in rats (Bhatia et al., 1973).

Examination of the A/G ratio gives a clear evidence of the toxic effect induced by present pesticide where it caused a significant decline in this ratio especially following repeated dosage.

For more identification of the impact of cyanophos on albino mice, our attention was directed towards the assay of activity of some serum enzymes like aminotransferases and alkaline phosphatase.

Aminotransferases are the most relevant liver enzymes whose serum levels are greatly affected in hepatocellular disease, particularly acute liver disease (Harper, 1969, Wilkinson, 1970 and 1976). These enzymes are found in high concentration in the liver (Molander et al.,

1957) and with liver cell damage such as necrosis or abnormal membrane permeability, these enzymes appear with increased level in the blood (Kallai et al., 1964) especially GPT whereas increased blood GOT level also points to myocardial infarction (Wilkinson, 1970 and Garb, 1971).

Cyanophos applied as a single dose, showed a highly significant escalation in sGPT level at 24 hrs post-treatment (+ 66 %) which was still apparent at 48 hrs (+ 45 %) after exposure. Restoration of the normal control level was achieved 96 hrs post-treatment and therefore denoting a complete recovery at 192 hrs post exposure.

The same effect holds true for sGOT level, however, the effect was less pronounced and recovery, as evidenced by serum level of this enzyme, was attained much earlier as compared to sGPT.

Repeated dosage of cyanophos has resulted in an increase in both serum aminotransferase enzymes, however, the effect on sGPT was much pronounced and was also duration-dependent. Our data are in agreement with those recorded by other researchers using different pesticides (Enan et al., 1982; Rawi, 1986, Abdel-Raheem et al., 1987 and El-Elaimy et al., 1988a). Other confirmations of the liver cell damage produced in response to pesticide intoxication came from histopathological alterations reported by other investigators (Atwal, 1973 and El-Eanhawy and El-Ganzuri, 1986) which came in favour of our present findings.

This is not surprising matter since liver is particularly the most susceptible organ to damage by different chemical compounds and foreign materials due to its primary role in the metabolism and its anatomical position as the first site of interaction with ingested substances for their biotransformation (Brodie et al., 1955) and detoxification (Conney, 1967).

Alkaline phosphatase which is thought to have a role in cellular transport (Russell et al., 1972) is also reported to be affected during liver dysfunction (Wilkinson, 1970). The analysis of serum alkaline phosphatase activity under the effect of cyanophos revealed the occurrence of highly significant decreases due to single and repeated dosage. It might be speculated that the toxicant could have induced lesions in the hepatic as well as extrahepatic tissues. These observations do not agree with those recorded by El-Elaimy et al. (1988b) who recorded an increase in serum alkaline phosphatase level in rats after oral or dermal application of organophosphorus insecticide. The possibility exists that the discrepancy between the present results and the previous work on rats may reside in differences concerning species used, route of application or in type of the test article used.

Alkaline phosphatase was also reported to have an important role in cellular proliferation and differentiation (Karaski, 1975) and as a regulator in gene transcription (Hwang and Cough, 1976). In this context the present data confirmed cyanophos teratogenic action in albino mice, as it has been reported before (Murphy, 1965). Recently, Komeil and Abd-Allah (1991) have reported similar inhibiting effect on alkaline phosphatase using another organophosphate member, methyl parathion. It is worthy to emphasise that repeated exposure to this type of pesticide potentiates its toxic effect, whereas the impact of a single dosage attenuates relatively during 8 days after exposure to the pesticide.

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## الملخص العربي

### التغيرات البيوكيميائية في دم الفأر الأبيض بعد المعاملة بالمبيد الفوسفوري (سيانوفوس)

تم دراسة التأثير السام للمبيد الفوسفوري (سيانوفوس) حيث قدرت الجرعة القاتلة لنصف عدد الأفراد المعاملة من نكور الفأر الأبيض ثم عوملت الذكور بربع الجرعة النصفية لمرة واحدة حقنا داخل الفراغ البريتوني وتم أخذ العينات بعد ٢٤ ، ٤٨ ، ٩٦ ، ١٩٢ ساعة من المعاملة وتم تكرار المعاملة مرتين أو أربع مرات وأخذت العينات بعد ٢٤ ساعة من آخر معاملة.

واتضح من النتائج مايلي:

- ١- الجرعة النصفية للمبيد = ٦٧٠ ملليجرام / كيلو جرام من وزن الجسم.
- ٢- انخفضت كمية الألمبيومين في حالة المعاملة مرة واحدة أو عند تكرار المعاملة بينما زادت كمية الجلوبيولين والبروتين خاصة عند المعاملة مرة واحدة.
- ٣- انخفضت نسبة الألمبيومين إلى الجلوبيولين إنخفاضاً معنوياً مقارنة بالحيوانات الغير معاملة.
- ٤- ارتفع نشاط أنزيمي GOT ، GPT وخاصة عند تكرار المعاملة بينما انخفض نشاط أنزيم الفوسفاتيز القلوي في الحالتين.