Aquatic Toxicology 165 (2015) 249–258

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Toxicity of environmental Gesagard to goldfish may be connected with induction of low intensity oxidative stress in concentration- and tissue-related manners

Nadia M. Mosiichuk[a], Viktor V. Husak[a], Ivan V. Maksymiv[a], Oksana Y. Hlodan[b], Janet M. Storey[c], Kenneth B. Storey[c], Volodymyr I. Lushchak[a,*]

[a] Department of Biochemistry and Biotechnology, Precarpathian National University named after Vassyl Stefanyk, 57 Shevchenko Str., Ivano-Frankivsk 76025, Ukraine
[b] Department of Human and Animal Anatomy and Physiology, Precarpathian National University named after Vassyl Stefanyk, 57 Shevchenko Str., Ivano-Frankivsk 76016, Ukraine
[c] Institute of Biochemistry, Carleton University, 1125 Colonel By Drive, Ottawa, Ontario K1S 5B6, Canada

ARTICLE INFO

Article history:
Received 11 April 2015
Received in revised form 27 May 2015
Accepted 9 June 2015
Available online 16 June 2015

Keywords:
Goldfish
Gesagard
Prometryn
Oxidative stress
Antioxidant enzymes

ABSTRACT

Prometryn is a selective herbicide commonly used in agriculture as the commercial preparation, Gesagard. Goldfish (*Carassius auratus*) exposure for 96 h to 0.2, 1, or 5 mg L^{-1} Gesagard 500 FW (corresponding to 0.1, 0.5, and 2.5 mg L^{-1} of prometryn) on indices of oxidative stress (lipid peroxides, protein carbonyls, and thiol content) and activities of antioxidant and related enzymes in gills, liver, and kidney was studied. Gills appeared to be the most resistant to Gesagard treatment, reacting to only the highest concentration of herbicide with enhanced levels of low molecular mass thiols and activities of glutathione *S*-transferase (GST) and glutathione reductase. Goldfish exposure to 0.2–5 mg L^{-1} Gesagard resulted in enhancement of carbonyl protein level and activity of superoxide dismutase (SOD), but reduced the lipid peroxide (LOOH) content and activity of glutathione peroxidase in liver. Kidney appeared to be the main target organ of Gesagard toxicity, showing the greatest number of parameters affected even under low concentrations of herbicide. An increase in the content of L-SH and activity of SOD was accompanied with decreased activities of catalase, GST, and glucose-6-phosphate dehydrogenase and reduced levels of LOOH in kidney of Gesagard treated fish. The treatment also induced various histological changes in goldfish liver and kidney which could be related to their dysfunction. The present study indicates that Gesagard induced oxidative stress of differing intensities in the three goldfish tissues and demonstrated that kidney would be the best target organ to analyze, reveal, and monitor Gesagard effects on fish.

1. Introduction

Triazine herbicides are a major class of toxic substances used around the world for weed control to enhance agricultural production and yield. However, their massive use has unavoidably contributed to deteriorating water quality in rivers and lakes frequently leading to pesticide accumulation in aquatic organisms (Akerblom, 2004). Prometryn [2,4-bis(isopropylamino)-6-(methylthio)-*s*-triazine] is a selective herbicide of the *s*-triazine chemical family and is utilized as a pre- or post-emergence controller of annual grasses and broad-leaf weeds (US EPA, 1996). Its herbicidic effects on target plants are based on the inhibition of photosynthetic transport of electrons at the photosystem II receptor site and inhibition of oxidative phosphorylation (Wakabayashi and Böger, 2004). Prometryn was first registered in the United States in 1964 as an herbicide for the control of weeds in cotton, celery, pigeon peas, and dill crops. Nowadays, prometryn is banned in many European countries due to its potential for bioaccumulation in organisms. At the same time, large areas of China, Australia, Canada, New Zealand, South Africa, and the United States are still treated with this herbicide (Dikić, 2014).

According to laboratory data, prometryn is a persistent chemical and is stable to hydrolysis or photolysis in water and soil (US

Abbreviations: AChE, acetylcholine esterase; CP, carbonyl protein groups; GPx, glutathione peroxidase; GST, glutathione-*S*-transferase; GR, glutathione reductase; G6PDH, glucose-6-phosphate dehydrogenase; H-SH, high molecular mass thiols; LOOH, lipid peroxides; L-SH, low molecular mass thiols; ROS, reactive oxygen species; SOD, superoxide dismutase.

* Corresponding author. Fax: +38 0342 714683.
E-mail address: lushchak@pu.if.ua (V.I. Lushchak).

http://dx.doi.org/10.1016/j.aquatox.2015.06.007
0166-445X/

EPA, 1996; Erickson and Turner, 2002). Average soil half-life (abiotic degradation) in shallow aerobic soil is up to 274 days and in anaerobic soil half-life can be up to 316 days. In water, average hydrolysis half-life is 28 days, but there are records from lake, river, and ground waters where prometryn was persistent up to 70 days depending on environmental physical–chemical properties, pH, and organic components. It is usually stable to hydrolysis at 20 °C in neutral, slightly acidic, or slightly alkaline water. In water, it can bind to suspended solids (especially organic) or sediment. Photodegradation half-life in seawater is between 55 and 70 days (US EPA, 1996; Dikić, 2014).

Due to the large dosage that was previously used and its long residual time, prometryn is still found in surface and groundwater of European countries. For example, prometryn concentrations of 0.078–4.40 $\mu g\,L^{-1}$ were measured in surface water in Greece (Vryzas et al., 2011) whereas, in surface waters of Western France, prometryn was detected at concentrations from 0.1 to 0.44 $\mu g\,L^{-1}$ (Caquet et al., 2013). The highest environmental concentration of prometryn (0.51 $\mu g\,L^{-1}$) was detected in Czech rivers (Stará et al., 2014). Prometryn concentrations in surface water in the United States of America were 0.021 $\mu g\,L^{-1}$ in South Florida (Pfeuffer, 2014) and 0.861 $\mu g\,L^{-1}$ in California (Smalling and Orlando, 2011). The herbicide was also found in surface water in China (Qi et al., 2015).

Fish can serve as bio-indicators of environmental pollution and can play significant roles in assessing potential risks associated with contamination in aquatic environments resulting from agricultural production. According to previous studies, prometryn is moderately toxic to fish. Acute toxicity 96 h LC_{50} values have been reported for several species: 2.9 $mg\,L^{-1}$ for rainbow trout (*Oncorhynchus mykiss*), 7.9 $mg\,L^{-1}$ for bluegill sunfish (*Lepomis macrochirus*), 5.1 $mg\,L^{-1}$ for sheepshead minnow (*Cyprinodon variegatus*), 4 $mg\,L^{-1}$ for goldfish (*Carassius auratus*), and 8 $mg\,L^{-1}$ for common carp (*Cyprinus carpio*) (Stará et al., 2013).

Numerous studies have demonstrated that exposure to triazine herbicides affects the antioxidant defenses of fish, causing an imbalance between production and elimination of reactive oxygen species (ROS) and resulting in oxidative stress and tissue damage (Velíšek et al., 2011a; Paulino et al., 2012; Husak et al., 2014; Maksymiv et al., 2015; Nwani et al., 2010; Blahova et al., 2013). In the case of prometryn, it was reported that chronic exposure (for 35 days) of common carp larvae and embryos to prometryn at concentrations 0.51–1200 $\mu g\,L^{-1}$ shows no influence on most oxidative stress parameters (Stará et al., 2012a). However, in adult carp, chronic exposure (for 14–60 days) to prometryn at concentrations 0.51–80 $\mu g\,L^{-1}$ resulted in significant changes in antioxidant enzyme activities in tissues, but with no observed oxidative damage to the cells (Stará et al., 2013). To date, however, there have been no studies of the effects of acute exposure to prometryn on oxidative stress parameters and antioxidant status in fish tissues. Therefore, the present study investigated the effects of goldfish exposure for 96 h to the prometryn-containing herbicide, Gesagard 500 FW, on indices of oxidative stress (lipid peroxides, protein carbonyls, and thiol content) and the activities of antioxidant and associated enzymes in gills, liver, and kidney.

