The effects of atrazine exposure on early life stages of common carp (*Cyprinus carpio*)

Lucie Chromcová [1], Jana Blahová [1], Lucie Plhalová [1], Dana Živná [1], Stanislava Štěpánová [1], Eva Prášková [1], Miroslav Prokeš [2], Lenka Zelníčková [1], Miša Škorič [1], Zdeňka Svobodová [1]

1 University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
2 Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, v.v.i., Brno, Czech Republic

Correspondence to: Mgr. Lucie Chromcová,
Department of Veterinary Public Health and Animal Welfare,
Faculty of Veterinary Hygiene and Ecology,
University of Veterinary and Pharmaceutical Sciences Brno,
Palackého tř. 1/3, 612 42 Brno, Czech Republic
tel: +420 541 562 780; fax: +420 541 562 790; e-mail: chromka.l@seznam.cz

Submitted: 2013-06-21 Accepted: 2013-08-30 Published online: 2013-11-10

Key words: **embryo-larval toxicity test; LOEC; oxidative stress; TBARS; defense enzymes**

Neuroendocrinol Lett 2013; **34**(Suppl.2):95–101 **PMID:** 24362100 NEL341013A15

Abstract

OBJECTIVES: Atrazine and its chloro-s-triazine metabolites are found in surface water and groundwater of the Czech Republic, although their use has been banned since 2005. The objective of the study was to determine the toxic effects of atrazine at an environmentally relevant concentration of atrazine, 0.3 μg.L^{-1} and at concentrations of 30, 100, and 300 μg.L^{-1} on morphometric and condition characteristics, development of early life stages, and antioxidant defense enzymes of common carp.

METHODS: The embryo-larval toxicity test was performed according to the OECD Guidelines 210 (Fish, Early-life Stage Toxicity Test).

RESULTS: Atrazine exposure showed no effect on morphometric and condition characteristics or histology. Exposure at 0.3 μg.L^{-1} was associated with significantly increased activity of glutathione peroxidase, glutathione S-transferase, superoxide dismutase, and catalase compared to control. Activity of glutathione reductase was slightly higher at a concentration of 0.3 μg.L^{-1}, with significantly lower (p<0.05) activity observed in groups exposed to 30, 100, and 300 μg.L^{-1} compared to the group exposed to 0.3 μg.L^{-1}. The level of oxidized lipids was slightly higher in groups exposed to atrazine at 100 and 300 μg.L^{-1} compared to controls.

CONCLUSIONS: Atrazine has a significant influence on the biotransformation enzyme and oxidative defense enzymes of early life stages of common carp. The lowest observed effect concentration (LOEC) was 0.3 μg.L^{-1}.

Abbreviations:

ANC4.5	- Acid Neutralizing Capacity
CAS	- Chemical Abstracts Service Registry Number
CAT	- Catalase
COD_{Mn}	- Chemical Oxygen Demand
FCF	- Fulton's condition factor
GPx	- Glutathione peroxidase
GR	- Glutathione reductase
GST	- Glutathione S-transferase
I	- Inhibition of specific growth
LC_{50}	- Median lethal concentration
LOEC	- Lowest observed effect concentration
OECD	- Organization for Economic Cooperation and Development
ROS	- Reactive oxygen species
SD	- Standard deviation
SE	- Standard error
SGR	- Specific growth rate
SOD	- Superoxide dismutase
TBARS	- Thiobarbituric acid reactive substances
TL	- Total length
W	- Weight

ORIGINAL ARTICLE

INTRODUCTION

Atrazine (6-chloro-*N*2-ethyl-*N*4-isopropyl-1,3,5-triazine-2,4-diamine) is a selective systemic herbicide belonging to the s-triazine family that inhibits photosynthesis. It was first registered in the United States in 1958 and has been used in agriculture to kill weeds in crops including maize, sorghum, sugarcane, winter wheat, pasture, hay, and minor crops (guava, macadamia nuts) and to kill grassy weeds and submerged vegetation in stagnant and slow running waters (Hussein *et al.* 1996; Kimbrough & Litke 1996; U.S. EPA 2001; WHO 2010). Atrazine and its chloro-s-triazine metabolites (deethylatrazine, deisopropylatrazine, and diaminochlorotriazine) are present in surface water and groundwater as a result of the use of atrazine as a pre-emergent or early post-emergent herbicide (WHO 2010).

This herbicide continues to be one of the most widely used in the United States, China, Brazil, and Argentina (Zhou *et al.* 2008; Svartz *et al.* 2012; Xing *et al.* 2012a). In compliance with the European Commission Decision 2004/248/EC, the Czech Republic has banned use of atrazine since 1 August 2005. According to the Czech Hydrometeorological Institute, this substance is a current contaminant of rivers in the Czech Republic detectable at concentrations ranging from 0.3 to 1 $\mu g.L^{-1}$.

