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Histological Effects of Low Atrazine Concentration on Zebra Mussel (*Dreissena polymorpha* Pallas)

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The s-triazine herbicide atrazine (2-chloro-4-ethylamino-6-isopropyl-amino-s-triazine) is one of the most used pesticides in the world. The greatest quantity of atrazine is used for weed control in corn, sorghum, sugarcane and other crops. It is one of the most frequently detected pesticides in surface- and ground waters in Europe and North America due to its widespread use, low chemical reactivity, relative mobility and high persistence in the environment. The highest concentrations were detected in streams and rivers in agricultural regions, after applications in spring and early summer (Bintein and Devillers 1996; Kolpin et al. 1998; Solomon et al. 1996). Such frequent appearance of atrazine in natural waters has generated a lot of research into its toxicity in aquatic systems, especially on green alga and macrophita (Schober and Lampert 1977; Peterson et al. 1994; Tang et al. 1997; Howe et al. 1998). The effects of atrazine in environmentally realistic concentrations on freshwater animals are less studied. The best known are the effects on fishes. The concentrations of 1.5, 3.0 and 6.0 mg/L of atrazine inhibit biochemical activity of alkaline phosphatase in carp liver, kidneys and heart. In higher concentrations atrazine caused vacuolisation of hepatocytes with fibrosis and necrosis of surrounding tissues. Gill damages were also noted (Nešković et al. 1993.). Depending on the concentration, atrazine induced progressive degradation of kidney tubular and intertubular tissue (Nešković et al. 1993.) and interferes with electrolyte balance in fishes (Prasad and Reddy 1994; Wiegand et al. 2000). The concentration of 0.1 mg/L of atrazine during 10 days causes significant irreversible lysis of kidney cells in the snail *Physa acuta* (Rosés et al. 1999). In environmentally realistic concentrations it also affects biochemical processes in the freshwater snail *Lymnaea palustris* (Baturo et al. 1995; Baturo and Lagadic 1996), while in *Physa acuta* and *Ancylus fluviatilis*, it causes changes in feeding behaviour (Rosés et al. 1999). It is known that environmentally realistic concentrations of atrazine have negative impact on feeding, growth and egg production in freshwater molluscs, cladoceran and leeches (Schober and Lampert 1977; Streit and Peter 1978).

The purpose of this work was to study the effects of low atrazine concentrations on the freshwater mussel *Dreissena polymorpha*, as assessed by changes in mortality and histological alterations.

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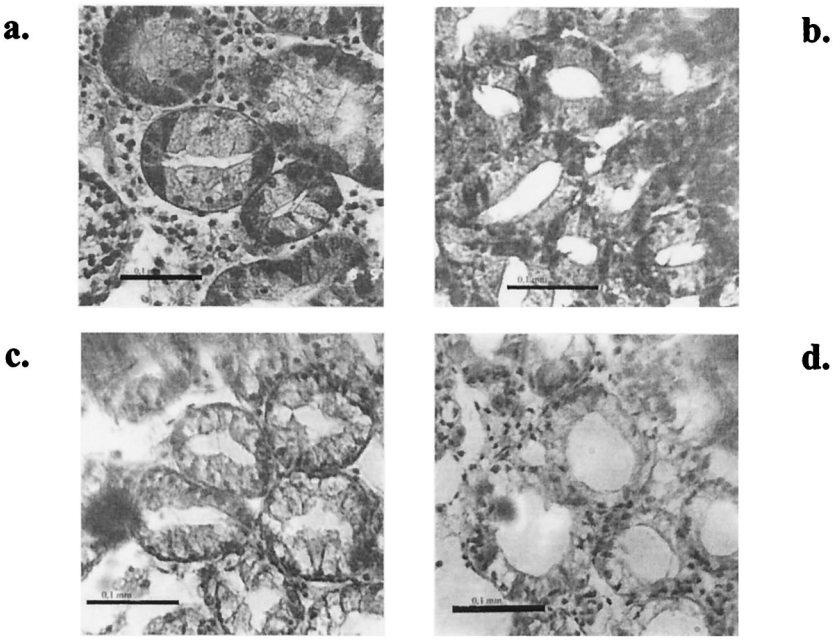

Figure 1. Hepatopancreas of zebra mussel, *Dreissena polymorpha* Pallas. Fixed and stained with haematoxylin-eosin. a. control (14th day of experiment); b. slight widening of tubular lumen and local damages of intertubular connective tissue on 21st day of treatment with 0.003 mg/L of atrazine; c. further widening of tubular lumen, vacuolisation of hepatocytes, partial loss of recognizability of digestive and lateral cells and further damages of intertubular connective tissue on 14th day of treatment with 0.05 mg/L of atrazine; d. total necrosis of connective tissue, strong widening of tubular lumen, shortening and vacuolisation of cells and total loss of recognizability of digestive and lateral cells noticed on 21st day of treatment with 0.5 mg/L of atrazine. Magnification 140x.

