ABSTRACT

Due to use of various pesticides has aggravated the problem of pollution to aquatic environment, especially for the fishes. Nuvan was found to be toxic to the fish, which was found to be increasing with the increase of its concentration. Experiments were conducted the effect of the organophosphorous pesticide, Nuvan on some of the blood biochemical parameters in *Clarias batrachus*. The 96 hours LC$_{50}$ of Nuvan of this fish was determined as 0.07ml/L. Blood sample were analyzed for biochemical parameters such as blood glucose, blood urea, serum cholesterol, SGOT and SGPT at 24, 48, 72, and 96 hours. There was significant ($P<0.05$) reduction in cholesterol content in the test groups compared to the control fish. Due to inhibition of lipid synthesis and increased utilization of stored lipid as a source of energy to conduct regular metabolic function. The blood glucose, blood urea were significantly higher ($P<0.05$) in Nuvan treated groups compared to the control fish. There was also significant ($P<0.05$) increased the SGOT and SGPT level in the fish exposed to Nuvan, as compared to the control group. Due to hepatocellular destruction, renal damage and caused by pesticidal Nuvan, which leads to the liver dysfunction. The result of the biochemical blood indicate a marked neurotoxic effect of Nuvan and shows that this changes could be used as biomarkers for aquatic pollution.

Keywords : Nuvan, *Clarias batrachus*, Biochemical parameters, Organophosphorous pesticide.

INTRODUCTION

Use of pesticide for control of disease in agriculture and aquaculture has increased enormously during the last two decades. Even sub-lethal concentration of a pesticide may still cause fish mortality in the exposed population after a sufficiently long time of exposure (Ghosh and Shrotri, 1992). The surface run-off from the agriculture lands carries the pesticides into the aquatic ecosystem, which enter into the organisms through food webs and also through contact in water (Edwards, 1973). Organophosphorous pesticide, which comprise about 80% of the agricultural pesticides, are being used for the control of agricultural pest and vector borne diseases, contaminate the aquatic environment through the agricultural run-off (Young and Nicholson, 1957).

Nuvan an organophosphate is a quick killing pesticide. Nuvan is first introduced in the 1960s. Nuvan is an insecticide in the organic phosphate chemical family. A Nuvan insecticide is structurally similar to acetylcholine, a potent neurotransmitter. At the enzyme site, they compete with acetylcholine and bind with cholinesterase, thus inhibiting is normal function. The EPA has classified it as toxicity class I - highly toxic, because it may cause cancer and there is only a small margin of safety for other effects. Nuvan is available as an aerosol and soluble concentrate. Nuvan is a colorless to amber liquid with a mild chemical odour. Dilute Nuvan breaks down rapidly in the presence of moisture.
When used properly, Nuvan is not hazardous to non-target organisms, and will not persist in the environment. The principal, direct routes of entry for Nuvan into waters include industrial effluents and accidental discharges (e.g. from pesticide manufacturing plants, formulation plants and marketing outlets), use in salmon fisheries, disposal of unused insecticide and the cleaning of application and mixing equipment. Nuvan may also indirectly enter the aquatic environment via spray drift during application and in land run-off. Nuvan is rapidly degraded in water both chemically and biologically. The main degradation process is hydrolysis. Persistence in water is low (days) and depends on the pH and temperature (Jones and Stewart, 1996). The vapour pressure of Nuvan is relatively low, so it is therefore unlikely that volatilisation is a significant removal process from the aquatic environment. Adsorption is not expected to be a significant removal pathway due to the low octanol-water and soil organic carbon-water partition coefficients.

Nuvan is highly toxic by inhalation, dermal absorption, and ingestion, because Nuvan is volatile; inhalation is the most common route of exposure. As with all organophosphates, Nuvan is readily absorbed through the skin. Acute illness from Nuvan is limited to the effects of cholinesterase inhibition. Compared to poisoning by other organophosphates, Nuvan causes a more rapid onset of symptoms, which is often followed by a similarly rapid recovery. This occurs because Nuvan is rapidly metabolized and eliminated from the body. Alcoholic beverages may enhance the toxic effects of Nuvan. High environmental temperatures or exposure of Nuvan to light may enhance its toxicity. Nuvan is mildly irritating to skin. Concentrates of Nuvan may cause burning sensations, or actual burns. Symptoms of acute exposure to organophosphate or cholinesterase-inhibiting compounds may include the following: numbness, tingling sensations, headache, dizziness, nausea, abdominal cramps, sweating, blurred vision, difficulty breathing or respiratory depression, slow heartbeat. Very high doses may result in unconsciousness, incontinence and convulsions or fatality. Nuvan primarily affects the nervous system through cholinesterase inhibition, the blockage of an enzyme required for proper nerve functioning.