Degradation of atrazine can take place through photolysis and by microorganisms in surface water followed by hydrolysis to the chloro-substituent (half-life is >100 days at 20 °C), but degradation decreases with increasing water depth. Atrazine has low solubility and can be stable and persist in groundwater. The presence of atrazine and its metabolites in surface water is most likely to be intermittent, particularly in flowing water, but groundwater contamination will usually be persistent (Burnside *et al.* 1963; Howard 1989; WHO 2010). Owing to its persistence in the environment, minimal, but essentially chronic, effects are associated with atrazine exposure in certain aquatic systems (Hussein *et al.* 1996; Solomon *et al.* 1996). The issue of contamination by atrazine has increased in importance in recent years (Du Preez *et al.* 2005; Murphy *et al.* 2006).

In aquatic ecosystems, the ecotoxicological relevance of atrazine has been widely studied in various aquatic animals, including fish (Huber 1993; Solomon *et al.* 1996; Elia *et al.* 2002; Phyu *et al.* 2006; Dong *et al.* 2009; Mhadhbi & Beiras 2012; Mhadhbi *et al.* 2012; Xing *et al.* 2012a). Most studies have focused on acute toxicity of atrazine and its adverse effects on fish: reduced sperm production; increased renal excretion of Na^+, Cl^-, and protein in rainbow trout and carp; disruptions of normal behavior; damage to kidney and gill epithelium; and decreased ability to withstand warm temperatures (Fischer-Scherl *et al.* 1991; Neskovic *et al.* 1993; Steinberg *et al.* 1995; Hussein *et al.* 1996). Critical development of tissues and organs in embryos and larvae can be easily disrupted by exposure to toxic compounds (Foekema *et al.* 2008; Kammann *et al.* 2009; Praskova *et al.* 2011). Early life stages of fish are generally regarded as the most sensitive to toxic agents (Hutchington *et al.* 1998; Wiegand *et al.* 2001; Hostovsky *et al.* 2012; Svartz *et al.* 2012) and are ideal models for determining responses to environmental contaminants. The prevalence of triazine pesticides in the aquatic environment, and their potential for adverse effects, makes them strong candidates for toxicological studies. The objective of the present study was to determine the effects of atrazine on early life stages of common carp with respect to morphometric and condition characteristics, histopathology, and antioxidant defense enzymes.

MATERIAL AND METHODS

Experimental protocol

Embryo-larval toxicity testing was performed according to OECD guidelines 210 (Fish, Early-life Stage Toxicity Test). Fertilized eggs of common carp were obtained from Rybnikarstvi Pohorelice a.s. Eggs were produced according to standard methods of artificial reproduction (Kocour *et al.* 2005).

A semi-static trial with solution replacement twice daily was used. Replacement was conducted with care to avoid interfering with development of embryos and larvae. Temperature range was 21.3–22.9 °C, pH was 8.1–8.9, and dissolved oxygen saturation was >60%; conditions were monitored daily.

Eggs were collected 24 h post-fertilization and groups of 100 were transferred into each of fifteen 900 ml crystallization dishes. Three groups of 100 eggs were exposed to atrazine (Sigma–Aldrich, Czech Republic; chemical purity – 98.9%) at the reported environmental concentration in the Czech Republic of 0.3 $\mu g.L^{-1}$ and three to each of the concentrations of 30, 100, 300 $\mu g.L^{-1}$ and in atrazine-free tap water as control. Concentrations during the test did not decrease <80% of the nominal concentration.

Day 1 post-fertilization was designated as the beginning of the embryo-larval toxicity test, and the test was terminated at 33 days, when all larvae in the control group had become juveniles. Larvae were fed *ad libitum* twice daily on fresh *Artemia salina nauplii*. The hatching rate and mortality were monitored also twice daily. Dead embryos and larvae were removed and recorded.

Sampling was done on days 6, 12, 19, 26, and at the conclusion of the test on day 33. From each group, 30 specimens (day 6 and 12), 15 specimens (day 19 and 26), and 45 specimens (day 33) were collected and stored in 4% formaldehyde. Samples were used for monitoring developmental stages, morphological abnormalities, and morphometric and condition characteristics: total length (TL) (stereomicroscopically to 0.01 mm using a micrometer), weight (W) (to 0.1 mg), Fulton's condition factor (FCF), specific growth rate (SGR), and inhibition of specific growth (I).

Sample specimens were assigned to developmental stages according to Penaz *et al.* (1983): nine embryonic (E1–E9), six larval (L1–L6), and two juvenile (J1–J2) stages.

Water parameters

The basic physical and chemical parameters of the dilution water used in the embryo-larval toxicity test were, acid neutralizing capacity ($ANC_{4.5}$), 3.6–3.7 mmol.L^{-1}; chemical oxygen demand (COD_{Mn}), 1.4–1.9 mg.L^{-1}; total ammonia, below the limit of determination (<0.04 mg.L^{-1}); nitrates, 12.1–13.6 mg.L^{-1}; nitrites, below the limit of determination, <0.01 mg L^{-1}; Cl^-, 17.5 to 18.5 mg L^{-1}; and Σ Ca + Mg, 3.06 mmol L^{-1}.