MATERIALS AND METHODS

Adult specimens of zebra mussels (*Dreissena polymorpha* Pallas) were collected from Drava river, downstream of Dubrava dam reservoir, in northern Croatia. The experiment was carried out in five glass aquaria, each containing 15 L of dechlorinated tap water. Based on previously published data (Jayachandran et al. 1994; Kolpin et al. 1998), environmentally realistic atrazine concentrations chosen were: 0.003, 0.05, 0.5 and 5 mg/L. One aquarium was used as a control and received dechlorinated tap water only. There were 150 mussels per aquarium. Acclimatisation of animals to laboratory conditions lasted for 24hr. The experiment lasted for 21 days. The test solutions and control water were renewed daily (semi-static test). Temperature of the water was 20.12(±0.84)°C. Mortality was checked daily and dead mussels were removed immediately. Animals were not fed during exposure. For histological analyses, in each experiment 4 live specimens were removed from each exposure concentration and control on the 7th, 14th and 21st days of the experiment. The animal soft tissue was placed in Bouin's fixative for 24hr. For light microscopy observations, fixed mussel tissue

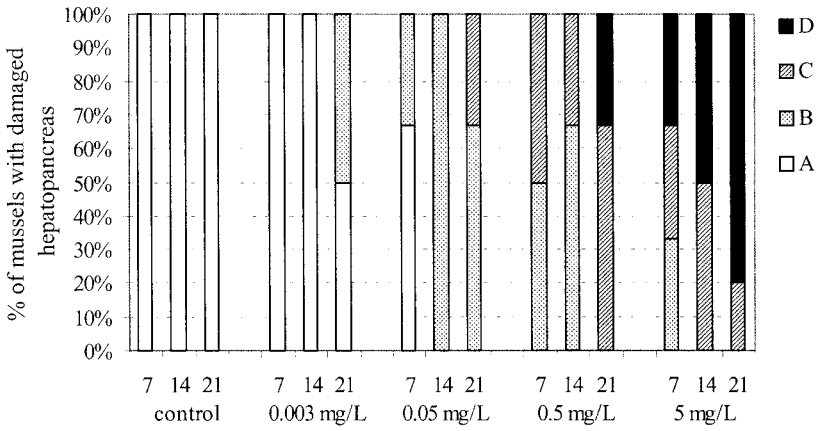

Figure 2. Intensity of histopatological changes in hepatopancreas of zebra mussels treated with atrazine: A – no changes; B - slight widening of tubular lumen and local damages of intertubular connective tissue; C - further widening of tubular lumen, vacuolisation of hepatocytes, partial loss of recognizability of digestive and lateral cells, and stronger damages of intertubular connective tissue; D - total necrosis of connective tissue, progressive widening of tubular lumen, shortening and vacuolisation of cells and total loss of recognizability of digestive and lateral cells.

was dehydrated, embedded in paraffin and cut with a microtome into 6 to 8 μm thick slices. The sections were stained with haematoxylin and eosin and mounted in Canada balsam. The experiment was carried out three times successively during the spring and summer. Mortality data are presented as a mean value of the results obtained in all replicates. Histological effects are analyzed in comparison to the parallel control.

RESULTS AND DISCUSSION

After day 21 of the experiment, the mean mortality rate of the control animal group was less than 5% (4.22% or 6.3±0.58 animals per experiment). The mean mortality rate of mussels treated with 0.003, 0.05 and 0.5 mg/L of atrazine for 21 day was 4.67% (or 7.00±0.00 animals per experiment), 5.33% (or 8.00±1.00 animals per experiment) and 5.11% (or 7.67±1.53 animals per experiment) respectively, and it didn't much differ from the control. Slightly higher mortality (8.22% or 12.33±1.53 animals per experiment) was observed at the end of the treatment with 5 mg/L of atrazine. The mortality rate was almost twice as much as in the control group and in groups treated with lower atrazine concentrations, which may be caused by mild toxic effect of this concentration. The low mortality of animals treated with atrazine (< 10%) showed that tested concentrations are sublethal for this species.