2. Materials and methods

2.1. Reagents

Phenylmethylsulfonyl fluoride (PMSF), 1-chloro-2,4-dinitrobenzene (CDNB), reduced glutathione (GSH), oxidized glutathione (GSSG), β-nicotinamide adenine dinucleotide phosphate (NADP), β-nicotinamide adenine dinucleotide reduced (NADH), glucose-6-phosphate (G6P), ethylenediamine-tetraacetic acid (EDTA), xylenol orange, cumene hydroperoxide, ferrous sulphate, 2,4-dinitrophenylhydrazine (DNPH), 5,5-dithiobis(2-nitrobenzoic acid) (DTNB), hydrogen peroxide (H_2O_2), NaCl, KH_2PO_4, NaCl, Tris(hydroxymethylaminomethane), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), pyruvic acid, glutathione reductase from baker's yeast, and β-nicotinamide adenine dinucleotide phosphate reduced (NADPH) were purchased from Sigma–Aldrich Corporation (USA). Gesagard 500 FW was purchased from Syngenta AG (Switzerland). All other reagents were of analytical grade.

2.2. Animals and experimental conditions

Specimens of goldfish (*C. auratus* L.) weighing 80–100 g were obtained from a local commercial fish farm (Halych district, Ivano-Frankivsk region, Ukraine) in September 2013. Fish were acclimated to laboratory conditions for four weeks in a 1000 L tank under natural photoperiod in aerated and dechlorinated tap water at 19.0–20.0 °C, pH 6.9–7.1, 8.1–8.6 $mg\,L^{-1}$ O_2 and hardness (determined as Ca^{2+} concentration) 38–40 $mg\,L^{-1}$. Fish were fed commercial pellets of CarpCo Excellent for Cyprinids (Koi Grower, The Netherlands), containing 36% protein, 7% fat, 3.6% cellulose, 8.7% ash, 1% phosphorus and vitamins C, A, D_3 and E. Fish were fed during the acclimation period (four weeks), but were fasted for 1 day prior to and during experimentation.

Experiments were carried out in 120 L glass aquaria (containing 100 L of water), in a static mode with or without the addition of the commercial herbicide Gesagard 500 FW (Syngenta AG, Switzerland) which contains prometryn (6-methylsulfanyl-2-*N*,4-*N*-di(propan-2-yl)-1,3,5-triazine-2,4-diamine) at a concentration of 500 $g\,L^{-1}$. Groups of seven fish were placed in aquaria with different nominal concentrations of Gesagard: 0.2, 1, and 5 $mg\,L^{-1}$, which corresponds to 0.1, 0.5, and 2.5 $mg\,L^{-1}$ of prometryn, respectively. Animals were exposed to these conditions for 96 h (no mortality occurred during exposures). Fish in the control group were maintained in the same manner, but Gesagard was omitted. Aquarium water was not changed over the 96 h course to avoid stressing the animals. Levels of dissolved oxygen, temperature and pH were monitored every 24 h. After exposure, fish were sacrificed by transspinal transsection without anesthesia and tissues (gills, liver, and kidney) were dissected, rinsed in ice-cold 0.9% NaCl, dried by blotting on filter paper, frozen, and stored in liquid nitrogen until use. All experiments were conducted in a strict accordance with the Ethics Committee of Precarpathian National University.

2.3. Determination of oxidative stress indices in goldfish tissues

2.3.1. Assay of lipid peroxides

The lipid peroxide (LOOH) content was assayed by the FOX (ferrous-xylenol orange) method (Hermes-Lima et al., 1995). For that, tissue samples were homogenized (1:5, w:v) using a Potter–Elvehjem glass homogenizer in 96% cold (4 °C) ethanol and centrifuged (5000 × *g*, 15 min, 4 °C). Aliquots of the supernatants were used for the assay as described previously (Lushchak et al., 2005). The content of LOOH was expressed as nanomoles of cumene hydroperoxide equivalents per gram wet mass of tissue.

2.3.2. Measurement of protein carbonyl groups

Carbonyl groups of proteins in tissues were determined as described previously (Lushchak et al., 2005). Tissue samples were homogenized (1:10, w:v) in homogenization medium (50 mM potassium phosphate buffer, pH 7.0, 0.5 mM EDTA, 1 mM PMSF) and centrifuged (16,000 × *g*, 15 min, 4 °C). Supernatants were removed and 0.25 ml aliquots were mixed with 0.25 ml of 40% trichloroacetic acid (TCA) (final TCA concentration 20%) and centrifuged (5000 × *g*, 5 min, 20 °C). Protein carbonyl (CP) levels were measured in

the resulting pellets by reaction with 2,4-dinitrophenylhydrazine (DNPH) (Lenz et al., 1989). The values were expressed as nanomoles of CP per milligram of protein.

2.3.3. Estimation of thiol-containing compounds

Free thiols were measured spectrophotometrically by the Ellman procedure with 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) at 412 nm (Ellman, 1959). Using supernatants prepared as above for the CP assays, total thiol concentration was measured as described previously (Lushchak and Bagnyukova, 2006b). For determination of low-molecular mass thiols (L-SH), aliquots of supernatants were mixed with trichloroacetic acid (TCA) to reach a final TCA concentration of 10%, centrifuged (16,000 × *g*, 5 min, 4 °C) to remove pelleted protein and the final supernatants were used for the assay. Thiol concentrations were expressed as micromoles of SH-groups per gram wet weight of tissue. The high-molecular mass thiol (H-SH) content was calculated by subtracting the L-SH concentration from total thiol concentration.

2.4. Assay of enzyme activities and protein

2.4.1. Determination of activities of antioxidant and associated enzymes

Tissue homogenization and centrifugation was carried out as described above for CP assays and supernatants were collected. The activities of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione-*S*-transferase (GST), glutathione reductase (GR), and glucose-6-phosphate dehydrogenase (G6PDH) were measured in supernatants as described earlier (Lushchak et al., 2005). One unit of SOD activity was defined as the amount of enzyme (per milligram protein) that inhibited quercetin oxidation reaction by 50% of maximal inhibition. Inhibition values for SOD activity were calculated using an enzyme Kinetics computer program (Brooks, 1992). One unit (U) of catalase, GST, GR, GPx, and G6PDH activity is defined as the amount of enzyme consuming 1 μmol of substrate or generating 1 μmol of product per minute. Activities were expressed as international units (or milliunits) per milligram soluble protein (U/mU mg protein^{-1}).

2.4.2. Assay of acetylcholinesterase activity

Acetylcholinesterase (AChE) activity was determined according to the method of Ellman et al. (1961) with minor modifications and adaptation of the procedure to microplate determination. The reaction mixture contained 100 mM potassium phosphate buffer (pH 8.0), 0.3 mM DTNB, and 10 μl supernatant. After 10 min incubation at 25 °C the mixture was supplemented with 30 μl of 75 mM acetylthiocholine iodide (final volume 0.3 ml) and reaction rate was immediately measured at 412 nm. The enzyme activity was calculated using a molar extinction coefficient of 13.600 M^{-1} cm^{-1}. One unit of AChE activity was defined as the amount of enzyme producing 1 μmol of thionitrobenzoate per minute and was expressed per milligram soluble protein (mU mg protein^{-1}).

2.4.3. Assay of protein

Soluble protein concentration was measured with the Coomassie Brilliant blue G-250 (Bradford, 1976) using bovine serum albumin as a standard.