Morphometric and condition characteristics

Fulton's condition factor was calculated at each sampling time.

$$FCF = \frac{W.10^5}{TL^3}$$

W= weight (mg), TL = total length (mm).

The mean specific growth rate (SGR) was calculated for each group beginning on day 6 (the first sampling time) and on day 33 (completion of the test).

$$SGR = \frac{\overline{\ln W_2} - \overline{\ln W_1}}{T_2 - T_1} \cdot 100$$

(W_1 = weight (mg) at time T_1 (6 day), W_2 = weight (mg) at time T_2 (33 day), T_1 = first sampling time, T_2 = end of the test).

The inhibition of specific growth rate (*I*) was calculated as follows:

$$I\ [\%] = \frac{SGR\ (contol)\ -\ SGR\ (group)}{SGR\ (control)} \cdot 100$$

Histology

Thirty additional specimens from each group were taken for histological examination at the end of the test. Samples were fixed in buffered 4% formaldehyde, dehydrated, embedded in paraffin wax, sectioned (cross section) at 4 μm, and stained with hematoxylin and eosin. Histology of skin, gill, kidney, and liver was assessed by light microscopy.

Evaluation of antioxidant defense enzymes and lipid peroxidation

At the end of the test, a further 30 specimens from each test and control group were killed and weighed, homogenized with phosphate buffer (pH 7.2), and stored at –85 °C until analysis. The catalytic concentrations of glutathione S-transferase (GST), glutathione reductase (GR), catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) were measured spectrophotometrically (Habig *et al.* 1974; Carlberg & Mannervik 1975; Aebi 1984; Flohe & Gunzler 1984; Ewing & Janero 1995). Protein concentrations were determined by a Bicinchoninic Acid Protein Essay Kit (Sigma–Aldrich, St. Louis, MO, USA) using bovine serum albumin as standard (Smith *et al.* 1985). To assess lipid peroxidation in the samples, malondialdehyde was measured by lipid peroxidation – thiobarbituric acid reactive substances (TBARS) method by Lushchak *et al.* (2005).

Determination of atrazine

Gas chromatography with ion trap tandem mass spectrometry (GC/IT-MS) was used for the determination of atrazine. A sample preparation was based on simple liquid-liquid extraction into cyclohexane.

Separation, identification and quantification were based on the GC/IT-MS method. A Varian 450-GC gas chromatograph (Varian Inc., USA) and VF-5ms (30 m × 0.25 mm) column were used for separation. A Varian 220-MS (Varian Inc., USA) ion trap mass spectrometer was used for identification and quantification. Chromatographic and MS conditions were based on the method described by Perreau & Einhorn (2006). All solvents were of GC/MS-grade purity (Chromservis, s.r.o., CZ). A certified standard of atrazine was purchased from Dr. Ehrenstorfer GmbH (Germany).

The detection limit (3σ) for atrazine was 0.04 μg.L^{-1}. Expanded uncertainty was 6.3% on the condition that the coefficient of expansion was k=2.

Statistical analysis

Statistical analysis was conducted with Statistica 8.0 for Windows (StatSoft, Inc., Tulsa, OK). A normality test of all of the datasets obtained for the parameters investigated was conducted with the Shapiro–Wilk test. An analysis of variance (ANOVA) was used to evaluate results of oxidative stress parameters. When significant differences were found, a conservative Tukey's test was conducted as a post hoc test to determine differences among groups. Data of cumulative mortality, morphometric and condition parameters, and hatching did not show normal distribution, and data were subjected to a Kruskal-Wallis ANOVA. Significant differences among pairs of groups were evaluated using the multi-sample rank sums test.

RESULTS

Hatching

Hatching began on day 3 in all groups. On the first day of hatching, treatment groups showed significantly higher (p<0.05) hatching rates compared to the control group (8%) in this order: 30% (100 μg.L^{-1}), 28% (30 μg.L^{-1}), 26% (0.3 μg.L^{-1}), and 20% (300 μg.L^{-1}). Hatching was completed on day 6.

Mortality rate

Cumulative mortality in the control group and all exposed groups was similar, below 23%.

Early ontogeny

The developmental stages observed at the sampling times in all tested concentrations and controls did not show significant differences in early ontogeny.

Morphological anomalies

The control and environmental concentration showed morphological anomalies in fewer than 5% of specimens. Anomalies (edema, yolk sac deformity, axial and/or lateral curvature of the spine – lordosis, scoliosis, and shortened body) were observed in approximately 25% of the fish in the 30 μg.L^{-1}, 100 μg.L^{-1}, and 300 μg.L^{-1} group on day 6.

Histopathology

No lesions of skin, gill, or liver were observed in any group.

Morphometric and condition characteristics

Atrazine exposure did not significantly affect morphometric or condition characteristics of early life stages of common carp. Significant differences appeared only in TL (mm) after 6 and 12 days of atrazine exposure (Table 1).

Tab. 1. Morphometric and condition characteristics of carp embryos and larvae at 6, 12, and 33 days exposure to atrazine. TL_6, TL_{12}, TL_{33} - median total body length, W_6, W_{12}, W_{33} - median fish weight, FCF_{33} - median Fulton's condition factor, SGR - specific growth rate, I - inhibition of specific growth.

