Acute toxicity and impact of an organophosphate pesticide, chlorpyrifos on some haematological parameters of an Indian minor carp, *Labeo bata* (Hamilton 1822)

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**ABSTRACT**

Chlorpyrifos (CPF) is a widely used organophosphate pesticide and toxic to aquatic organisms including fish. In the present study, an attempt was performed to estimate the 24h, 48h, 72h, and 96h LC$_{50}$ value of chlorpyrifos for *Labeo bata* and the resultant values were found to be 257.03µg L$^{-1}$, 208.92µg L$^{-1}$, 177.82µg L$^{-1}$ and 109.64µg L$^{-1}$ respectively indicating tremendous toxicity of chlorpyrifos to the studied fish. The fish were exposed to chlorpyrifos in two sublethal concentrations, 30.8µg L$^{-1}$ and 61.6µg L$^{-1}$ for 24h, 48h, 72h and 96h and effect of CPF on the haematological parameters of the fish were studied. The result showed that CPF could cause anaemia with significant (p<0.001) decrease in RBC count, haemoglobin (Hb), packed cell volume (PCV) and mean corpuscular volume (MCV) in the pesticide exposed fish. However, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) did not vary significantly. White blood cell (WBC) significantly increased (p<0.001) in CPF exposed fish as a protective response. These results suggest that CPF is toxic and has disruptive effect on the blood of fish.

**Keywords:** Chlorpyrifos, acute toxicity, haematological parameter, *Labeo bata*.

1. **Introduction**

The application of synthetic fertilizers, insecticides and pesticides are increasing now-a-days with increasing demand of agricultural food material throughout the world. There is a high chance of aquatic ecosystem to be contaminated, located in industrial or agricultural areas, through runoff or ground water leaching of a variety of chemicals (Todd and Leuwen, 2002). Agricultural pesticides contaminate the aquatic environment which can affect aquatic organism in different ways (Ventura et al., 2008).

In India, more than 70% of the chemical formulation used in agricultural practice ultimately affects non-target organisms (Bhatnagar et al., 1992). In the beginning of 1980’s chlorinated pesticides have been fully replaced by organophosphate pesticides (Svoboda et al., 2001). Organophosphate pesticide is the most preferable because of their low cumulative ability and short term persistence in the environment. Chlorpyrifos (CPF), one of the broad-spectrum organophosphate (OP) pesticides is widely used to control arthropod pests but has severe adverse effect on aquatic organisms (Sparling and Fellers, 2007). There are information on the effect of chlorpyrifos based pesticides on haematological parameters in many fish species as for example the effect of termifos on African catfish *Clarias garepinus* (Nwani et al., 2013), diazinon on common carp, *Cyprinus carpio* (Svoboda et al., 2001), and European catfish *Silurus glanis* (Köprücü et al., 2006), chlorpyrifos on *Cyprinus carpio* (Yonar et al., 2012) and the effect of methyl-parathion on *Heteropneustes fossilis* (Nath and Banerjee,
1996). Many works have been done on the effect of OP pesticides on the biochemical, physiological and haematological parameters. However information on the median tolerance limit of CPF and its effect on haematological parameters for an Indian minor carps are not available.

Accordingly, in the present study an effort has been taken to estimate median tolerance limit of CPF and to examine its short term sublethal effect on some haematological parameters of an Indian minor carp, *Labeo bata* (Hamilton, 1822). *Labeo bata* selected for the present study because it is an important cultivable fish in India and used as a protein supplement for poor people. This species is also a very important model organism for toxicity tests because of its availability throughout the year and easy acclimatization to laboratory condition.

2. Material and methods

2.1 Experimental fish

The Indian minor carp, *Labeo bata* (Hamilton 1822) (Cypriniformes, Cyprinidae), a freshwater teleost (average 15-18cm in length and 30-40g in weight) was chosen for this experiment. The fish (n=110) was obtained from local freshwater bodies of Santiniketan, West Bengal, India (Lat. 23°39′N, Long. 87°42′E). The fish were acclimatized in the laboratory conditions for 15 days during which the fish were fed with adequate amount of pellet feed. Laboratory care of fish and their use for experiments were adopted according to the guideline of the departmental animal ethic committee.