The results of histological analysis of treated animals were consistent in all three experiments and showed that atrazine has the strongest effect on the hepatopancreas. The mildest effect was at the lowest concentration (0.003 mg/L),

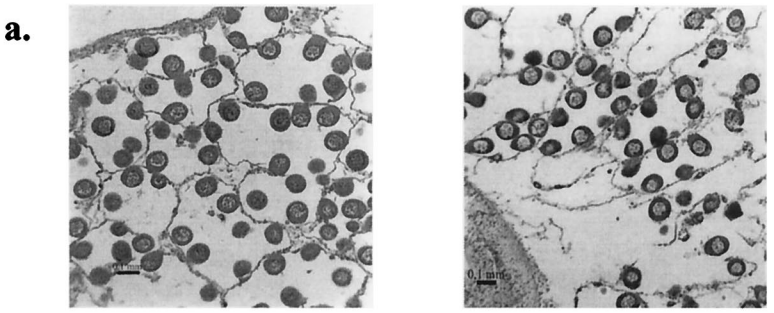

Figure 3. Ovaries of zebra mussel, *Dreissena polymorpha* Pallas. Fixed and stained with haematoxylin-eosin. a. control (21st day of experiment); b. connective tissue damage noticed after 14 days of treatment with 5 mg/L of atrazine. Magnification 35x.

as expected. After 21 days only, this concentration caused the slight widening of tubular lumen and local damages of intertubular connective tissue in half of the treated animals (Figure 1b).

Atrazine at 0.05 mg/L caused damage of the same intensity after 7 days of treatment. After 21 days at this concentration some animals showed stronger changes in the hepatopancreas, such as further widening of tubular lumen, vacuolisation of hepatocytes, partial loss of recognizability of digestive and lateral cells (that build tubules of hepatopancreas), and further damage of intertubular connective tissue (Figure 1c). All animals treated with 0.5 and 5 mg/L of atrazine on the 7th day of the experiment had a damaged hepatopancreas. Depending on exposure, the damage was progressively greater (Figure 2). Intensive damage, like total necrosis of connective tissue, progressive widening of tubular lumen, shortening and vacuolisation of cells and total loss of recognizability of digestive and lateral cells (Figure 1d), were noted on the 21st day of treatment with 0.5 mg/L of atrazine. Changes of similar intensity were also noticed at the 7th day of the treatment with 5 mg/L of atrazine. With longer exposure to this concentration of atrazine the number of the animals with such strong damage increased (Figure 2).

From our results it is clearly seen that intensity of hepatopancreas tissue damage depended upon atrazine concentration and duration of exposure. It is known that atrazine causes changes in metabolism of proteins (Gojmerac et. al. 1996), so damage of digestive and lateral tubular cells established in this study can be caused by changes in protein metabolism. As an alkaline stain, haematoxylin binds with acid molecules in the cell and darkly colours the cytoplasm of lateral cells, making the difference between digestive and lateral cells visible. During our experiment, depending on the concentration and duration of exposure, the recognizability of those two types of tubular cells in the hepatopancreas of treated animals progressively disappeared. The cause can be disturbances of protein synthesis and changes in pH of lateral cells. Very strong damage, like those caused by the highest test concentrations (0.5 and 5 mg/L), probably also caused dysfunction of the hepatopancreas. Although the hepatotoxicity of atrazine wasn't determinate in herbivorous invertebrates until now (Rosés et al. 1999), a strong