Increasing attention has been given during the past decades to the protection of the aquatic environment against pollution both nationally and internationally, in the fresh water field. The presence or absence of fish has been widely used as a biological indicator of the degree of the pollution. Therefore, present investigation was to access the toxic effect of Nuvan on blood glucose, blood urea, serum cholesterol, SGOT and SGPT of *Clarias batrachus*.

**Materials and Methods**

Healthy living specimens of common air breathing freshwater fish *Clarias batrachus* of uniform length (12 to 15cm) and weight (55 to 70gm) were collected from local freshwater pond at Agra (U. P.). The fishes were acclimatized to laboratory condition for one week. The fishes were fed once daily with boiled chicken egg and water was changed every day. Fishes were kept in glass aquarium capacity 20 liter, having non chlorinated tap water aquaria bath 1% KmnO₄, solution for disinfection. All experiment were conducted at laboratory temperature and fishes not fed during the experiment. Dead fish was removed from aquaria and mortality were recorded.

Nuvan from Syngenta India Ltd. was used for present study. Five aquaria were set up for each concentration and each aquaria contained six fish in 20L dechlorinated tap water. The data was analysed statistically by log dose/ probit regression line method (Finny, 1971) which was calculated 0. 07ml/ L. Sub-lethal dose of Nuvan was applied to four aquaria for 24, 48, 72, 96 hours. Simultaneously a control was run parallel to the treated ones. The blood were collect from the live fish from each group after completion of exposure time. Blood glucose, blood urea, cholesterol, SGOT and SGPT estimated by the method of GOD/ POD kit, Coulambe and Favreen, Wybenga and Pileggi, Reitman and Frankel, & Reitman and Frankel data were analysed statistically by student ‘t’ test (Fisher, 1950).
Results and Discussion

Comparative data on biochemical components of control and experimental fishes for blood glucose, blood urea and serum cholesterol has been given in table I and graph I & II and for enzymes SGOT and SGPT in table II and graph III respectively.

Table – I
Blood Glucose, Blood Urea and Serum Cholesterol levels in Blood of *Clarias batrachus* after Nuvan intoxication.

<table>
<thead>
<tr>
<th>Treatment time of Nuvan</th>
<th>Blood Glucose mg/dl Mean ± S.Em.</th>
<th>Blood Urea mg/dl Mean ± S.Em.</th>
<th>Serum Cholesterol mg% Mean ± S.Em.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>32.50±0.42</td>
<td>11.94±0.278</td>
<td>222.33±0.80</td>
</tr>
<tr>
<td>24 Hours</td>
<td>33.51±0.50&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>12.47±0.320&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>221.26+0.35&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>48 Hours</td>
<td>34.45±0.71&lt;sup&gt;*&lt;/sup&gt;</td>
<td>12.98+0.387&lt;sup&gt;*&lt;/sup&gt;</td>
<td>218.00±1.00&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>72 Hours</td>
<td>37.53±1.14&lt;sup&gt;**&lt;/sup&gt;</td>
<td>13.96+0.501&lt;sup&gt;**&lt;/sup&gt;</td>
<td>211.66+1.05&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>96 Hours</td>
<td>44.66±1.98&lt;sup&gt;***&lt;/sup&gt;</td>
<td>16.33±0.988&lt;sup&gt;***&lt;/sup&gt;</td>
<td>203.33±1.07&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Graph – I
Blood glucose and blood urea levels in blood of *Clarias batrachus* after nuvan intoxication.

Results are expressed as mean ± S.Em.  
* Significant (*p < 0.05 or *p = 0.02)  
** Highly significant (*p ≤ 0.01)  
*** Very highly significant (*p ≤ 0.001)
Serum cholesterol levels in blood of *Clarias batrachus* after nuvian intoxication.

