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# Effect of Organophosphate Pesticide, Nuvan on Serum Biochemical Parameters of Fresh Water Catfish *Heteropneustes fossilis* (Bloch.)

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## Abstract

*In the present study, effects of various concentrations of Nuvan (2, 2 dichlorovinyl dimethylphosphate) exposures on days 7, 15, 30 and 60 for many serum biochemical parameters in the freshwater teleost fish, Heteropneustes fossilis, were photometrically investigated. The 96h $LC_{50}$ value of Nuvan was estimated by using log-dose probit regression line method. On the basis of $LC_{50}$ value, the sub-lethal concentrations were determined as 0.26 mg/L, 0.32 mg/L and 0.43 mg/L which are 1/25, 1/20 and 1/15 of $LC_{50}$ respectively. Well acclimated fishes from both control and treated group were sacrificed after 7, 15, 30 and 60 days and blood samples were collected. Various biochemical parameters such as Serum total Protein, Serum Albumin, Serum Creatinine, Serum Bilrubin and Serum Urea has been studied as diagnostic tools. In general significant effects ($p<0.05$) from different concentrations and time of exposure were observed in exposed fishes. It was found that significant alterations in all the biochemical parameters were dose dependant as well as duration dependent. Results indicated that serum total protein and Serum Albumin decreased significantly where as Serum Creatinine, Bilurubin and Serum Urea increased with increase in Nuvan concentration and time of exposure when compared with control groups.*

**Key words:** *Heteropneustes fossilis*, Biochemical parameters, Nuvan.

## Introduction

Pesticides by their nature are toxic compounds and as such besides controlling pests they also have potentialities of affecting the life and environment adversely. Organophosphate pesticides are finding increasing use in recent years, because of their low persistence, repeated applications of this pesticide are being practiced for the control of pests in agriculture fields and there by large quantities find their way into water bodies. Nuvan (2, 2 dichlorovinyl dimethylphosphate) is one of the organophosphate pesticides extensively used in agriculture practice throughout the world. However, little information is available on its long term effects on blood using biochemical parameters as biomarkers. Pesticides are one of the most potentially harmful chemical liberated into the environment in an unplanned manner. Though they have contributed considerably to the welfare of humans their adverse effect on non-target organism are breathtaking. The major sources of environmental contamination by these chemical are agriculture practices, usage in public health program and industrial discharges[1].

Aquatic pollution due to pesticide needs considerable attention because of its harmful effects on aquatic organisms which may cause fish mortality. The surface run-off from the agriculture lands carries the pesticide into the aquatic ecosystem, which enter the organisms through food webs and also through contact water. Water pollution by pesticides has resulted in the marked increase in the incidences of mass mortality and adversely affects the fish life[2]. Even sub-lethal concentration of pesticide can still cause fish mortality in the exposed population after a sufficiently long time of exposure[3].

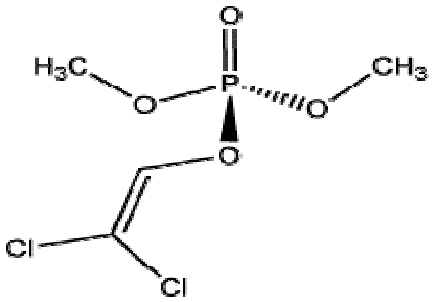
This study investigated the acute and chronic effects of Nuvan on various blood biochemical parameters of freshwater fish, *Heteropneustes fossilis*. This study also aimed to evaluate dose and duration dependent effects of Nuvan in the experimental fish.

## Material and Methods

**Experimental fish:** The healthy and active specimens of *Heteropneustes fossilis* (Singhii) selected as a model for the present investigation were procured from local outlets of river Yamuna in the month of December, when room temperature ranged 18°C-25°C. The mean weight of fishes used was 60 ± 5.

**Maintenance of experimental fish:** The fishes were transferred to the departmental laboratory and were put in to the stock aquarium containing tap water for two weeks so that they become acclimated to the new laboratory conditions. The fishes were carefully analyzed and treated with 0.2% KMno4 solution for 2 minutes before stocking to get rid of any dermal infection. The water was changed every alternate day. Fishes were fed properly with commercial food manufactured by Aerosol Chemicals Private Ltd India. The temperature of the experimental room was maintained constant (21 ± 2˚C) and lighted for 12 hours. The mean weight of fishes used was 60 ± 5. The water used throughout the experiment has pH value of 7.1 ± 0.1 and total hardness of 230 ± 2 $CaCO_3$ mg/L.