2.5. Histological examination

Liver and kidney samples of the control and treated fish were fixed in 10% neutral-buffered formalin, and then the samples were processed for routine wax histological evaluation (dehydrated and embedded in paraffin). Sections of 5 μm were prepared and stained with hematoxylin and eosin stains as described by Luna, (1968) and Bernet et al. (1999). Histological samples of respective tissue were prepared from four fishes from each experimental group with two replicates of each sample and the changes described were similar in all the slides that were assessed.

Table 1
The concentrations of high molecular mass (H-SH) and low molecular mass (L-SH) thiols (μmol gwm^{-1}) in gills, liver and kidney of goldfish, exposed to control conditions or 0.2, 1, and 5 mg L^{-1} of Gesagard for 96 h.

Tissue	Parameter	Fish group			
		Control	0.2 mg L^{-1}	1 mg L^{-1}	5 mg L^{-1}
Gill	H-SH	6.45 ± 0.46	6.15 ± 0.65	5.53 ± 0.48	5.28 ± 0.41
	L-SH	0.50 ± 0.09	0.48 ± 0.06	0.49 ± 0.08	0.70 ± 0.05*
Liver	H-SH	3.95 ± 0.19	3.86 ± 0.15	3.60 ± 0.26	3.78 ± 0.45
	L-SH	2.26 ± 0.21	1.92 ± 0.12	2.33 ± 0.19	2.00 ± 0.14
Kidney	H-SH	5.94 ± 0.28	5.93 ± 0.52	5.24 ± 0.40	6.10 ± 0.67
	L-SH	1.82 ± 0.14	1.99 ± 0.18	2.49 ± 0.25*	2.62 ± 0.27*

Data are presented as means ± S.E.M, $n = 7$.
* Significantly different from the control group with $P < 0.05$.

2.6. Statistical analysis

Statistical analysis was performed by Mynova software (version 1.3) using ANOVA followed by the Dunnett's test to compare multiple experimental treatments to the single control value. Data are presented as means ± S.E.M. The probability value of $P < 0.05$ was considered to be statistically significant.

3. Results

The $LC_{50}{}^{96}$ value (half-lethal concentration after 96 h exposure) for goldfish exposed to prometryn was determined to be 4 mg L^{-1} (Erickson and Turner, 2002). Therefore, we chose to expose goldfish to Gesagard at concentrations of 0.2, 1, or 5 mg L^{-1}, which corresponds to 0.1, 0.5, or 2.5 mg L^{-1} of prometryn, respectively. Animal exposure to these concentrations resulted in no mortality during the experiment, suggesting that these concentrations were clearly sublethal.

3.1. Levels of oxidative stress markers

The concentration of CP groups in tissues of control goldfish was highest in gills (17.0 ± 1.3 nmol mg protein^{-1}), intermediate in kidney (4.04 ± 0.68 nmol mg protein^{-1}), and lowest in liver (1.65 ± 0.27 nmol mg protein^{-1}). Fish exposure for 96 h to 0.2–5 mg L^{-1} Gesagard significantly increased CP levels in liver by 61–75%, but did not affect this parameter in gills or kidney (Fig. 1A).

Among control goldfish, the concentration of lipid peroxides (LOOH) was lowest in kidney (35.2 ± 6.8 nmol gwm^{-1}), whereas in gills and liver it was similar at 58.4 ± 4.6 and 64.3 ± 9.0 nmol gwm^{-1}, respectively. Exposure to Gesagard had no effect on LOOH concentration in gills, whereas exposure to 0.2 and 5 mg L^{-1} of the herbicide caused a substantial decline by 27 and 29%, respectively, in liver lipid peroxide level (Fig. 1B). In kidney, the LOOH content was lower by 35 and 43% in fish exposed to 1 and 5 mg L^{-1} of Gesagard, respectively, than in controls (Fig. 1B).

The sulfhydryl group content of proteins (high molecular mass thiols, H-SH) was lowest in liver of control goldfish, about 61–66% of the value in gills and kidney (Table 1). None of the tissues showed substantial changes in H-SH content during goldfish exposure to any of the Gesagard concentrations used. By contrast, the concentration of L-SH, which is believed to be represented mainly by glutathione, was lowest in gills of control goldfish, just 22–27% of the value in liver and kidney (Table 1). Goldfish exposure to 0.2–5 mg L^{-1} Gesagard did not affect L-SH in liver, but in the other two tissues Gesagard at its highest concentration (5 mg L^{-1})

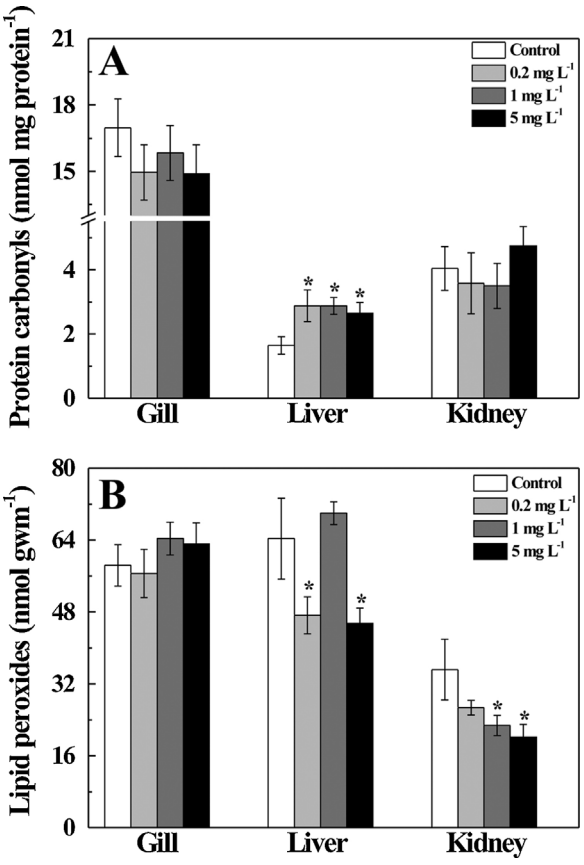

Fig. 1. The level of carbonyl proteins (nmol mg protein^{-1}) (A) and lipid peroxides (nmol gwm^{-1}) (B) in gills, liver and kidney of goldfish, exposed to control conditions or 0.2, 1, and 5 mg L^{-1} of Gesagard for 96 h. Data are presented as means ± S.E.M, n = 5–7. *Significantly different from the control group (P < 0.05) using ANOVA followed by a Dunnett's test.

increased L-SH content by 39% in gills and by 44% in kidney compared to controls (Table 1).

3.2. Activities of antioxidant and associated enzymes

The activity of superoxide dismutase (SOD), a first line antioxidant enzyme, in control goldfish was highest in liver (184 ± 14 U mg protein^{-1}) and lower in kidney and gills (81.3 ± 4.2 and 56.7 ± 3.0 U mg protein^{-1}, respectively) (Fig. 2A). Fish exposure to Gesagard did not affect SOD activity in gills, but increased it in liver and kidney. In liver, a significant enhancement by 28% occurred in fish treated with 5 mg L^{-1} herbicide, whereas in kidney the activity was elevated by 36 and 47% after fish exposure to 0.2 or 1 mg L^{-1} Gesagard, respectively.

The activity of the second primary antioxidant enzyme, catalase, was 10.1 ± 0.6, 60.6 ± 10.3 and 14.3 ± 1.3 U mg protein^{-1} in gills, liver and kidney of control goldfish, respectively. In kidney, catalase activity was diminished by ~20% in all Gesagard-treated animals, whereas in liver and gills no statistically significant differences from control values were found (Fig. 2B). The activities of glutathione peroxidase (GPx) were 116 ± 17, 201 ± 23, and 180 ± 21 mU mg protein^{-1} in control goldfish gills, liver, and kidney, respectively. In gills and kidney, Gesagard did not affect GPx activity at any of the concentrations used, whereas in liver GPx activity was 38% lower thsn in controls in fish exposed to 1 and 5 mg L^{-1} of herbicide (Fig. 2C).