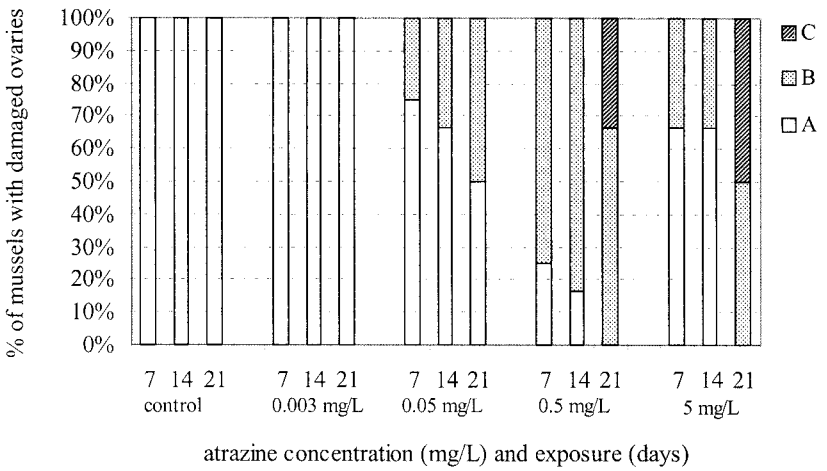

Figure 4. Intensity of histopatological changes in ovaries of zebra mussels treated with atrazine: A – no changes; B - mild necrotic changes of the connective tissue; C - strong necrosis on the peripheral parts of the gland, floccules completely separated.

effect on the hepatopancreas was expected. The hepatopancreas is an organ similar to the liver and pancreas in vertebrates and besides digestion, has a significant role in general metabolism and detoxication processes. Depending on dosage and the duration of exposure, atrazine interferes with glycogen and lipid metabolism in the liver of mammals and birds and causes intestinal hepatitis and steatosis (Čurić et al. 1999; Srebočan et al. 1975). Hepatotoxicity of atrazine has been determined in fishes also. Nešković and others (1993) noted that subacute exposure to 1.5, 3 and 6 mg/L of atrazine inhibits biochemical activities of alkaline phosphatase in carp liver. Concentrations of 3 and 6 mg/L caused vacuolisation of carp hepatocytes followed by fibrosis and necrosis of surrounding tissue (Nešković et al. 1993). In our study similar but stronger effects were determined on zebra mussel hepatopancreas at lower concentrations of atrazine (0.05, 0.5 and 5 mg/L), which implies that freshwater invertebrates are more sensitive to atrazine. In the study of Rosés and colleagues (1999) after 10 days of exposure to 0.1 mg/L of atrazine no damage was found on the hepatopancreas in the snails *Physa acuta* and *Ancylus fluviatilis*. In our experiment, similar atrazine concentrations (0.05 and 0.5 mg/L) caused visible and relatively strong effects on histological features of the hepatopancreas, which shows that *Dreissena polymorpha* is more sensitive to atrazine than snails. This can be explained by different feeding habits of these species. *Dreissena polymorpha* an intensive filterer, probably has greater intake of atrazine and other xenobiotics dissolved in water.

At the end of the experiment, we did not notice any degenerative changes on the gills of animals treated with 0.003, 0.05, 0.5 and 5 mg/L of atrazine. Histological build of the gills was no different from the control. Regarding their position and function, gills are in direct contact with water and all the xenobiotics in it. A number of studies confirmed that this organ is very sensitive to many toxicants. Local hyperplasia of epithelium was noticed in gills of carp exposed to 0.1 and 3

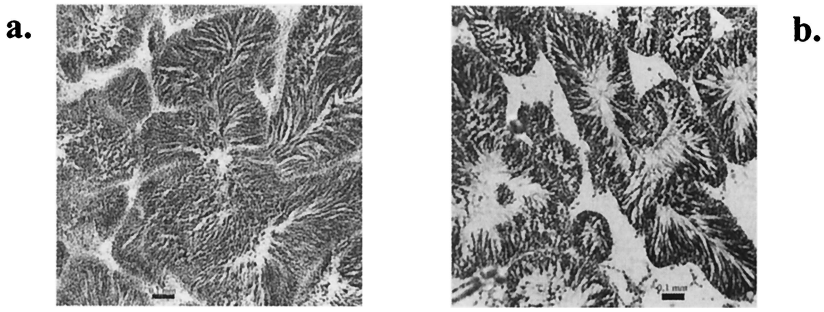

Figure 5. Testes of zebra mussel, *Dreissena polymorpha* Pallas. Fixed and stained with haematoxylin-eosin. a. control (21st day of experiment); b. necrotic changes of the connective tissue noticed on the 7th day of treatment with 0.5 mg/L of atrazine. Magnification 35x.

mg/L of atrazine. In fishes exposed to 6 mg/L of atrazine, damage was more progressive (Nešković et al. 1993).