Results are expressed as mean ± S.Em.  
NS = Non significantly (*p* ≥ 0.05)  
** Highly significant (*p* ≤ 0.01)  
*** Very highly significant (*p* ≤ 0.001)

<table>
<thead>
<tr>
<th>Treatment time of Nuvian</th>
<th>SGOT (IU/L)</th>
<th>SGPT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± S.Em.</td>
<td>Mean ± S.Em.</td>
</tr>
<tr>
<td>Control</td>
<td>156.49±0.69</td>
<td>46.73±0.50</td>
</tr>
<tr>
<td>24 Hours</td>
<td>153.16±1.01*</td>
<td>49.00±1.21**</td>
</tr>
<tr>
<td>48 Hours</td>
<td>154.66±1.10*</td>
<td>50.33±1.35***</td>
</tr>
<tr>
<td>72 Hours</td>
<td>156.33±1.33**</td>
<td>52.01±1.36***</td>
</tr>
<tr>
<td>96 Hours</td>
<td>158.83±1.39***</td>
<td>54.50±1.60***</td>
</tr>
</tbody>
</table>
Graph – III

SGOT and SGPT levels in blood of *Clarias batrachus* after nuvan intoxication.

Results are expressed as mean ± S.E.m.  
NS = Non significantly (*p* ≥ 0.05)  
* Significant (*p* < 0.05 or *p* = 0.02)  
** Highly significant (*p* ≤ 0.01)  
*** Very highly significant (*p* ≤ 0.001)

The blood glucose level can be an indicator of biological stress caused by pollutants such as pesticides and metal (Silbergeld, 1974). Hyperglycemic condition in naturally as well as experimentally stressed fishes may due to impairment in the hormone level in blood involved in the carbohydrate metabolism. Nuvan is the inhibitor of acetylcholinesterase. Inhibition of cholinesterase in adrenal medulla which stimulates the break down of glycogen to glucose and increase corticosteroid level which increase the blood glucose. Catecholamine might deplete glycogen reserves in stressed fish by stimulating glycogenolysis and gluconeogenesis (Singh and Srivastava, 1992; Singh and Agrwal, 1993). Umminger (1977) observed blood glucose has direct correlation to metabolism. Rao et al. 1986 observed increased blood glucose in *Clarias batrachus* exposed to pesticide. Thus hyperglycemia in *Clarias batrachus* seems to be due to reduced insulin secretion, increased corticosteroid and also stimulation of glyconeogenesis.

The elevated blood urea level reflects the deficient glomerular filtration rate (GFR) of kidney this result agreed with that of Jyothi and Narayan (1997), pesticide (phorate) induced alterations of non-protein nitrogenous constituents of a freshwater catfish *Clarias batrachus*. The air-breathing walking catfish is a potential ureogenic species, due to having relatively high level of activity for all the key urea cycle enzymes in hepatic and also in extra-hepatic tissues. *Clarias batrachus* exposed to different concentrations of carbaryl (carbamate) exhibit many biochemical alteration in blood urea relates to renal failure, Luskova et al. (2002) have observed this result in *Cyprinus carpio*. Begum et al. (2004) have observed abnormal increase in blood urea as a result of liver and renal failure in *Clarias batrachus*. 
The elevated level of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) are markers of liver functions. SGOT and SGPT are the two important enzymes found in all tissues. SGOT and SGPT are enzymes liberated into the blood in pathological conditions and therefore are of clinical significance and their presence in blood plasma can give information on tissue injury or organ dysfunction. They catalyse the transfer of the amino group \((-\text{NH}_2\)) from glutamic acid to either oxaloacetic acid or pyruvic acid. Therefore increased level of serum transaminase related to distortion of normal metabolism which is due to extensive alteration in the liver histology and indicates liver damage. Increased SGOT and SGPT activity after pesticidal stress was reported by Joyce et al. 1989.

In the present study, the serum cholesterol has been found to be decreased after sub-lethal treatment of Nuvan in *Clarias batrachus*. Cholesterol content in the blood is linked to lipid metabolism and depends on the calorific value of the feed. Gill and Pant (1984) reported that the decrease in serum cholesterol was due to the cadmium stress affecting absorption of dietary cholesterol in fish. The reduced cholesterol level may be due to the inhibition of cholesterol biosynthesis in liver or due to reduced absorption of dietary cholesterol as reported by Jyantha Rao et al. (1984) and Kanagaraj et al. (1993). Shakoori et al. (1996) observed that the cholesterol decrease may due to utilization of deposits instead of glucose for energy purpose.

References