Activities of the second line antioxidant enzymes, namely glutathione-*S*-transferase (GST), glutathione reductase (GR), and glucose-6-phosphate dehydrogenase (G6PDH), responded differently to goldfish exposure to Gesagard. The activity of GST was highest in liver of control goldfish (2.42 ± 0.25 U mg protein^{-1}), intermediate in kidney (1.09 ± 0.14 U mg protein^{-1}), and lowest in gills (0.35 ± 0.01 U mg protein^{-1}). The three tissues showed differential responses by GST to treatment with Gesagard. Goldfish exposure to 1 and 5 mg L^{-1} of Gesagard resulted in higher GST activity than conrols in gills by 55% and 59%, respectively, whereas the herbicide had no effect on liver GST activity and in kidney GST activity was suppressed by 38–46% after exposure to the herbicide at any concentration (Fig. 3A).

Glutathione reductase activity in control fish was virtually the same in gills and liver, 20.6 ± 2.0 and 20.8 ± 1.3 mU mg protein^{-1}, respectively, whereas in kidney it was 31.09 ± 2.89 mU mg protein^{-1}. Fish exposure to the highest concentration (5 mg L^{-1}) of Gesagard resulted in higher GR activity in gills than in control gills by 52% (Fig. 3B), but activity was unchanged in the other two tissues (Fig. 3B).

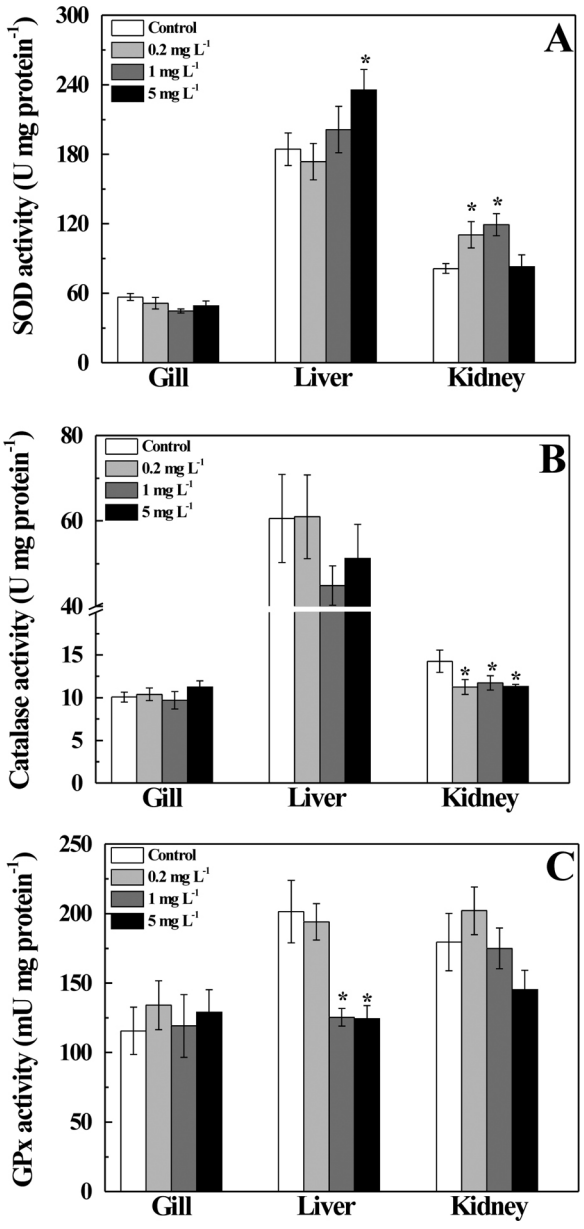

Fig. 2. The activities of superoxide dismutase (SOD, U mg protein^{-1}) (A), catalase (U mg protein^{-1}) (B), and glutathione peroxidase (GPx, mU mg protein^{-1}) (C) in gills, liver and kidney of goldfish, exposed to control conditions or 0.2, 1, and 5 mg L^{-1} of Gesagard for 96 h. Other information as in Fig. 1.

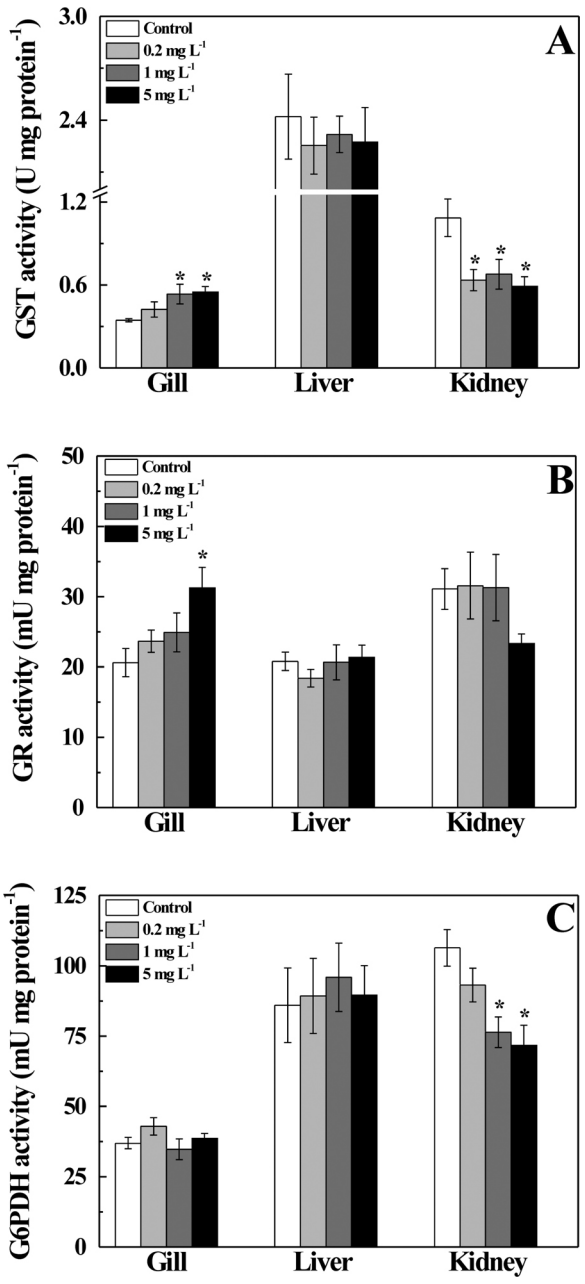

Fig. 3. The activity of glutathione-S-transferase (GST, mU mg protein^{-1}) (A), glutathione reductase (GR, mU mg protein^{-1}) (B), and glucose-6-phosphate dehydrogenase (G6PDH, mU mg protein^{-1}) (C) in gills, liver, and kidney of goldfish, exposed to control conditions or 0.2, 1, and 5 mg L^{-1} of Gesagard for 96 h. Other information as in Fig. 1.

The activities of G6PDH in control fish were 36.9 ± 2.0, 86.0 ± 13.2, and 106 ± 7 mU mg protein^{-1} in gills, liver, and kidney, respectively. Goldfish exposure to Gesagard did not affect the G6PDH activity in gills and liver, whereas it was reduced by 28% and 33% in kidney after exposure to 1 and 5 mg L^{-1} of herbicide, respectively (Fig. 3C).

3.3. Acetylcholinesterase activity

The activity of the AChE in gills of control fish was about 1.4-fold higher than that in liver and kidney (Table 2). Herbicide exposure had no effect on AChE activity in liver, whereas in gills and kidney treatment with the highest concentrations of Gesagard (5 mg L^{-1}) increased AChE activity by 53% and 44%, respectively, as compared to the controls.

Table 2
The activity of acetylcholinesterase (AChE) (mU mg protein^{-1}) in gills, liver, and kidney of goldfish, exposed to control conditions or 0.2, 1, and 5 mg L^{-1} of Gesagard for 96 h.