**Experimental chemical:** The technical grade insecticides Nuvan (2, 2 dichlorovinyl dimethylphosphate) selected for the present investigation is the trademark of AMVAC Chemical Corporation of India.




**Mean lethal concentration ($LC_{50}$):** The experimental fishes were divided into four groups (A, B, C and D) in four aquaria each contained 20L dechlorinated water. Each group consists of ten individuals. Different concentrations of Nuvan 2 mg, 5 mg, 9 mg and 18 mg were given. The mortality of fishes was noted after 96 hours. The data were analyzed statistically by log-dose probit regression line method[4]. Regression line was drawn on the basis of two variables, log-dose and empirical probit on the simple graph paper to determine the expected probit, necessary for LC50 determination. $LC_{50}$ for 96 hour of Nuvan to *Heteropneustes fossilis* was calculated as 6.45 mg/L as shown in the table 1 and table 2.

**Table-1**
**Percentage mortality of *Heteropneustes fossilis* after 96 hours of treatment with different concentrations of nuvan**

| Group | Concentration Mg/l | No. of fishes | Fish mortality | Percentage mortality |
|---|---|---|---|---|
| A | 2 | 10 | 2 | 10% |
| B | 5 | 10 | 4 | 40% |
| C | 9 | 10 | 7 | 70% |
| D | 18 | 10 | 10 | 100% |

**Table-2**
**Toxicity evaluation of nuvan to *Heteropneustes fosslis* (Bloch.) specifying $LC_{50}$ and regression equation**

| Experimental Fish | Experimental Chemical | Regression Equation | LC50 mg/l |
|---|---|---|---|
| Heteropneustes fossilis | Nuvan (Dichlorovos 76%) | Y = 5.22+3.41 (X-1.38) | 6.4 |

**Determination of sub-lethal concentrations:** After estimating the 96h $LC_{50}$ value, three different sub-lethal concentrations of Nuvan 0.26 mg/L, 0.34 mg/L and 0.43 mg/L which respectively corresponds 1/15, 1/20 and 1/25 of $LC_{50}$ was chosen to study their long-term and sub-acute effect on blood biochemical components.

**Maintenance of fishes:** Three sets of about twenty fishes were kept in three different aquaria each containing 20L test solution. Simultaneously a control set was run parallel to the treated ones. Feeding was stopped two days prior to the commencement of experiments to keep the test animals more or less in the same state of metabolic requirements. Fifteen to twenty control well as treated fishes were sacrificed after 7, 15, 30 and 60 days from each aquarium. Slime and water present on the body surface of the fishes were removed by using blotting paper.

**Blood collection and serum separation:** The blood was collected from their cut caudal vein in to a plain sterilized glass centrifuge tubes. For the separation of serum blood was allowed to clot in the tubes and then centrifuged at 2500 rpm for 30 minutes. The supernatant (serum) was separated and used for estimation of various biochemical parameters such as serum total protein, serum albumin, serum bilirubin, serum creatinine and serum urea.

**Methods of biochemical estimations:** Serum total protein was determined by Biurete method[5], Serum albumin by Dumas method[6]. Serum bilirubin was determined by modified Jendrassik and Grobe's method[7], Serum creatinine by Alkaline Picrate method and Serum urea by Coulambe and Favreen[8].

**Statistical Calculations:** Student's t-test was employed to calculate the significance of the difference between control and experimental means. P values of 0.05 or less were considered statistically significant[9].

## Results and Discussion

Changes in the level of Serum Total Protein in the *Heteropneustes fossilis* are shown in the figure-1. Serum Total Protein decreases significantly ($p< 0.05$) at 0.26 mg/L on 7th, 15th, 30th and 60th day of exposure however highly significant ($p\leq0.01$) and very highly significant ($p<0.01$) decrement of Serum Total Protein was observed at 0.32 mg/L and 0.43 mg/L on every autopsy.