Group	Control	0.3 μg.L^{-1}	30 μg.L^{-1}	100 μg.L^{-1}	300 μg.L^{-1}
TL_6 (mm)	6.26^{a}	6.12^{ab}	6.28^{ac}	6.14^{ab}	5.93^{b}
W_6 (mg)	1.60^{a}	1.50^{a}	1.50^{a}	1.50^{a}	1.40^{a}
TL_{12} (mm)	8.32^{a}	8.00^{ab}	7.73^{b}	7.81^{b}	7.68^{b}
W_{12} (mg)	5.10^{a}	3.90^{a}	4.05^{a}	3.80^{a}	3.90^{a}
TL_{33} (mm)	18.24^{a}	18.54^{a}	17.95^{a}	18.09^{a}	18.29^{a}
W_{33} (mg)	79.70^{a}	85.75^{a}	73.90^{a}	76.80^{a}	82.30^{a}
FCF_{33}	1.33^{a}	1.35^{a}	1.31^{a}	1.36^{a}	1.38^{a}
SGR	14.48	14.82	14.14	14.59	14.97
I (%)	–	–2.34	2.32	–0.75	–3.39

Significant differences among groups are indicated by different alphabetic superscripts (p<0.05).

Oxidative stress

Glutathione reductase

The highest activity of GR was found in the control and environmental concentration. Significantly lower (p<0.05) activity of GR was observed in groups exposed to 30, 100 and 300 μg.L^{-1} compared to the group exposed at 0.3 μg.L^{-1}, but no significant differences were found compared to the control (Table 2).

Catalase

The slight increase in CAT activity was observed at all tested concentrations compared to the control group, with significant difference (p<0.05) seen only at the environmental concentration of 0.3 μg.L^{-1} (Table 2).

Superoxide dismutase

Compared to the control group, slightly increased SOD activity was detected at 0.3 μg.L^{-1} and 30 μg.L^{-1} atrazine, with higher activity (p<0.05) at 0.3 μg.L^{-1}. Atrazine at 300 μg.L^{-1} showed a significant (p<0.05) reduction in SOD activity compared to the control group (Table 2).

Glutathione S-transferase

GST activity was significantly increased (p<0.05) in larvae exposed to atrazine at 0.3 μg.L^{-1} compared to the control. The lowest level (p<0.05) of GST activity was observed in groups exposed to 100 and 300 μg.L^{-1} compared to the group exposed at 0.3 and 30 μg.L^{-1}, but no significant differences were found compared to the control group (Table 2).

Lipid peroxidation – TBARS

The slight increase in TBARS values compared to the control group was observed with atrazine at 100 and 300 μg.L^{-1} (Table 2). A technical problem precluded measuring TBARS levels in the 0.3 and 30 μg.L^{-1} concentrations.

Glutathione peroxidase

A significant increase (p<0.05) in GPx activity compared to the control was found in all atrazine exposed groups (Figure 1).

Tab. 2. Activity of GR (nmol.min^{-1}.mg protein^{-1}), CAT (μmol.min^{-1}.mg protein^{-1}), SOD (U.mg protein^{-1}) and GST (nmol.min^{-1}.mg protein^{-1}), and content of TBARS (nmol.g^{-1} wet tissue) in common carp (mean ± SE) after atrazine exposure.

Group	Control	0.3 μg.L^{-1}	30 μg.L^{-1}	100 μg.L^{-1}	300 μg.L^{-1}
GR	6.04±0.56^{ab}	6.84±0.69^{a}	5.46±1.01^{b}	5.59±1.20^{b}	5.64±0.68^{b}
CAT	19.67±2.05^{a}	24.52±2.87^{b}	22.88±4.28^{ab}	21.33±4.07^{ab}	22.14±2.62^{ab}
SOD	3.17±0.41^{b}	3.71±0.17^{a}	3.34±0.28^{ab}	3.00±0.35^{bc}	2.81±0.13^{c}
GST	101.80±8.60^{bc}	118.99±7.00^{a}	109.07±11.70^{ac}	93.73±7.30^{b}	91.91±5.07^{b}
TBARS	17.80±10.06^{a}	–	–	22.98±3.71^{a}	18.60±4.37^{a}

Significant differences among groups are indicated by different alphabetic superscripts (p<0.05).