Tissue	Fish group			
	Control	0.2 mg L^{-1}	1 mg L^{-1}	5 mg L^{-1}
Gill	13.2 ± 1.1	16.4 ± 2.1	14.7 ± 2.5	20.2 ± 1.5*
Liver	9.76 ± 0.83	11.6 ± 1.0	7.95 ± 0.63	9.53 ± 0.92
Kidney	9.77 ± 0.81	10.3 ± 1.4	12.8 ± 1.2	14.0 ± 1.3*

Data are presented as means ± S.E.M, $n = 7$.
* Significantly different from the control group with $P < 0.05$.

3.4. Histopathology

In the present work, we observed pathological changes of liver of fish exposed to Gesagard at all concentrations used. The histological analysis of control fish showed normal histological liver structure (Fig. 4A). Exposure for 96 h to 0.14 mg L^{-1} Gesagard increased the number of dilated sinusoids (Fig. 4B). More pronounced changes occurred in liver of fish exposed to higher Gesagard concentrations. Dystrophy in hepatic cells was observed along with an increased number of dilated sinusoids at Gesagard concentrations of 1 and 5 mg L^{-1} (Fig. 4C, D).

Changes in kidney structure were also observed in goldfish exposed to Gesagard. At low concentration (0.2 mg L^{-1}) of the herbicide, we observed small hemorrhages and necrotic cells in kidney structure (Fig. 5B). At concentration 1 mg L^{-1} pesticide induced more substantial damages in fish kidney, particularly degeneration of epithelium cells, hemorrhages, and decrease in space between glomerulus and Bowman's capsule (Fig. 5C). The similar histological alterations were also observed in fish exposed to the highest herbicide concentration (5 mg L^{-1}) with additional hypertrophy of hematopoietic tissue and necrotic cells (Fig. 5D).

4. Discussion

In recent decades, there has been great interest in studying the toxicity of triazine pesticides on living organisms due to their widespread use in agriculture. Prometryn, along with terbutryn and simetryn, belongs to the group of methylthio-*s*-triazine herbicides (Fenoll et al., 2014). Prometryn is commonly used in agriculture as commercial preparations named Caparol and Gesagard. In phototrophic organisms, prometryn inhibits photosynthesis by acting as a photosystem II inhibitor and induces oxidative stress (Jiang and Yang, 2009). In non-target organisms, particularly mammals, the possible mechanisms of prometryn action include disruption of the tricarboxylic acid cycle and inhibition of oxidative phosphorylation (Brvar et al., 2008; Dikić et al., 2009a; Dikić et al., 2010). As shown previously, chronic oral exposure of mice to prometryn disrupted the operation of the immune system, markedly affecting lymph nodes and thymus, as well as altering blood biochemistry indicators (Dikić et al., 2009b; Dikić et al., 2010). Acute poisoning in humans was accompanied by metabolic acidosis (Brvar et al., 2008). Metabolism of prometryn in mammals primarily occurs via *N*-dealkylation, conjugation through the sulfur group, sulfur oxidation and disulfide formation, with 90–98% of prometryn residues being eliminated in the urine and feces within seven days (Maynard et al., 1999).

In aquatic organisms, enhanced ROS generation and development of oxidative stress are believed to be among the main mechanisms of pesticide-induced toxicity (Livingstone, 2003; Slaninova et al., 2009; Lushchak, 2011). Organic compounds can

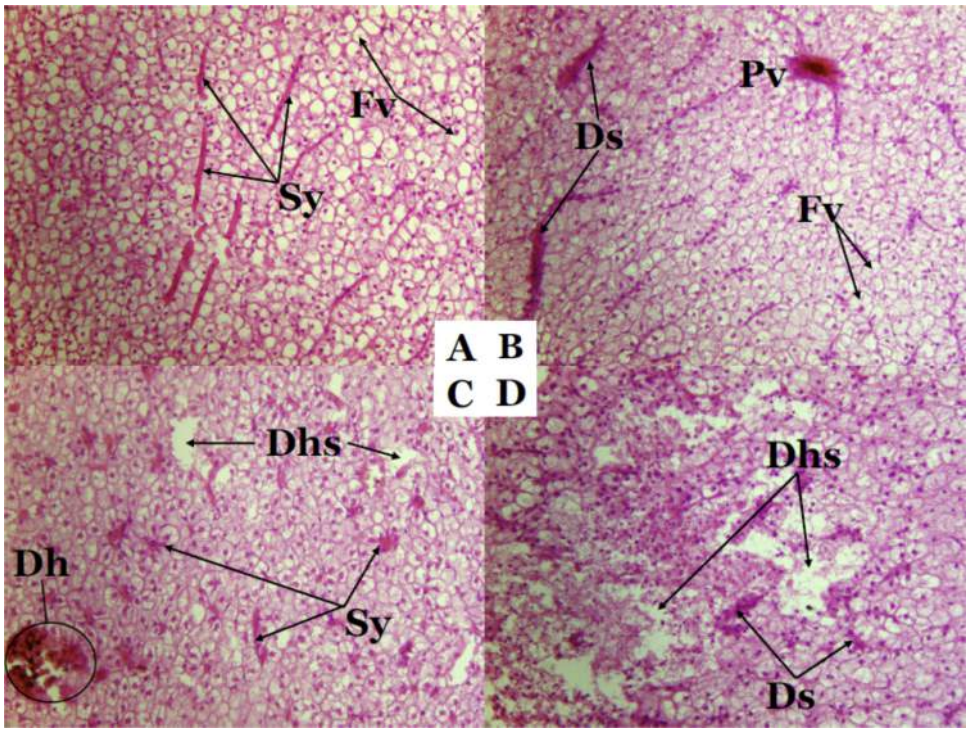

Fig. 4. Light micrographs of sections through liver of goldfish (*C. auratus* L.) showing histological structure of the control group (A), and animals treated with 0.2 (B), 1 (C) or 5 (D) mg L^{-1} of Gesagard for 96 h. Samples were stained with hematoxylin-eosin and photomicrographs were taken using 200 × magnification. Fv – fatty vacuolization; Sy – sinusoids; Ds – dilated sinusoids; Pv – portal vein; Dh – diffuse haemorrhage; Dhs – dystrophy of hepatic cells. These are representative pictures of sections prepared from multiple fish – at least four animals were tested per control or experimental groups.