2.2 Chemical

Tricel, 20% EC a commercial chlorpyrifos was used for the experiment.

2.3 Experimental water

The experiments were conducted in glass aquaria. All aquaria were filled with 40L filtered water and prior to experiment physico-chemical parameters of water were estimated following the methods of APHA, 1998. The water parameters were found as temperature 25-27º C, pH 7.73, dissolve oxygen 5.17mg L⁻¹, total alkalinity 197.5mg L⁻¹ and total hardness 142mg L⁻¹.

2.4 Experimental procedure for acute toxicity test

In order to perform the experiment, 11 aquaria were taken and previously acclimated 10 fish were transferred to each aquarium. In each set, one aquarium was kept as control and 10 aquaria were contaminated with ten different concentrations (100, 150, 200, 250, 300, 350, 400, 450, 500, and 550µg L⁻¹) of chlorpyrifos. Aquaria were kept under continuous aeration and without food. The experiment was continued for 96 hours. Mortality of the fish in each aquarium was recorded separately at 4h interval and dead fish were immediately removed. Accumulated mortality data were analyzed to determine 24h, 48h, 72h and 96h LC₁₀ to LC₉₀ values following the probit analysis method (Finney, 1971). These values were obtained by plotting a linear regression curve of the probit value of mortality percentage of fish against the log concentrations of CPF exposure.
2.5 Experimental set up, blood sampling and haematological assay

A separate experiment was conducted to examine the effect of CPF on the blood parameters. The fish were exposed to 30.8µg L⁻¹ and 61.6µg L⁻¹ of CPF for 24h, 48h, 72h, and 96h respectively. Blood samples of 500µL were collected into EDTA rinsed syringes from the fish of each set after stipulated period of experiment. The total count of erythrocytes (RBC, 10⁶/µL) and leucocytes (WBC, 10⁴/µL) were performed by haemocytometer. Haemoglobin (Hb, g/dL) concentration, packed cell volume (PCV, %), mean corpuscular volume (MCV, fL/cell), mean corpuscular haemoglobin (MCH, pg/cell), and mean corpuscular haemoglobin concentration (MCHC, g/dL) were estimated following the methods of Jain, 1993.

\[
\text{MCV (fL/cell)} = (\text{packed cell volume as percentage/RBC in millions}) \times 10 \\
\text{MCH (pg/cell)} = (\text{Hb in g}/\text{RBC in millions}) \times 10 \\
\text{MCHC (g/dL)} = (\text{Hb in g/packed cell volume as percentage})
\]

2.6 Statistics

The haematological parameters were represented as mean ± standard error of mean (SEM). The differences in the haematological parameters among the fish group exposed to different concentrations of CPF for different duration were subjected to one-way ANOVA followed by Duncan’s multiple range test to determine the significant difference at 5% probability level.

3. Results

3.1 Behavioural changes

The behaviours of *Labeo bata* were observed in the control and exposed sets. The fish exposed to CPF showed some abnormal behaviours. The group of fish which were exposed to 400µg/L⁻¹ and higher showed less activity, loss of equilibrium, motionlessness, erratic swimming and loss of aPPetite. The fish stood motionless in the aquarium bottom, hanging vertically.

3.2 Acute toxicity of chlorpyrifos

The mortality of the fish, *Labeo bata*, increased with the increasing concentrations of chlorpyrifos and duration of exposure. Table 1 represented the 24h, 48h, 72h and 96h LC₅₀ to LC₉₀ values of CPF for the experimental fish, *L. bata*. The LC₅₀ value of 24h, 48h, 72h, and 96h were determined as 257.03µg L⁻¹, 208.92µg L⁻¹, 177.82µg L⁻¹ and 109.64µg L⁻¹, respectively.