Treatment with the lowest concentration (0.003 mg/L) did not cause any changes on the gonads of both sexes. Higher concentrations of atrazine (0.05, 0.5 and 5 mg/L) caused damage of loose connective tissue and interstitial cells in both ovaries and testes. Mild necrotic changes of the ovarian connective tissue were noted in some animals treated with 0.05, 0.5 and 5 mg/L of atrazine for 7 and 14 days (Figure 4). On the 21st day, all animals treated with 0.5 and 5 mg/L of atrazine had the necrotic changes in ovarian connective tissue. In some of those animals, necrosis was so strong that on the peripheral parts of the gland the floccules were completely separated (Figures 3b and 4). The first changes on testes were noticed in a few animals treated with 0.05 mg/L of atrazine for 7 days (Figures 5 and 6). Local necrotic changes of the testicular connective tissue (Figure 5b) were noted on the 14thand 21st day at that atrazine concentration. Testes of all the animals treated with 0.5 and 5 mg/L were damaged by the 7th day. In some of those animals, besides the strong necrosis of connective tissue, disturbances in spermatozoa arrangement and collection in the center of the follicle were noted (Figure 6). Intensity of connective tissue damage in gonads was clearly dependent on atrazine concentration and duration of exposure. Atrazine did not cause any visible changes on gametes. From the previous studies it is clear that atrazine interferes with normal reproductive function in mammals and some freshwater invertebrates (Čurić et al. 1999; Schober and Lampert 1977; Streit and Peter 1978). Besides histopatological changes of gonads and reproductive cells, atrazine caused changes in metabolism of steroid hormones (Gojmerac et al. 1996; Kniewald et al. 2000). We are planning to study possible similar effects of atrazine on reproduction of zebra mussel, during the period of gonadal maturation and spawning.

Because of lifestyle, population density and effectiveness of filtration, *Dreissena polymorpha* Pallas is a very significant link in the food net of freshwater ecosystems (Stuijfzand et al. 1995). Since its natural area includes freshwater ecosystems in agricultural areas of Europe and North America, it is likely that

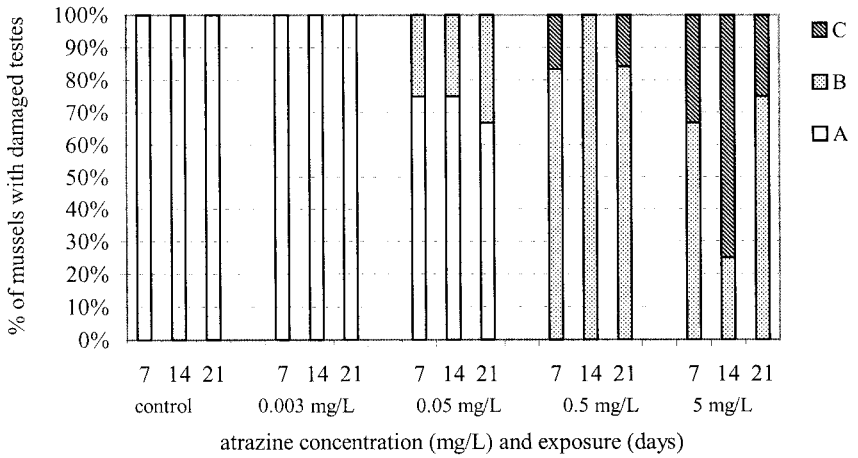

Figure 6. Intensity of histopatological changes in testes of zebra mussels treated with atrazine: A – no changes; B - local necrotic changes of the connective tissue; C - strong necrosis of connective tissue, disturbances in spermatozoa arrangement and collection in the centre of the follicle were noted.

this species will be exposed to atrazine in its natural habitat. Concentrations of atrazine that we used in our study are close to those frequently detected in surface and ground waters in Europe and the United States, where this herbicide is in heaviest use (Kolpin et al. 1998).

The lowest tested concentration of atrazine – 0.003 mg/L (3 μg/L or 3 ppb), is the US EPA maximum contaminant level (MCL) for this herbicide in USA drinking waters. The U.S. Geological Survey detected it in drinking water supplies throughout the midwest regularly (Kolpin et al. 1998). In this study we determine that even this concentration has mild hepatotoxic effects on *Dreissena polymorpha*.

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