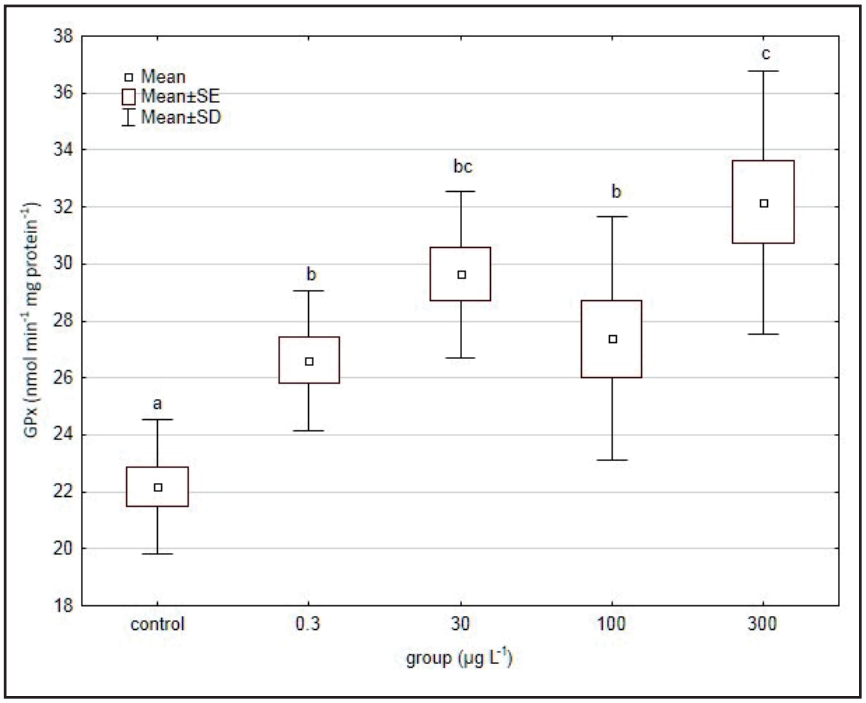

Fig. 1. GPx (nmol min^{-1} mg $protein^{-1}$) in common carp larvae after 33 days exposure to atrazine. Significant differences among groups are indicated by different alphabetic superscripts ($p<0.05$).

DISCUSSION

Acute toxicity tests on various species of fish have determined 48 h LC_{50} values for atrazine ranging from 10 to 100 mg.L^{-1} (Svobodova *et al.* 1987). Wiegand *et al.* (2000) found 48 h LC_{50} in early life stages of zebrafish (*Danio rerio*) to be 36.8 mg.L^{-1}. There are currently no reports of acute poisoning of fish by pesticides with the active ingredient of atrazine (Svobodova *et al.* 2011).

Atrazine and its metabolites remain in the aquatic environment, as these chemical substances are persistent in surface and groundwaters (Sehonova *et al.* 2012). In our study, the embryo-larval toxicity test used an environmentally relevant concentration of atrazine, 0.3 µg.L^{-1}, and concentrations of 30, 100, and 300 µg.L^{-1}. After 33 days of exposure, the range of concentrations did not differ in effects on the early developmental stages, mortality, morphometric and condition characteristics, and histopathology of common carp. Scahill (2008) obtained similar results, observing that atrazine (20–250 µg.L^{-1}) did not significantly affect timing of development, muscle contractions, embryo length, yolk diameter, or mortality of embryonic fathead minnows (*Pimephales promelas*) and the African clawed frog *(Xenopus laevis)*.

At day 6 of exposure, the carp embryos showed morphological anomalies at atrazine concentrations of 30, 100, and 300 µg.L^{-1} (edema, yolk sac deformity, lordosis, scoliosis, and body shortening). Similarly, Scahill (2008) described abnormal spine curvature, abnormal eye, and lack of pigmentation in atrazine exposed frogs and fish.

Our study found atrazine to have significant effects on biotransformation and oxidative enzymes and defense systems at 0.3 µg.L^{-1}. Atrazine and its metabolites remain in the organism and are associated with damage such as increased superoxide radical formation (Xing *et al.* 2012a). The oxygen free radicals and other reactive oxygen species potentially cause oxidative damage to membrane lipids, DNA, and proteins and induce changes in antioxidant enzyme levels (Halliwell & Gutteridge 1999; Avci *et al.* 2005; Valavanidis *et al.* 2006; Di Giulio & Meyer 2008; Slaninova *et al.* 2009; Hostovsky *et al.* 2012).

The present study showed significantly higher ($p<0.05$) activity of SOD, CAT, and GST in larvae exposed to 0.3 µg.L^{-1} atrazine compared to the control and slightly increased activity of these enzymes at 30 µg.L^{-1} atrazine (Table 2). Blahova *et al.* (2013) documented a slight increase of SOD and GST activity in zebrafish after subchronic exposure of atrazine in the environmental concentration of 0.3 µg.L^{-1}. Xing *et al.* (2012a) detected increase of SOD and CAT with atrazine at 4.28 µg.L^{-1} in common carp after 40 days exposure, and Jin *et al.* (2010) reported an increase ($p<0.05$) of SOD and CAT activity in ovary of zebrafish after 14 day exposure to atrazine at 10 µg.L^{-1} compared to a control group. Like other organisms, fish can combat elevated levels of ROS in their systems with protective ROS-scavenging enzymes such as SOD and CAT, which convert superoxide anions (O_2^-) to H_2O_2 and then to H_2O and O_2. Thus, it is possible that an increase in the activity of these enzymes contributes to the elimination of ROS induced by atrazine exposure from the cell (Jin

et al. 2010). However, our results showed that a higher concentration of atrazine used in our experiment (100 μg.L^{-1}) was associated with decreased activity of SOD, CAT, and GST. Similar results have been obtained in other studies, suggesting that organisms suffered serious damage due to weakening of their antioxidant capacity (Wiegand *et al.* 2001; Xing *et al.* 2012a, b; Blahova *et al.* 2013).