3.3 Effect of chlorpyrifos on haematological parameters

Table 2 represented the changes in haematological parameters of the CPF exposed fish, *Labeo bata*. RBC, Hb, PCV and MCV significantly decreased (p< 0.001), whereas MCH shows no significant changes. MCHC significantly (p< 0.001) declined when the fish exposed to low concentration of CPF but changes were not significant at the higher concentration. WBC significantly (p< 0.001) inclined along with the increase of concentration and exposure time.
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Table 1: LC$_{10}$ to LC$_{90}$ values of chlorpyrifos for *Labeo bata* at different exposure duration.

<table>
<thead>
<tr>
<th>Point</th>
<th>24 hour</th>
<th>48 hour</th>
<th>72 hour</th>
<th>96 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC$_{10}$</td>
<td>91.20</td>
<td>72.44</td>
<td>75.85</td>
<td>21.37</td>
</tr>
<tr>
<td>LC$_{20}$</td>
<td>131.82</td>
<td>104.71</td>
<td>102.32</td>
<td>37.15</td>
</tr>
<tr>
<td>LC$_{30}$</td>
<td>169.82</td>
<td>134.89</td>
<td>125.89</td>
<td>56.32</td>
</tr>
<tr>
<td>LC$_{40}$</td>
<td>208.92</td>
<td>169.82</td>
<td>151.35</td>
<td>79.43</td>
</tr>
<tr>
<td>LC$_{50}$</td>
<td>257.03</td>
<td>208.92</td>
<td>177.82</td>
<td>109.64</td>
</tr>
<tr>
<td>LC$_{60}$</td>
<td>316.22</td>
<td>257.03</td>
<td>213.79</td>
<td>151.35</td>
</tr>
<tr>
<td>LC$_{70}$</td>
<td>398.10</td>
<td>323.59</td>
<td>251.18</td>
<td>213.79</td>
</tr>
<tr>
<td>LC$_{80}$</td>
<td>512.86</td>
<td>426.57</td>
<td>316.22</td>
<td>323.59</td>
</tr>
<tr>
<td>LC$_{90}$</td>
<td>724.43</td>
<td>616.59</td>
<td>426.57</td>
<td>575.43</td>
</tr>
</tbody>
</table>

Table 2: Alterations in the haematological parameters of chlorpyrifos (CPF) exposed *L.bata*.