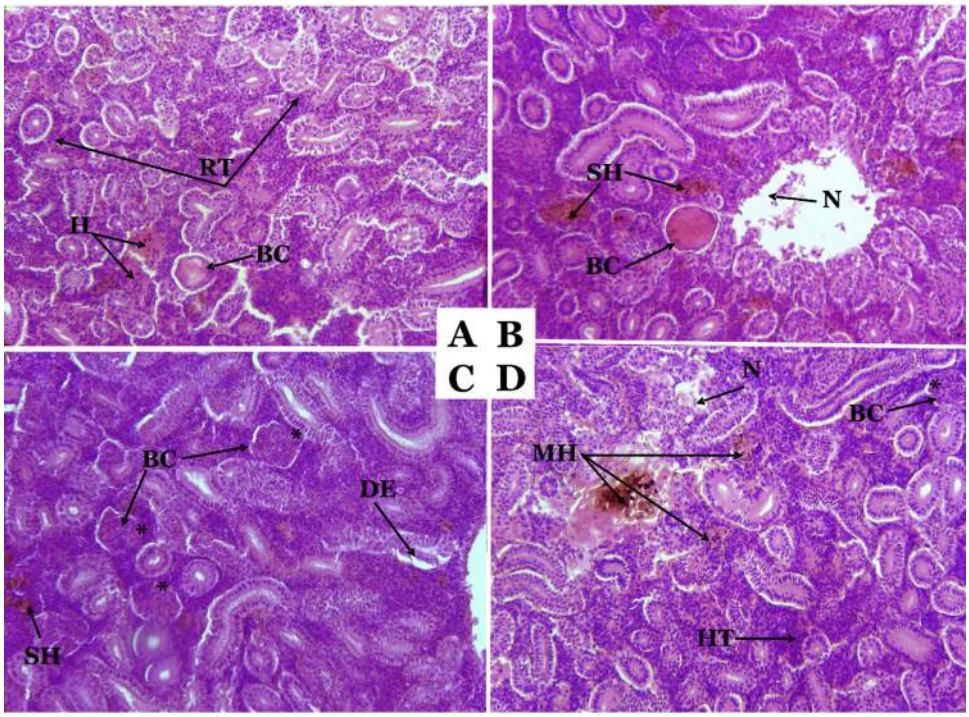

Fig. 5. Light micrographs of section through kidney of goldfish (*C. auratus* L.) showing histological structures of the control group (A), and animals treated with 0.2 (B), 1 (C) or 5 (D) mg L^{-1} of Gesagard for 96 h. Samples were stained with hematoxylin–eosin and photomicrographs were taken using 400× magnification. RT – renal tubules; H – hematopoietic tissue; SH – small hemorrhage; DE – degeneration of tubular epithelium; N – necrotic cells and nuclei of tubular epithelium; MH – multiple hemorrhage; HT – hypertrophy of intertubular hematopoietic tissue; BC – Bowman's capsule; * – asterisk indicates decrease in space between glomerulus and Bowman's capsule. These are representative pictures of sections prepared from multiple fish; kidney tissue from at least four animals was sectioned, stained and examined for each of the groups (control and three experimental groups).

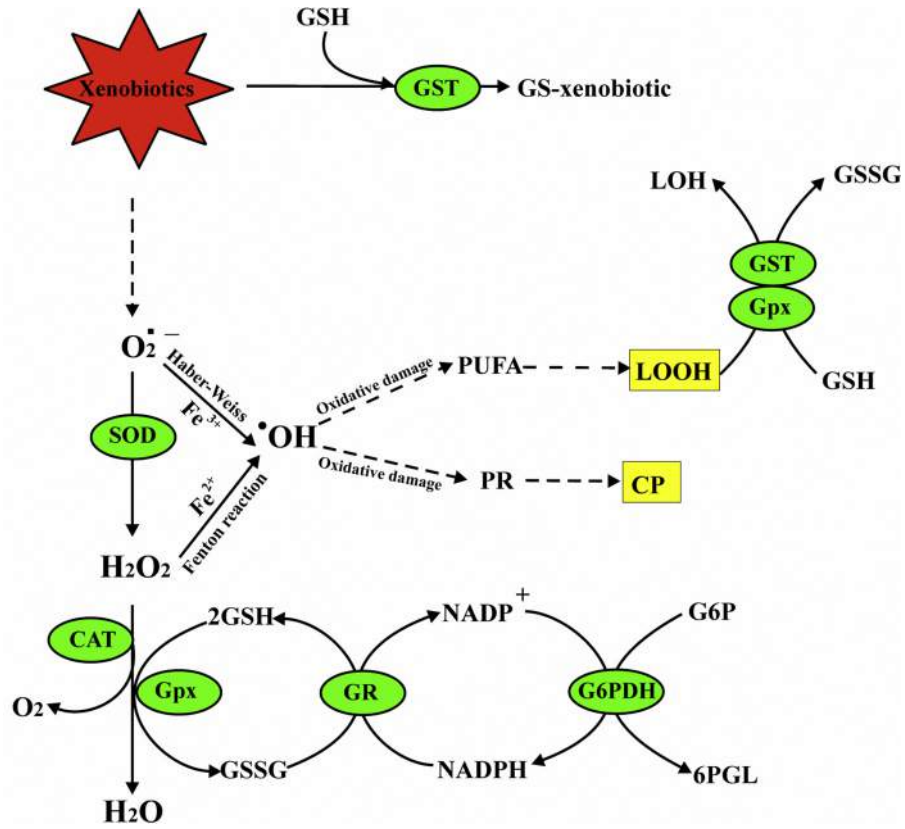

Fig. 6. ROS transformation in living organisms. Abbreviation: GSH, reduced glutathione; GSSG, oxidized glutathione; LOOH, lipid hydroperoxide; LOH, lipid hydroxide; G6P, glucose-6-phosphate; 6PGL, 6-phosphoglucolactone; PR, protein; CP, protein carbonyls. Enzymes involved: SOD, superoxidedismutase; CAT, catalase; GR, glutathione reductase; Gpx glutathione peroxidase; GST, glutathione-*S*-transferase; G6PDH, glucose-6-phosphate dehydrogenase.

stimulate ROS production by a variety of biochemical mechanisms including redox cycling of quinones, nitroaromatics, nitroamines, and bipyridyl herbicides; autoxidation of particular oxygenases (e.g., cytochrome P450s (CYPs); enzyme induction (e.g., CYPs, flavoprotein reductases); disruption of membrane electron transport by lipophilic contaminants; and depletion of antioxidant defenses (e.g., GSH involved in the biotransformation of organic contaminants) (Livingstone, 2003; Lushchak, 2011, 2014; Sies, 2015). Living organisms are equipped with a variety of defense mechanisms to eliminate ROS and other free radicals as well as the damage products resulting from their interactions with cellular components (Fig. 6). The antioxidant defense system includes the first line antioxidant enzymes such as SOD, catalase, and GPx, and second line (associated or auxiliary) antioxidant enzymes such as GST, GR, and G6PDH, as well as low molecular mass antioxidants such as glutathione (Lushchak, 2011, 2014; Sies, 2015).

Several toxicological studies have confirmed that acute or chronic exposure to triazine herbicides enhanced ROS generation and induced oxidative stress in fish (Elia et al., 2002; Nwani et al., 2010; Zhu et al., 2011; Paulino et al., 2012; Blahova et al., 2013). The effects of chronic exposure to prometryn in environmentally reasonable concentrations on oxidative stress parameters have been investigated to a significant extent in common carp and crayfish (Stará et al., 2012a; Stará et al., 2013; Stará et al., 2014). However, there is a scarcity of data on the effects of acute exposure to prometryn or its commercial formulations at sublethal concentrations. Therefore, we decided to fill this knowledge gap with an analysis of the effects of acute prometryn exposure on oxidative stress and antioxidant responses in the goldfish. We chose three tissues (gills, liver, kidney) and evaluated the toxicity of Gesagard on goldfish. Gills are primary organs that are directly exposed to water-borne pollutants, whereas liver and kidney are key organs involved in elimination, biotransformation, and excretion of xenobiotics from the body.

4.1. Reaction of gills to Gesagard exposure

Persistent hydrophobic chemicals may enter aquatic organisms through different routes: via direct uptake from water by gills or skin (bioconcentration), via uptake of suspended particles (ingestion), or via consumption of contaminated food (biomagnification). Gills are the first site of direct contact with water contaminants and they perform biotransformation and elimination of xenobiotics (Van der Oost et al., 2003). Previous research proved that uptake of triazine via fish gills was a main route of entry (Gunkel, 1981). Therefore, it might be predicted that goldfish gills would be the tissue most affected by Gesagard as compared with the other tissues investigated, namely liver and kidney. However, our experiments did not confirm this initial hypothesis. The main markers of oxidative stress, protein carbonyls (CP), and lipid hydroperoxides (LOOH), did not change in gills of goldfish exposed to any of the Gesagard concentrations used. Similarly, previous studies reported low sensitivity of gill tissue to triazine herbicides. In particular, chronic exposure to prometryn at concentration 0.51–80 $\mu g\,L^{-1}$ had no effect on the intensity of lipid peroxidation, measured as levels of thiobarbituric acid reactive substances (TBARS), in gills of common carp (Stará et al., 2013). Long term exposure to another methylthio-*s*-triazine pesticide, terbutryn, did not change TBARS levels, either, but increased CP content in gills of common carp (Velíšek et al., 2011a). Furthermore, acute (48 h) exposure of *Prochilodus lineatus* to atrazine did not change LOOH levels in

gills, whereas after subchronic (14 day) exposure, the level of LOOH increased (Paulino et al., 2012).