Glutathione reductase plays an important role in cell antioxidant protection in catalyzing the regeneration of glutathione. A decrease in this enzyme activity may result in glutathione depletion and a reduction in antioxidant defense (Stepanova *et al.* 2013). Higher GR activity was found only at 0.3 μg.L^{-1}, while other concentrations showed a reduction in GR activity. Significant differences (p<0.05) were found only between environmental levels 0.3 μg.L^{-1} and other concentrations (Table 2). Blahova *et al.* (2013) observed lower GR activity in zebrafish exposed to atrazine at 90 μg.L^{-1} compared to concentrations of 0.3 μg.L^{-1} and 30 μg.L^{-1}. Conversely, Nwani *et al.* (2010) reported GR activity unchanged in the liver of *Clarias punctatus* following exposure to atrazine at 4.24, 5.30, and 10.60 mg L^{-1}.

Activity of GPx converts H_2O_2 or other lipid peroxides to water or hydroxyl lipids, and, in the process, glutathione is converted to oxidized glutathione. A significant increase in GPx activity compared to the control was found in fish in all groups exposed to atrazine (Figure 1). Xing *et al.* (2012a) obtained similar results when, after a 40 day exposure of common carp to atrazine, GPx activity was highest at concentrations 4.28 and 42.8 μg.L^{-1}.

Lipid peroxidation determined by the formation of TBARS is one of the commonly used markers of oxidative stress. The TBARS level in the group exposed to 100 and 300 μg.L^{-1} was slightly increased over than of the control group (Table 2). Our results are in agreement with Nwani *et al.* (2010) and Blahova *et al.* (2013), who confirmed an elevated level of lipid peroxidation in liver of zebrafish exposed to atrazine at 90 μg.L^{-1}.

Exposure to atrazine demonstrated a significant effect on biotransformation and oxidative defense enzymes of early life stages of common carp, and, based on these results, LOEC = 0.3 μg.L^{-1} was determined. The determined LOEC value is equal to atrazine environmental concentration in the Czech Republic (0.3 μg.L^{-1}).

ACKNOWLEDGMENTS

This research was supported by GACR P502/12/P163. We would like to thank Lucidus Consultancy for manuscript improvement and English correction.

REFERENCES

1 Aebi H (1984). Catalase in vitro. Methods Enzymol. **105**: 121–126.
2 Avci A, Kaçmaz M, Durak I (2005). Peroxidation in muscle and liver tissues from fish in a contaminated river due to a petroleum refinery industry. Ecotoxicol Environ Saf. **60**: 101–105.
3 Blahova J, Plhalova L, Hostovsky M, Divisova L, Dobsikova R, Mikulikova I, et al (2013). Oxidative stress responses in zebrafish *Danio rerio* after subchronic exposure to atrazine. Food Chem Toxicol. pii: S0278-6915(13)00150-6. doi: 10.1016/j.fct.2013.02.041.
4 Burnside OC, Fenster CR, Wicks GA (1963). Dissipation and leaching of monuron, simazine and atrazine in Nebraska soils. Weeds. **11**: 209–213.
5 Carlberg I, Mannervik B (1975). Purification and characterization of the flavoenzyme glutathione reductase from rat liver. J Biol Chem. **250**: 5475–5480.
6 Di Giulio RT, Meyer JN (2008). Reactive oxygen species and oxidative stress. In: Di Giulio RT, Hinton EE, editors. The Toxicology of Fishes. Boca Raton: CRC Press, Taylor and Francis Group. p. 273–324.
7 Dong X, Zhu L, Wang J, Wang J, Xie H, Hou X, et al (2009). Effects of atrazine on cytochrome P450 enzymes of zebrafish (*Danio rerio*). Chemosphere. **77**: 404–412.
8 Du Preez L, Solomon KR, Carr JA, Giesy JP, Gross TS, Kendall RJ, et al (2005). Population structure of the African Clawed Frog (*Xenopus laevis*) in maize-growing areas with atrazine application versus non-maize-growing areas in South Africa. Afr J Herpetol. **54**: 61–68.
9 Elia AC, Waller WT, Norton SJ (2002). Biochemical responses of bluegill sunfish (*Lepomis macrochirus*, Rafinesque) to atrazine induced oxidative stress. Bull Environ Contam Toxicol. **68**: 809–816.
10 European Commission (2004) COMMISSION DECISION of 10 March 2004 concerning the non-inclusion of atrazine in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing this active substance (No. 2004/248/EC). Official Journal L **78**: 53–55.
11 Ewing JF, Janero DR (1995). Microplate superoxide dismutase assay employing a nonenzymatic superoxide generator. Anal Biochem. **10**: 243–248.
12 Fischer-Scherl T, Veeser A, Hoffmann RW, Kühnhauser C, Negele RD, Ewringmann T (1991). Morphological effects of acute and chronic atrazine exposure in rainbow trout (*Oncorhynchus mykiss*). Arch Environ Con Tox. **20**: 454–461.
13 Flohe L, Gunzler WA (1984). Assays of glutathione peroxidase. Methods Enzymol. **105:** 114–121.
14 Foekema EM, Deerenberg CM, Murk AJ (2008). Prolonged ELS test with the marine flatfish sole (*Solea solea*) shows delayed toxic effects of previous exposure to PCB 126. Aquat Toxicol. **90**: 197–203.
15 Habig WH, Pabst MJ, Jakoby WB (1974). Glutathione S-transferases. First enzymatic step in mercapturic acid formation. J Biol Chem. **249**: 7130–7139.
16 Halliwell B, Gutteridge JMC (1999). Free Radicals in Biology and Medicine. 3rd ed. Oxford: Oxford University Press. p. 617–783.
17 Hostovsky M, Blahova J, Plhalova L, Stepanova S, Praskova E, Marsalek P, et al (2012). Oxidative stress parameters in early developmental stages of common carp (*Cyprinus carpio* L.) after subchronic exposure to terbuthylazine and metribuzin. Neuroendocrinol Lett. **33**: 101–106.
18 Howard PH (1989). Handbook of environmental fate and exposure data for organic chemicals pesticides. Chelsea: Lewis publisher. p. 5–13.
19 Huber W (1993). Ecotoxicological relevance of atrazine in aquatic systems. Environ Toxicol Chem. **12**: 1865–1881.
20 Hussein SY, El-Nasser MA, Ahmed SM (1996). Comparative studies on the effects of herbicide atrazine on freshwater fish *Oreochromis niloticus* and *Chrysichthyes auratus* at Assiut, Egypt. B Environ Contam Tox. **57**: 503–510.
21 Hutchington TH, Solbe J, Kloepper-Sams P (1998). Analysis of the ecetoc aquatic toxicity (EAT) database III-Comparative toxicity of chemical substances to different life stages of aquatic organisms. Chemosphere. **36**: 129–142.