<table>
<thead>
<tr>
<th>Haematological parameters</th>
<th>Concentration of CPF (µg L$^{-1}$)</th>
<th>24h</th>
<th>48h</th>
<th>72h</th>
<th>96h</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (10$^6$/µL)</td>
<td>0 (Control)</td>
<td>3.508±0.020$^{ax}$</td>
<td>3.46±0.22$^{ax}$</td>
<td>3.462±0.345$^{ax}$</td>
<td>3.522±0.328$^{ax}$</td>
</tr>
<tr>
<td></td>
<td>30.8</td>
<td>2.8±0.027$^{by}$</td>
<td>2.678±0.022$^{by}$</td>
<td>2.372±0.055$^{by}$</td>
<td>2.126±0.055$^{by}$</td>
</tr>
<tr>
<td></td>
<td>61.6</td>
<td>2.71±0.013$^{by}$</td>
<td>2.638±0.036$^{by}$</td>
<td>2.286±0.034$^{by}$</td>
<td>1.746±0.026$^{by}$</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>0 (Control)</td>
<td>10.406±0.038$^{ax}$</td>
<td>10.51±0.235$^{ax}$</td>
<td>10.428±0.116$^{ax}$</td>
<td>10.474±0.122$^{ax}$</td>
</tr>
<tr>
<td></td>
<td>30.8</td>
<td>8.524±0.195$^{by}$</td>
<td>8.448±0.149$^{by}$</td>
<td>7.282±0.023$^{by}$</td>
<td>6.306±0.066$^{by}$</td>
</tr>
<tr>
<td></td>
<td>61.6</td>
<td>8.204±0.103$^{by}$</td>
<td>8.208±0.072$^{by}$</td>
<td>6.954±0.013$^{by}$</td>
<td>5.798±0.077$^{cz}$</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>0 (Control)</td>
<td>30.104±0.100$^{ax}$</td>
<td>30.084±0.091$^{ax}$</td>
<td>30.04±0.138$^{ax}$</td>
<td>30.106±0.189$^{ax}$</td>
</tr>
<tr>
<td></td>
<td>30.8</td>
<td>26.236±0.189$^{by}$</td>
<td>26.086±0.250$^{by}$</td>
<td>22.54±0.119$^{by}$</td>
<td>18.356±0.131$^{by}$</td>
</tr>
<tr>
<td></td>
<td>61.6</td>
<td>24.2±0.150$^{by}$</td>
<td>24.2±0.146$^{by}$</td>
<td>20.38±0.029$^{by}$</td>
<td>14.216±0.093$^{cz}$</td>
</tr>
<tr>
<td>MCV (fL/cell)</td>
<td>0 (Control)</td>
<td>85.826±4.644$^{ax}$</td>
<td>88.382±5.956$^{ax}$</td>
<td>89.8±7.676$^{ax}$</td>
<td>88.224±7.524$^{ax}$</td>
</tr>
<tr>
<td></td>
<td>30.8</td>
<td>93.732±2.119$^{ax}$</td>
<td>97.42±1.126$^{ax}$</td>
<td>95.23±2.306$^{ax}$</td>
<td>86.53±6.960$^{ax}$</td>
</tr>
<tr>
<td></td>
<td>61.6</td>
<td>89.52±1.982$^{ax}$</td>
<td>91.81±1.008$^{ax}$</td>
<td>89.24±1.267$^{ax}$</td>
<td>81.458±0.724$^{ax}$</td>
</tr>
<tr>
<td>MCH (pg/cell)</td>
<td>0 (Control)</td>
<td>30.466±0.923$^{ax}$</td>
<td>31.538±0.451$^{ax}$</td>
<td>30.772±0.826$^{ax}$</td>
<td>30.97±1.096$^{ax}$</td>
</tr>
<tr>
<td></td>
<td>30.8</td>
<td>30.274±0.437$^{ax}$</td>
<td>31.008±0.432$^{ax}$</td>
<td>30.45±0.655$^{ax}$</td>
<td>33.21±2.595$^{ax}$</td>
</tr>
<tr>
<td></td>
<td>61.6</td>
<td>30.274±0.437$^{ax}$</td>
<td>31.008±0.432$^{ax}$</td>
<td>30.45±0.655$^{ax}$</td>
<td>33.21±2.595$^{ax}$</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>0 (Control)</td>
<td>34.564±0.170$^{ax}$</td>
<td>34.938±0.839$^{ax}$</td>
<td>34.71±0.447$^{ax}$</td>
<td>34.792±0.469$^{ax}$</td>
</tr>
<tr>
<td></td>
<td>30.8</td>
<td>32.492±0.766$^{ax}$</td>
<td>32.374±0.348$^{ax}$</td>
<td>32.30±0.145$^{ax}$</td>
<td>34.408±2.195$^{ax}$</td>
</tr>
<tr>
<td></td>
<td>61.6</td>
<td>33.818±0.414$^{ax}$</td>
<td>33.896±0.308$^{ax}$</td>
<td>34.108±0.343$^{ax}$</td>
<td>40.702±6.932$^{ax}$</td>
</tr>
<tr>
<td>WBC (10$^4$/ µL)</td>
<td>0 (Control)</td>
<td>22.247±0.082$^{ax}$</td>
<td>22.148±0.246$^{ax}$</td>
<td>21.738±0.340$^{ax}$</td>
<td>21.872±0.284$^{ax}$</td>
</tr>
<tr>
<td></td>
<td>30.8</td>
<td>22.82±0.048$^{by}$</td>
<td>23.128±0.168$^{by}$</td>
<td>23.34±0.144$^{by}$</td>
<td>23.96±0.111$^{ax}$</td>
</tr>
<tr>
<td></td>
<td>61.6</td>
<td>23.306±0.188$^{ax}$</td>
<td>23.744±0.100$^{ax}$</td>
<td>24.328±0.155$^{ax}$</td>
<td>24.61±0.173$^{ax}$</td>
</tr>
</tbody>
</table>

The letters a, b and c in each row denote significant (p<0.001) differences in a blood parameter of a particular group due to variation exposure period. The letters x, y and z in each column denotes significant (p<0.001) differences in a blood parameter due to concentration of CPF exposure.

