Acute toxicity and some haematological and biochemical changes in giant sturgeon (Huso huso) exposed to diazinon

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Abstract
Acute toxicity and the effect of diazinon on some haematological parameters of giant sturgeon (Huso huso) weighing 14±2 g was studied under static water quality conditions at 22±1°C. The values of 5.89, 5.65, 5.60 and 4.99 mg diazinon /L were calculated as LC₅₀ after 24, 48, 72 and 96 hours, respectively, while those of LC₅₀ were calculated to be 6.64, 6.32, 6.18 and 5.63 mg/L, respectively. After 96 hours of exposing the fish to LC₅₀ concentration of the toxicant (5.6 mg/L), haematological indices of erythrocyte count (RBC), haematocrit (PCV), haemoglobin (Hb), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), leucocyte count (WBC) lymphocyte and eosinophil counts were significantly lower than the control group (P<0.05), while there was a significant increase in values of neutrophils and leucocyte and MCV of the test group compared to the control group (P<0.05). Also, the levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and total protein were significantly lower in fish exposed to the toxicant compared to control fish (P<0.05), while aspartate aminotransferase (AST) level was insignificantly higher in experimental fish (P>0.05).

Introduction
The increased use of insecticides and pesticides in the agricultural industry has caused many problems associated with their effects on the environment. The leaching of pesticides into waters may cause a serious deterioration in aquatic animals. Toxic chemicals can affect fish at various levels, from the morphological structure of organs to the molecular structure and activity of important enzymes (e.g. Keizer et al., 1991; 1993; Sancho et al., 1992a; 1992b; 1993; 1997; Tsuda et al., 1997; Bailey et al, 2000). Diazinon [O-O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate] is a widely used pesticides in many regions including north Iran. This organophosphorous chemical also possesses a wide range of insecticidal activity, and has been used from the early 1950s (e.g. Sovoboda et al. 2001; Luskova et al. 2002; Eisler, 1986; Moore & Waring, 1996). The toxic inhibitory function on the activity of the enzymes acetylcholinesterase and co-ATPase is well recognized (Goodman et al., 1979; Ansari et al., 1987; Ansari & Kumar, 1988; Keizer et al, 1993). Although the susceptibility of different fish species to various organophosphorous insecticides including diazinon has been reported in the literature (e.g. Sancho et al. 1993; Dutta et al., 1997; Wall, 2000; Luskova et al., 2002;
Svoboda et al., 2001), no attention has been invested to elucidate the blood-pesticide relationship in valuable sturgeon species, particularly with reference to organophosphorous formulations. The present study was undertaken to evaluate (A) acute toxicity of diazinon to giant sturgeon, (B) haematological indices of the affected fish and (C) biochemical parameters of blood plasma.

**Materials and Methods**

1. **Acute toxicity**
   Juveniles of giant sturgeon (*H. huso*) with 14±2 g mean body weight were used for the acute toxicity test using the procedure described in the OECD Direction and Methodical Manual. Diazinon (Maccidal EC 600) was used in the form of 60 % emulsion. Five concentrations of 5, 5.5, 6, 6.5 and 7 mg/L and a negative control were used in the basic test. Ten fish in 3 replicates were used for each concentration and for the negative control. Each group was kept in 160 L aquaria. The test was performed without any water exchange for 96-hours using water quality conditions consisting of pH 7.5- 8.0, NH₃<0.01 mg/L, NO₂<0.1 mg/L, CaCO₃ 145 mg/L, PO₄ 0.285mg/L, water temperature of 22±1ºC and dissolved oxygen of 8±1 mg/L. The LC₅₀ and LC₅₀ values in the respective time intervals were determined by probit analysis with 95% confidence.