The resistance of gill proteins and lipids to oxidative modification might be connected with a high potential of the antioxidant system to eliminate ROS. However, we did not observe any changes in gill in the activities of first-line defense enzymes such as SOD, catalase, and GPx in response to Gesagard exposure. Similarly, (Stará et al., 2012a) did not observe alterations in the activities of SOD and catalase in tissues of carp at early life stages when exposed to 0.51–1.2 $\mu g\,L^{-1}$ of prometryn for 35 days. Later, the same authors reported that SOD activity decreased in gills of common carp after long-term exposure to 0.51–80 $\mu g\,L^{-1}$ of prometryn, whereas catalase activity was unchanged (Stará et al., 2013). In addition, acute (48 h) exposure to atrazine did not impact the activities of SOD, catalase and GPx in the gills of *P. lineatus* (Paulino et al., 2012).

In many cases, free thiols offer a key defense against oxidative stress in living organisms (Van der Oost et al., 2003; Lushchak, 2011, 2012, 2014). In animal tissues, low molecular mass thiols (L-SH) are represented mainly by the tripeptide glutathione (GSH) and the free amino acid cysteine (Lushchak, 2012). In this study, we observed an increase in L-SH concentration in gills of goldfish exposed to the highest concentration of Gesagard, potentially attributable to increased GSH biosynthesis. The level of free low molecular mass thiols also depends on GR activity, which was substantially increased in gills in response to 5 $mg\,L^{-1}$ of Gesagard exposure. But GR only reduces oxidized glutathione and cannot increase its total pool due to which increase in total L-SH concentration is supposed to be attributed to *de novo* synthesis. Importantly, GSH is also needed for conjugation with xenobiotics via the reaction catalyzed by GST (Lushchak, 2012). The increased GST activity in gills of fish exposed to 1 and 5 $mg\,L^{-1}$ of Gesagard suggests that the detoxification pathway for prometryn in the gills may involve the direct conjugation of prometryn to GSH since its chemical structure favors such conjugation. At the same time, the lack of increase in GPx activity could be a mechanism for preserving the GSH content in the cell above a critical level favoring the conjugation of prometryn to GSH by GST (Paulino et al., 2012). Oropesa et al. (2009) also reported an increase in GSH level in gills of common carp after chronic exposure to simazine, as a result of as an adaptive response to this stressful situation.

Acetylcholinesterase (AChE) is widely known as a specific biomarker of pesticide intoxication due to inactivation of the enzyme (Assis et al., 2011). Instead, we observed an increase AchE activity in gills of goldfish exposed to 5 $mg\,L^{-1}$ of Gesagard. One can suggest that an increase in AChE activity in response to Gesagard may be an adaptive response to acute and really not intensive exposure, whereas decreases in AChE activity would be expected after more extended or extensive exposure. A similar effect was reported for tissues of fish exposed to heavy metals (Bainy et al., 2006).

4.2. Gesagard-induced changes in liver

Liver is the primary organ for bioaccumulation and detoxification of xenobiotics, as well as a site of multiple oxidative reactions and ROS generation in substantial amounts (Van der Oost et al., 2003). This tissue has been extensively studied in regards to the toxic effects of triazines. For example, liver was viewed as a primary organ for atrazine metabolism in zebrafish (Jin et al., 2010). Velisek and colleagues did not observe any changes in the activities of enzymes (ethoxyresorufin-*O*-deethylase, GST) and levels of cofactors (cytochrome P450, GSH) involved in xenobiotic biotransformation in liver of common carp after chronic exposure to terbutryn or simazine (Velíšek et al., 2011a; Stará et al., 2012b). Previously, we showed that short-term exposure to the metribuzin-containing herbicide Sencor altered liver histology, metabolism, and free radical processes (Maksymiv et al., 2015). The present study demonstrates that Gesagard enhanced ROS-induced modification of proteins in goldfish liver. At the same time, the pesticide decreased the level of lipid peroxides in liver that could result either from their decreased production or enhanced degradation under goldfish exposure to Gesagard. Lipid hydroperoxides are not only a marker of oxidative damage to lipids – they are also possibly involved in triggering the up-regulation of activities of antioxidant enzymes (Valavanidis et al., 2006; Lushchak and Bagnyukova, 2006a). As a result, an enhanced antioxidant response could suppress the intensity of lipid peroxidation (Bagnyukova et al., 2007). However, only minor changes were observed in the activities of antioxidant and associated enzymes in liver of goldfish exposed to Gesagard. For example, SOD activity increased in liver only in fish exposed to the highest concentration of Gesagard. Concurrently, catalase activity showed tendency to decrease in fish exposed to Gesagard, whereas GPx activity was significantly suppressed in liver of the 1 and 5 $mg\,L^{-1}$ treatment groups. The latter enzyme is involved in ROS detoxification and plays an important role in defense against lipid peroxidation in aquatic organisms (Lushchak, 2011, 2012, 2014). The decrease of GPx activity might be related to enhanced superoxide production or to a direct action of the pesticide or its derivatives on enzyme synthesis (Monteiro et al., 2006). With these results one may suggest a scenario of oxidative stress development in liver of goldfish at least at the highest concentration of Gesagard used. The activities of GST, GR, and G6PDH were unaffected by fish exposure to Gesagard, suggesting that their high constitutive activities in liver are probably sufficient to deal with any oxidative stress arising from Gesagard exposure. Of the three tissues studied, liver also possessed the highest level of L-SH in control goldfish, which could contribute to alleviating the toxic effects of the pesticide.

Similar findings were reported in the liver of carp exposed to prometryn at doses of 0.51 to 80 $\mu g\,L^{-1}$ over 14–60 days (Stará et al., 2012a). In that study, SOD and GR activities were unchanged over the experimental course, whereas catalase activity decreased after 60 day exposure of fish to 80 $\mu g\,L^{-1}$ of prometryn. Prolonged exposure of common carp to terbutryn significantly increased SOD activity and decreased GR activity but had no effect on GPx activities in liver (Velíšek et al., 2011a). Earlier we found that acute exposure of goldfish to the metribuzin-containing herbicide Sencor enhanced SOD activity, but reduced catalase, GPx, and GR activities in liver (Maksymiv et al., 2015).

In our study, histology of fish liver exposed to Gesagard showed dystrophy in hepatic cells and dilated sinusoids. The histological alterations in the liver tissue resulting from acute Gesagard exposure might reduce its functional efficacy. In contrast to our observation, no histological anomalies were demonstrated in liver of carps subjected to long-term exposure to terbutryn, simazine, and prometryn (Velisek et al., 2011α, 2012, 2013).