22 Jin Y, Zhang X, Shu L, Chen L, Sun L, Qian H (2010). Oxidative stress response and gene expression with atrazine exposure in adult female zebrafish (*Danio rerio*). Chemosphere. **78**: 846–854.

23 Kammann U, Vobach M, Wosniok W, Schäffer A, Telscher A (2009). Acute toxicity of 353-nonylphenol and its metabolites for zebrafish embryos. Environ Sci Pollut Res. **16**: 227–231.

24 Kimbrough RA, Litke DW (1996). Pesticides in streams draining agricultural and urban areas in Colorado. Environ Sci Technol. **30**: 908–916.

25 Kocour M, Gela D, Rodina M, Linhart O (2005). Testing of performance in common carp *Cyprinus carpio* L. under pond husbandry conditions I: top crossing with Northern mirror carp. Aquac Res. **36**: 1628–1628.

26 Lushchak VI, Bagnyukova TV, Lushchak OV, Storey JM, Storey KB (2005). Hypoxia and recovery perturb free radical processes and antioxidant potential in common carp (*Cyprinus carpio*) tissues. Int J Biochem Cell Biol. **37**: 1319–1330.

27 Mhadhbi L, Beiras R (2012). Acute Toxicity of seven selected pesticides (alachlor, atrazine, dieldrin, diuron, pirimiphos-methyl, chlorpyrifos, diazinon) to the marine fish (Turbot, *Psetta maxima*). Water Air Soil Poll. **223**: 5917–5930.

28 Mhadhbi L, Toumi H, Boumaiza M, Aloui N (2012). Toxicity of three selected pesticides (Alachlor, Atrazine and Diuron) to the marine fish (turbot *Psetta maxima*). Afr J Biotechnol. **11**: 11321–11328.

29 Murphy MB, Hecker M, Coady KK, Tompsett AR, Higley EB, Jones PD, et al (2006). Plasma steroid hormone concentrations, aromatase activities and GSI in ranid frogs collected from agricultural and non-agricultural sites in Michigan (USA). Aquat Toxicol. **77**: 153–166.

30 Neskovic NK, Elezovic I, Karan V, Poleksic V, Budimir M (1993). Acute and subacute toxicity of atrazine to carp (*Cyprinus carpio* L.). Ecotoxicol Environ Saf. **25**: 173–182.

31 Nwani CHD, Lakra WS, Nagpure NS, Kumar R, Kushwaha B, Srivastava SK (2010). Toxicity of the herbicide atrazine: effects on lipid peroxidation and activities of antioxidant enzymes in the freshwater fish *Channa punctatus* (Bloch). Int J Env Res Pub He. **7**: 3298–3312.

32 OECD (1992). Guideline for testing of chemicals 210. Fish, early-life stage toxicity test, p. 18.

33 Penaz M, Prokes M, Kouril J, Hamackova J (1983). Early development of the carp, *Cyprinus carpio*. Acta Sci Nat Brno. **17**: 1–39.