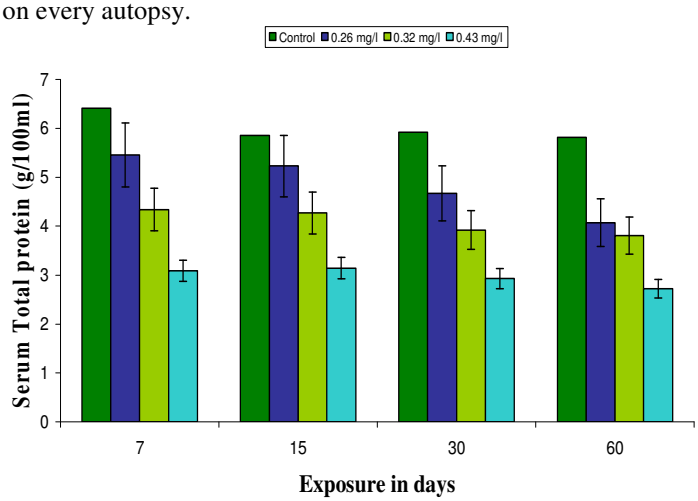
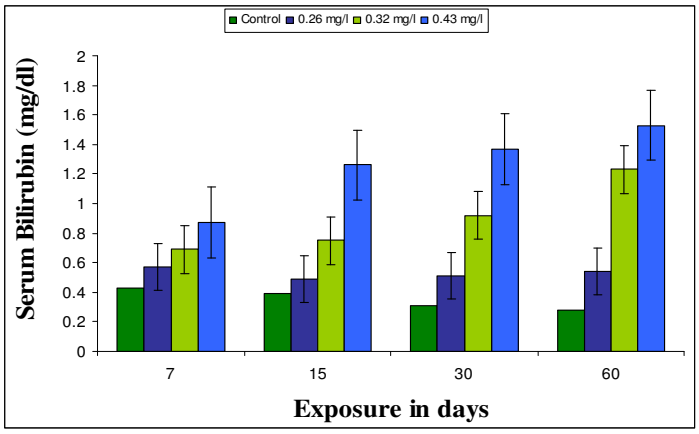



**Figure-1**
**Histogram showing alteration in the serum total protein of *Heteropneustes fossilis* exposed to 0.26 mg/L, 0.32 mg/L and 0.43 mg/L of nuvan for 7, 15, 30 and 60 days.**

Like Serum Total Protein, serum albumin also decreases with increasing concentration of nuvan and duration of exposure

(figure-2). Significant ($p< 0.05$) and highly significant ($p\leq0.01$) changes were observed at 0.26 mg/L and 0.32 mg/L on 7th, 15th, 30th and 60th day of exposure, however at 0.43 mg/L very highly significant ($p<0.01$) fall in serum albumin level was observed.

**Figure-2**
**Alteration in the serum albumin of *Heteropneustes fossilis* exposed to 0.26 mg/L, 0.32 mg/L and 0.43 mg/L of nuvan for 7, 15, 30 and 60 days**




Serum bilirubin in the treated fish showed an increasing trend when compared with the control set (figure-3). There is a significant ($p< 0.05$) elevation of serum bilirubin at 0.26 mg/L on 7th, 15th, and 30th day of exposure however at the same concentration there is highly significant ($p\leq0.01$) increment of serum bilirubin on 60th day of exposure. At 0.32 mg/L there is highly significant ($p\leq0.01$) enhancement of serum bilirubin on 7th and 15th day of exposure, however there is very highly significant elevation of the same on 30th and 60th day of exposure. At 0.43 mg/L there is very highly significant ($p<0.01$) elevation of serum bilirubin almost at every autopsy in the treated fish when compared with the control set.

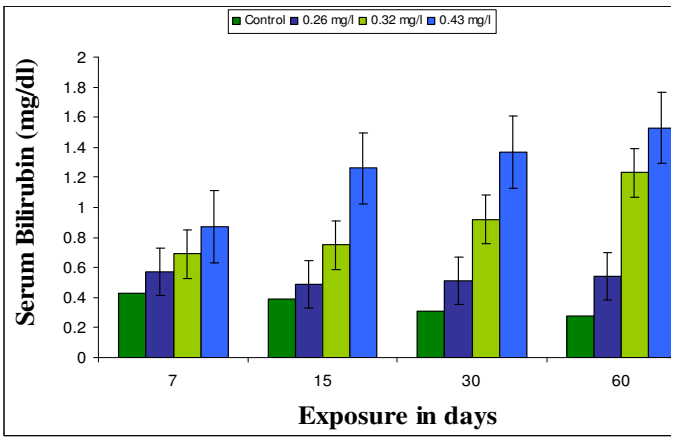



**Figure-3**
**Alteration in the serum total bilirubin of *Heteropneustes fossilis* exposed 0.26 mg/L, 0.32 mg/L and 0.43 mg/L of nuvan for 7, 15, 30 and 60 days**

There is a remarkable elevation of serum creatinine and serum urea in the treated fish when compared with the control sets (figure-4 and figure-5). Serum creatinine and serum urea increases significantly ($p< 0.05$) at 0.26 mg/L on 7th, 15th and 30th day of exposure, however at the end of experiment there is a highly significant ($p\leq0.01$) elevation of the parameter. At 0.32 mg/L and 0.43 mg/L there is very highly significant ($p<0.01$) elevations observed almost throughout the experiment as shown in the respective figures.