2. **Haematology**
   The haematological study was carried out using giant sturgeon of 14±2 g body weight at the end of the 96-hour acute toxicity test with diazinon in concentration of 5.63 mg/L. At the same time, the control group was examined haematologically. The test was performed under the conditions described above, each tank containing 15 specimens of giant sturgeon in 2 replicates. The survival fish were mixed and blood samples were obtained by tail vein puncture from 10 random fish and transferred to heparinized and non-heparinized tubes. At the same time of blood sampling, the appropriate smears were prepared for Geimsa staining. The smears were first air-dried, then fixed in 96% ethanol for 30 minutes and stained by Giemsa staining for 30 minutes. The smears were examined for leucocyte differential count under a compound microscope (Klont, 1994). The haematological parameters examined were erythrocyte count (RBC), haematocrit (PCV), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), leucocyte count (WBC) and differential leucocyte count (Klont, 1994).

3. **Plasma biochemistry**
   The non-heparinized blood samples were centrifuged for 15 minutes at 400 g and separated sera were used to determine the levels of the following factors: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and total protein using an automatic analyzer machine (Eppendorf, Epos 5060 and optimized tests of Bochring Manheim GmBH by spectrophotometry).

4. **Data analysis**
   Results were processed statistically by means of the analysis of variance (ANOVA).
Results
1. Acute toxicity
The results of LC$_5$ and LC$_{50}$ in the respective time intervals are shown in Table 1. The values of 5.89, 5.65, 5.60 and 4.99 mg/L were calculated as LC$_5$ after 24, 48, 72 and 96 hours, respectively, while those of LC$_{50}$ were calculated to be 6.64, 6.32, 6.18 and 5.63 mg/L, respectively. The affected fish were paralyzed and showed restlessness, which started when the fish came into contact with the diazinon bath. An increased reaction to the toxicant was displayed by cramp-like movements of fins and mouth, followed by loss of coordination and orientation in the water column. Fish then turned onto the side and swam in half-circles followed by sudden movements and fin tremor. Darkening of the dorsal body surface and cessation of respiratory movements were also observed shortly before the fish died.

2. Haematology
Results of erythrocyte profile are shown in Table 2. After an acute exposure to diazinon, fish had significantly lower values of RBC, PCV, Hb, MCH and MCHC ($P<0.05$) than those in the control group, while the MCV value was significantly lower in control group ($P<0.05$). Results of the leucocyte profiles are shown in Table 3. The values of WBC, lymphocyte and eosinophil counts were significantly lower for fish exposed to diazinon than fish in the control group ($P<0.05$), while

<table>
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* Indicating values are significantly different between control and experiment.
there was a significant increase in numbers of neutrophils and leucocytes of the test group compared to the control group (P<0.05).

3. **Plasma biochemistry**

Results of diazinon effect on biochemistry of plasma are shown in Table 4. The levels of ALT, ALP, LDH and total protein were significantly lower in fish exposed to diazinon compared to control fish (P<0.05). However, there was no difference in the level of AST between the experimental and the control groups (P>0.05).

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<th>Unit</th>
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Table 3. Leucocyte profile of *H. huso* following exposing to diazinon at 5.63 mg/L at 22°C.

* Indicating values are significantly different between control and experiment.

Table 4. The effect of diazinon at concentration 5.63 mg/L on enzyme activities and total protein (TP) (concentrations of blood plasma of *H. huso* at 22°C.

* Indicating values are significantly different between control and experiment.
Discussion
During the 96-hour acute toxicity test of diazinon on juveniles of *H. huso* weighing about 14 g no mortality occurred in the control group. Based on the calculated 96-hour LC$_{50}$ values (5.63mg/l) obtained in this study, diazinon can be classified as a moderate toxic substance for *H. huso*. In the studies by Khoshbavar-Rostami and Soltani (2002) the 96-hour LC$_{50}$ of diazinon on *Acipenser nudventris* of about 12 g was calculated at 4.6mg/l using similar water quality conditions used in this study. Therefore, it seems that *H. huso* might be more resistant to diazinon than *A. nudventris*. The specific sensitivity of fish to diazinon may be associated with different ability of absorption, acetylcholinesterase inhibition and detoxification as mentioned by other workers (e.g. Eisler, 1986; Keizer et al., 1991; Oh et al. 1991). Also, the obtained LC$_{50}$ may vary depending upon the water quality parameters during the experiment, species and size/weight of experimental fish, which makes it difficult to compare the acute toxicity results among the different species of fish. Similar behavioural abnormalities have been reported in other species such as carp exposed to acute toxicity of diazinon (Rahman et al., 2002; Sobodova et al., 2001).