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4. Discussions

The present study assessed the toxicity of a widely used organophosphate pesticide, chlorpyrifos with the evaluation of its effect on some blood parameters of an Indian minor carp, *Labeo bata*. The 24h, 48h, 72h and 96h LC₁₀ to LC₉₀ values of CPF were determined for adult *L.bata* and 24h, 48h, 72h and 96h LC₅₀ values of CPF for the fish were found as 257.03µg L⁻¹, 208.92µg L⁻¹, 177.82µg L⁻¹ and 109.64µg L⁻¹ respectively. The acute toxicity of CPF varied among different fish species and different age groups. The 96h LC₅₀ of CPF for common carp were reported as 160µg L⁻¹ (HalaPPa and David, 2009) and 203µg L⁻¹ (Banaee et al., 2013). Whereas, 96h LC₅₀ of CPF for *Oreochromis mossambicus*, *Gambusia affinis* and *Channa punctatus* were estimated as 154µg L⁻¹ (Rao et al., 2003), 297µg L⁻¹, (Rao et al., 2005), and 0.811mg L⁻¹ (Ali et al., 2008) respectively. The 96h LC₅₀ value of chlorpyrifos-methyl for *O. niloticus* larvae was 0.92 mg L⁻¹ (Ali, 2005). The result of present study and available reports indicated that chlorpyrifos was highly toxic to *L.bata*.

The haematology is an important tool to assess the health status of fish. The blood indices vary with the variation of environmental conditions (Ramaswamy and Reddy, 1978), reproductive activities and chemical stress (Srivastava and Agrawal 1981). The result showed that RBC count, haematoaocrit percentages and haemoglobin decreased significantly and indicates that chlorpyrifos can cause anaemia to the fish. Decrease of RBC and haemoglobin may be due to either increased rate of erythrocyte destruction or inhibition of RBC formation and haemoglobin synthesis (Jenkins et al., 2003; Ramesh and Saravanan, 2008). Similar findings of decreased RBC, PCV and Hb have been recorded due to pesticide exposure as for example in cypermethrin and carbofuran exposed *Labeo rohita* (Adhikari et al., 2004), and in chlorpyrifos exposed *C. carpio* (Ramesh and Saravanan, 2008). The decrease in MCHC indicates the hypochromic condition. The hypochromic anaemia was reported in lindane exposed *C. carpio* (Saravanan et al., 2011). The declined haemoglobin content as well as binding of chlorpyrifos with haemoglobin rapidly reduced the amount of oxyhaemoglobin in blood and released of free reactive oxygen radicals (Seth and Saxena, 2003). Anaemia associated with erythropenia was reported for several freshwater fish species (Svoboda et al., 2001 and Gbem et al., 2003). The decrease of RBC and haemoglobin established a condition of erythropenia and stress that caused haemolysis. The significant decrease of RBC, haemoglobin, PCV and MCV in this study might be a result haemolyses of RBC. Stimulation of lymphopoiesis and enhanced release of lymphocytes from lymphomyeloid tissue under toxic stress might lead to an increase in WBC number (El-Sayed et al., 2007) as observed in 4 tetr-octylphenol exposed *C. dimerus* (Va’quez and Nostro, 2014). Similarly, the significant increase of WBC in chlorpyrifos exposed fish *L. bata* in the present study might be due to a protective response against the stress.

5. Conclusion

The organophosphate pesticide, chlorpyrifos is highly toxic to the Indian minor carp, *Labeo bata* as revealed from the acute toxicity test and estimated 24h, 48h, 72h and 96h LC₅₀ value as 257.03µg L⁻¹, 208.92µg L⁻¹, 177.82µg L⁻¹ and 109.64µg L⁻¹ respectively. Chlorpyrifos is disruptive even at a low concentration to the haematological parameters and survival of fish. This toxic hazard of chlorpyrifos should be taken in consideration during its use near a fish habitat.
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6. References


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