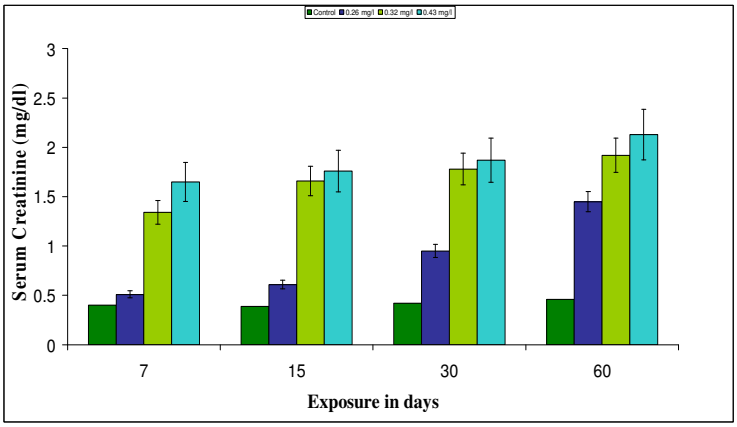



**Figure-4**
**Alteration in the serum creatinine of *Heteropneustes fossilis* exposed 0.26 mg/L, 0.32 mg/L and 0.43 mg/L of nuvan for 7, 15, 30 and 60 days**

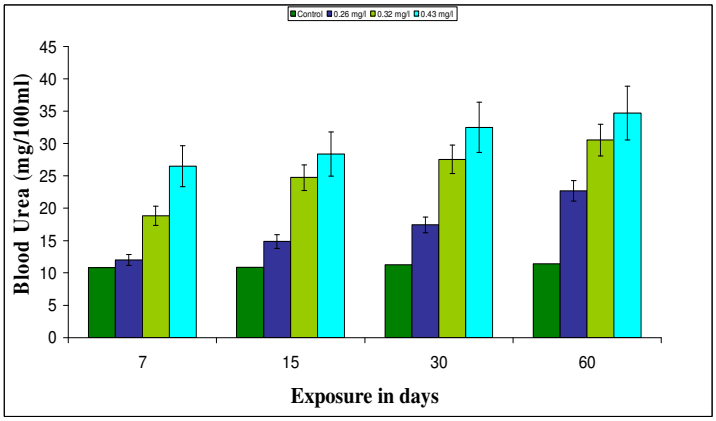



**Figure-5**
**Histogram showing alteration in the serum Urea of *Heteropneustes fossilis* exposed to 0.26 mg/L, 0.32 mg/L and 0.43 mg/L of nuvan for 7, 15, 30 and 60 days**

**Discussion:** Depletion of serum total protein in the present investigation may be due to the inhibition of RNA synthesis disturbing the protein metabolism. This is also supported by the study in which it was observed that when Nuvan exposure increased further decrease in total protein level may be due to inhibition of metabolizing enzymes in presence of toxicant[10]. Significant decrease in serum protein observed in present investigation was in agreement with the work of

Ravichandran[11]. Significant depletion of total protein in *Oreochromis niloticus* and *hrysichthytes auratus* after acute exposure to atrazine[12]. Similar trend of protein decrement was also observed by in Indian catfish, *Mystus vittatus* after Nuvan exposure[13]. In the present study there was hypoproteinemia after 60 days of exposure to Nuvan which may be due to the liver damage where most plasma protein synthesis usually occurs, this result agreed with that of Singh and Sharma[14]. Similar results were also reported in *Labeo rohita* fingerlings after quinalphos exposure[15]. This is supported by the findings in rainbow trout exposed to cypermethrin[16].