Significant decrease was observed in the levels of RBC, PCV, Hb, MCH, MCHC, WBC, lymphocyte and eosinophil counts of *H. huso* exposed to acute toxicity of 96-hour LC$_{50}$ diazinon compared to control fish (P<0.05), while MCV value and the numbers of neutrophils were observed to be significantly increased in experimental fish compared to control one (P<0.05). Similar results on RBC, Hb and PCV were found by Sobodova et al. (2001), while values of MCV, MCHC of common carp exposed to acute level of diazinon at 19-21°C were identical to the control group (P>0.05). Results of other published studies show that other substances of organophosphorous pesticides including diazinon affect haematopoesis, thus inducing anaemia in the exposed fish (e.g. Anees, 1978, Benarji & Rajendranath, 1990, Khattak & Hafeez, 1996, Tavares et al., 1999). A significant decrease of leucocyte count (WBC) and the occurrence of lymphopenia and neutrophilia characterize the leucocyte profile of *H. huso* after the acute exposure to diazinon at 22°C. Similar results were observed by Svoboda et al. (2001) after exposing common carp to the acute effect of diazinon at 19-21°C. Occurrence of lymphopenia and neutrophilia in fish exposed to various toxicants including diazinon have been reported by a number of other authors e.g. Siwicki, Cossarini-Dunier & Demael, 1990; Schwalger et al., 1993, Thakur and Sahai, 1993, Alkahem, 1994 and Svobodova et al., 1996. Therefore, a decrease in non-specific immunity of fish can be expected after acute exposure to organophosphorous pesticides due to decreased leucocyte count, lymphopenia and granulocytosis. The changes in differential leucocyte count observed here also indicate a decreased level of non-specific immunity in fish after acute exposure to toxic substances.

Significantly lower levels of LDH, ALT, ALP and total protein were measured in *H. huso* exposed to acute level of diazinon. Similar results were found for the level of LDH, AST, ALP and total protein following the exposure of *Channa punctata* and common carp to an acute level of diazinon for 96 hours (Sastry & Shavma, 1980; Luskova et al., 2002). Signifi-
cant reduction in the concentration of LDH in the blood plasma of experimental *H. huso*, compared with the control group, indicates a decrease in the glycolytic process due to the lower metabolic rate as a result of the effect of diazinon. Luskova *et al.* (2002) observed similar results when common carp were exposed to acute level of diazinon. However, other authors reported an increase in plasma LDH concentration in various fish species (e.g. Gill *et al.*, 1990, Ceron *et al.*, 1997, Sancho *et al.*, 1997). It has been suggested that ALP is inhibited after 96 hours of exposure to a toxicant and then it is either reduced to its normal values or an increased activity is observed (Sasry & Sharma (1980). A reduction in the level of ALP supports the assumption that the liver tissue of the experimental *H. huso* was remarkably affected by acute level of diazinon, as histological examination of affected fish showed necrosis of hepatocytes (data not shown). Also, decreased levels of ALT in experimental fish indicate that diazinon may damage parenchymal tissues or skeletal musculature. However AST was increased in experimental fish following exposure to diazinon compared to fish in the control group. Exposure of *H. huso* to diazinon also caused a significant decrease in protein concentration in the blood plasma compared to the control group. Similar findings were reported for species of eel, *Cyprinon watsoni* and common carp, which were exposed to fenitrothion, malathion and diazinon, respectively (Khattak & Hafeez, 1996, Sancho *et al.*, 1997, Luskova *et al.* 2002). Therefore, because of heavy use of diazinon as pesticide and/or insecticide in the borders of the Caspian Sea, which is a major aquatic environment for sturgeon species, it is recommended to frequently monitor the level of such toxic chemicals in the rivers entering into the sea and in the sea itself.

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**References**