34 Perreau F, Einhorn J (2006). Determination of frequently detected herbicides in water by solid-phase microextraction and gas chromatography coupled to ion-trap tandem mass spectrometry. Anal Bioanal Chem. **386**: 1449–1456.

35 Phyu YL, Warne M, St J, Lim RP (2006). Toxicity and bioavailability of atrazine and molinate to the freshwater fish (*Melanotenia fluviatilis*) under laboratory and simulated field conditions. Sci Total Environ. **356**: 86–99.

36 Praskova E, Voslarova E, Siroka Z, Macova S, Plhalova L, Bedanova I, et al (2011). Comparison of acute toxicity of ketoprofen to juvenile and embryonic stages of *Danio rerio*. Neuroendocrinol Lett. **32**: 117–120.

37 Scahill JL (2008). Effects of atrazine on embryonic development of fathead minnows (*Pimephales promelas*) and *Xenopus laevis*. Bios. **79**: 139–149.

38 Sehonova P, Kodes V, Leontovycova D, Svobodova Z (2012). Assessment of pesticide residues in surface waters in the Czech Republic. [(Zhodnocení výskytu reziduí pesticidů v povrchových vodách České republiky.) (In Czech with English abstract.)] Bulletin VUHR Vodnany. **48**: 5–19.

39 Slaninova A, Smutna M, Modra H, Svobodova Z (2009). A review: Oxidative stress in fish induced by pesticides. Neuroendocrinol Lett. **30**: 2–12.

40 Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, et al (1985). Measurement of protein using bicinchoninic acid. Anal Biochem. **150**: 76–85.

41 Solomon KR, Baker DB, Richards RP (1996). Ecological risk assessment of atrazine in North American surface waters. Environ Toxicol Chem. **15**: 31–76.

42 Steinberg CEW, Lorenz R, Spieber OH (1995). Effect of atrazine on swimming behavior of zebrafish, *Brachydanio rerio*. Water Res. **29**: 981–985.

43 Stepanova S, Praskova E, Chromcova L, Plhalova L, Prokes M, Blahova J, et al (2013). The effects of diclofenac on early life stages of common carp (Cyprinus carpio). Environ Toxicol Phar. **35**: 454–460.

44 Svartz GV, Herkovits J, Perez-Coll CS (2012). Sublethal effects of atrazine on embryo-larval development of Rhinella arenarum (Anura: Bufonidae). Ecotoxicology. **21**: 1251–1259.

45 Svobodova Z, Gelnarova J, Justyn J, Krupauer V, Macova J, Simanov L, et al (1987). Toxicology of aquatic animals. [(Toxikologie vodních živočichů.) (In Czech.)] Praha: SZN., p.231.

46 Svobodova Z, Sehonova P, Kaut J, Pazourova M (2011). Analysis of surface water accidental pollution and fish kills in the Czech Republic in the period 1989–2010. [(Analýza příčin havarijního znečištění povrchových vod a následných úhynů ryb v České republice v období 1989–2010.) (In Czech with English abstract.)] Bulletin VUHR Vodnany. **47**: 47–56.

47 U.S. EPA (2001). Drinking water exposure assessment for atrazine and various chloro-triazine and hydroxy-atrazine degradates. Memo from H. Nelson, Environmental Fate and Effects Division, *et al.* to Pam Noyes, Special Review and Reregistration Division. Washington, D.C., p. 8.

48 Valavanidis A, Vlahogiannia T, Dassenakisb M, Scoullosb M (2006). Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol Environ Saf. **64**: 178–189.

49 WHO (2010). Atrazine and its metabolites in drinking-water. Background document for development of WHO Guidelines for drinking-water quality. WHO Press, Geneva, Switzerland.

50 Wiegand C, Pflugmacher S, Giese M, Frank H, Steinberg C (2000). Uptake, toxicity, and effects on detoxication enzymes of atrazine and trifluoroacetate in embryos of zebrafish. Ecotoxicol Environ Saf. **45**: 122–131.

51 Wiegand C, Krause E, Steinberg C, Pflugmacher S (2001). Toxicokinetics of atrazine in embryos of the zebrafish (*Danio rerio*). Ecotoxicol Environ Saf. **49**: 199–205.

52 Xing H, Li S, Wang Z, Gao X, Xu S, Wang X (2012a). Oxidative stress response and histopathological changes due to atrazine and chlorpyrifos exposure in common carp. Pestic Biochem Phys. **103**: 74–80.

53 Xing H, Wang X, Sun G, Gao X, Xu S, Wang X (2012b). Effects of atrazine and chlorpyrifos on activity and transcription of glutathione S-transferase in common carp (*Cyprinus carpio* L.). Environ Toxicol Phar. **33**: 233–244.

54 Zhou QX, Xie GH, Pang L (2008). Rapid determination of atrazine in environmental water samples by a novel liquid phase microextraction. Chinese Chem Lett. **19**: 89–91.