Decreased protein level may be attributed to stress mediated immobilization of these compounds to fulfill an increased element for energy by the fish to cope with environmental condition exposed by the toxicant[17]. The finding in *Cyprinus carpio* after exposure to pesticide monocrotphos displayed the similar results[18]. These findings are also supported by results observed in *Monosex tilapia* treated with cabofuran[19]. Similar results were recorded in *Cyprinus carpio* subjected to Chlorpyrifos[20]. The effect of indofil toxicity was noted on the total serum protein content of *Channa punctatus* (Bloch.)[21]. Recently similar findings were interpreted in *Clarias gariepinus* on paraquat dichloride toxicity[22].

Remarkable fall of serum albumin (hypoalbuminemia) in the treated fish can be attributed to the liver damage. This in agreement with our study and the reduced level of albumin is observed in the Rock fish exposed to cypermethrin[23]. Similar findings were reported in *Mystus vittatus* exposed to metasystox and sevin regarding serum albumin[24]; however similar findings were estimated in Sheet fish after exposing it to 2-phenoxy ethanol[25]. Depletion of serum albumin was found in Nile Tilapia, *Oreochromis mossambicus* exposed to benomyl[26]. Recently, similar results of decreasing serum albumin were observed in *Channa punctatus* under indofil toxicity[21], it was witnessed the same in *Channa punctatus* exposed to sub-lethal concentration of Nuvan[27]. Similar findings were also observed recently in *Clarias gariepinus*[22].

Total billirubin showed significant increase during the experimental periods. This result may be attributed to the great damage of hepatocytes, obstruction of the bile duct or a reluctant haemolysis. Hyperbilirubenaemia is a characteristic of jaundice. Thus the elevated levels of serum billirubin suggest liver damage. In support of the present results, a rise in serum billirubin level was observed in *Tilapia mossambica* treated with phosphamidon and suggested liver damage and impaired liver function[28]. Similar findings were also reported in the serum of freshwater fish *Clarias batrachus* after sub-chronic exposure to pesticides[29]. These findings are supported by the observations in *Monosex tilapia* treated with cabofuran[19]. No change in the total bilirubin was observed in *Clarias gariepinus* after paraquat dichloride toxicity[22], however decreased levels of serum total bilirubin levels were recorded in *Clarias albopunctatus* due to roundup toxicity[30] and elevated levels in *Clarias gariepinus* after jatropa extract toxicity[31].

Creatinine also showed some sort of significant increase after Nuvan exposure which might be due to the experimental pesticide exerts harmful effects on kidney tissues. These findings are supported by similar results in *Monosex tilapia* treated with cabofuran[19]. Similar findings were also reported in *Jundia ramdia quelen* after sublethal toxicity of cypermthrin who used serum creatinine as a diagnostic feature for renal function test and studied haematological and serum biochemical values[32]. They reported the similar increasing trend in serum creatinine attributed this to the renal damage as a result of Nuvan toxicity. Depletion of serum creatinine was observed in *Clarias gariepinus* after paraquat dichloride toxicity[22], however elevated levels of serum urea levels were recorded in *Clarias albopunctatus* due to roundup toxicity[30] and in *Clarias gariepinus* after jatropa extract toxicity[31].

Increase in blood urea in the experimental fish is due to the inability of damaged kidney to filter urea up to normal levels. Identical results in response to the activity of blood urea were recorded in *Clarias batrachus* under the stress of rogor who studied the haematological and biochemical anomalies[33]. The alteration of blood urea in freshwater fish, *Mystus vittatus* was investigated after chronic exposure to metasystox and sevin and reported that blood urea showed similar increasing trend which is in agreement to the present study[24]. Similar findings were also reported in *Jundia ramdia quelen* after sublethal toxicity of cypermethrin who used blood urea as a diagnostic feature for renal function test and studied haematological and serum biochemical values[32] however present investigation was fully supported by studying the blood biochemistry of *Channa punctatus* (Bloch.) after Nuvan toxicity[27]. They reported the similar increasing trend in blood urea attributed this to the renal damage as a result of Nuvan toxicity. Depletion of serum urea was observed in *Clarias gariepinus* after paraquat dichloride toxicity[22], however elevated levels of serum urea levels were recorded in *Clarias albopunctatus* due to roundup toxicity[30] and in *Clarias gariepinus* after jatropa extract toxicity[31].

## Conclusion

It is concluded that fishes exposed to nuvan exhibited dose and duration dependent alterations in many biochemical components such as serum protein, serum albumin, serum bilurubin, serum creatinine and urea. Nuvan is a highly hepatotoxic organophosphate which damages the liver more predominantly. It affects the target as well as non target organisms including man.

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