4.3. Effects of Gesagard on the fish kidney

Kidney is a target organ for certain toxicants since, it actively transforms xenobiotics and is a major route for their excretion. Thus, pollution with some xenobiotics is highly likely to affect kidney. Triazines are mainly excreted in mammals and fish by the kidney (Gunkel, 1981; Bradway and Moseman, 1982) and have direct effects on kidney structure and function in freshwater fish (Velíšek et al., 2011b; Husak et al., 2014). We found that kidney appeared to be the target organ of Gesagard toxicity, because most of the parameters studied were affected in kidney of goldfish exposed to this herbicide. Similar to the effects seen in liver, acute Gesagard treatment significantly decreased the levels of lipid peroxides in goldfish kidney. Lipid peroxides can be degraded by phospholipid hydroperoxide glutathione peroxidase and/or GST (Hermes-Lima, 2004; Lushchak, 2012, 2014). However,

we did not observe any changes in GPx activity in goldfish kidney, whereas GST activity was reduced in pesticide treatment groups, suggesting that in our case these enzymes might not responsible for decreased LOOH. Protein carbonyl levels were unaffected by Gesagard exposure in kidney, possibly because of good endogenous defense systems that prevent ROS-promoted protein oxidation. In particular, this effect may be linked with the increase in L-SH content in kidney of goldfish exposed to Gesagard, since a similar tendency was observed in gills. With respect to antioxidant enzymes, our results showed a significant increase of SOD activity in the kidney of goldfish exposed to Gesagard, which suggests that a rise in the $O_2^{\bullet -}$ generation rate might have taken place. In contrast, catalase activity was reduced in all experimental groups. This reduction might be due to high superoxide anion levels that might inactivate catalase. Catalase activity also depends on the level of NADPH. It has been demonstrated that NADPH protects catalase from inactivation, because each of the four monomers of catalase contains an NADPH-binding site which should be occupied to provide full enzymatic activity (Kirkman and Gaetani, 1984; Kirkman et al., 1999). Decreased NADPH level correlated with a loss of catalase activity in human erythrocytes (Gaetani et al., 1989). The role of G6PDH in antioxidant defense is peripheral but important, because this enzyme is one of the main cellular sources of NADPH synthesis required for antioxidant defense (Winzer et al., 2002; Lushchak, 2014). We observed decreased G6PDH activity in kidney of Gesagard treated fish, which correlated with the decrease in catalase activity. Activity of GR in kidney did not respond to treatment of fish with low Gesagard concentrations, but a downward trend was observed at the highest concentration of herbicide. Antioxidant enzymes, such as catalase, GST, G6PDH, and GR are sensitive to damage by ROS, leading to their inactivation (Lushchak et al., 2005). It could be suggested, therefore, that this scenario took place in our experiment suggesting that goldfish exposure to Gesagard induced oxidative stress in animal kidney.

Triazine pesticides have been reported previously to modify the activities of various antioxidant enzymes in kidney. For example, subchronic exposure of common carp to atrazine reduced activities of SOD, catalase, and GPx in kidney (Xing et al., 2012). Exposure to the metribuzin-containing herbicide Sencor decreased the activities of catalase, GPx, and GR (but not SOD and GST) in kidney of goldfish after 96 h exposure (Husak et al., 2014). Similar to gills, in kidney of goldfish we also observed increased AchE activity at the highest concentration of herbicide. That may reflect an adaptive response to pesticide insult by this enzyme.

In addition to disturbances to antioxidant system, kidney structure is altered in goldfish exposed to Gesagard. We observed necrotic cells, degeneration of epithelium cells, hemorrhages, and decrease in space between glomerulus and Bowman's capsule in the organ. Velisek et al. (2013) also observed concentration-dependent structural changes in tubular epithelium of caudal kidney of carps after chronic exposure to prometryn. They included the shedding of cellular fragments into the lumina, shedding of tubular epithelial cells, and the presence of hyaline casts as well as inflammatory infiltration and interstitial hemorrhaging (Velisek et al., 2013). Long-term exposure to simazine at concentrations of 0.06–4 $\mu g\,L^{-1}$ caused severe hyaline degeneration of the epithelial cells of renal tubules of the caudal kidney, but no histopathological changes were demonstrated in caudal kidney of carp following long-term exposure to terbutryn at concentrations of 0.02, 0.2, and 2 $\mu g\,L^{-1}$ (Velisek et al., 2011b, 2012).

5. Conclusions

The present study demonstrated that 96 h exposure to the prometryn-contained herbicide Gesagard perturbed ROS homeostasis in goldfish gills, liver, and kidney and caused histopathological changes in kidney and liver. However, pesticide-induced changes varied considerably between the tissues with an unexpectedly weak response by gills which are in direct contact with the water-borne pesticide. In gills, only four of the 11 parameters measured were increased at the highest Gesagard concentration used: L-SH levels and activities of GST, GR, and AChE, indicative of a relatively weak antioxidant defense response in this organ. So, Gesagard stress on gills may be categorized as low intensity (Lushchak, 2014; Sies, 2015). Of the three tissues tested, goldfish liver possesses the most powerful antioxidant defense system as evidenced by levels of L-SH and activities of SOD, catalase, GPx, GST, and G6PDH. Despite intensification of selected signatures of oxidative stress in liver (increased CP levels; decreased GPX activity), damage may be controlled by the existing antioxidant defenses even allowing for a reduction in LOOH levels in liver of pesticide-exposed fish. Clearly, however, liver showed several signatures of low intensity oxidative stress response to Gesagard (Lushchak, 2014; Sies, 2015). Finally, perturbations of ROS-related processes in response to goldfish exposure to Gesagard were most pronounced in kidney among the three tissues analyzed. Decreased activities of catalase, GST and G6PDH were seen but might be counteracted by increased L-SH levels and SOD activity. These data suggest that kidney experienced the greatest response to Gesagard, with an oxidative stress level between weak and intermediate in intensity (Lushchak, 2014; Sies, 2015). Collectively, the results of this research allow us to conclude that oxidative stress is one of the mechanisms responsible for the toxicity of Gesagard to fish. Indeed, the same mechanism of Gesagard toxicity may also be at work in other groups of non-target organisms. Comparison of our data on acute fish exposure to Gesagard with information in the literature about long term pesticide exposures suggests that in the latter case lower doses/concentrations over longer timeframes may induce changes similar to those reported here for acute exposures to higher toxicant levels. Finally, our work shows that kidney was the most responsive organ to Gesagard exposure which may make this organ the best tissue to utilize to reveal and monitor Gesagard effects on fish.

Acknowledgement

The research received partial support from a discovery grant from the Natural Sciences and Engineering Research Council of Canada to KBS.

References

Akerblom, N., 2004. Agricultural pesticide toxicity to aquatic organisms. In: A Literature Review. Sveriges Lantbruks Univ., Uppsala, pp. 31.

Assis, C.R.D., Carvalho Jr, L.B., Bezerra, R.S., 2011. Fish cholinesterases as biomarkers of organophosphorus and carbamate pesticides. In: Stoytcheva, M. (Ed.). Pesticides in the Modern World - Pests Control and Pesticides Exposure and Toxicity Assessment. ISBN: 978-953-307-457-3, InTech, 10.5772/20777.

Bagnyukova, T.V., Lushchak, O.V., Storey, K.B., Lushchak, V.I., 2007. Oxidative stress and antioxidant defense responses by goldfish tissues to acute change of temperature from 3 to 23 °C. J. Therm. Biol. 32, 227–234.

Bainy, A.C.D., de Medeiros, M.H.G., Di Mascio, P., de Almeida, E.A., 2006. *In vivo* effects of metals on the acetylcholinesterase activity of the *Perna perna* mussel's digestive gland. Biotemas 19, 35–39.

Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P., Wahli, T., 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. J. Fish Dis. 22, 25–34.

Blahova, J., Plhalova, L., Hostovsky, M., Divisova, L., Dobsikova, R., Mikulikova, I., Stepanova, S., Svobodova, Z., 2013. Oxidative stress responses in zebrafish Danio rerio after subchronic exposure to atrazine. Food Chem. Toxicol. 61, 82–85.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Bradway, D.E., Moseman, R.F., 1982. Determination of urinary residue levels of the *N*-dealkyl metabolites of triazine herbicides. J. Agric. Food Chem. 30, 244–247.

Brooks, S.P.J., 1992. A simple computer program with statistical tests for the analysis of enzyme kinetics. Biotechniques 13, 906–911.
Brvar, M., Okrajsek, R., Kosmina, P., Staric, F., Kaps, R., Kozelj, G., Bunc, M., 2008. Metabolic acidosis in prometryn (triazine herbicide) self-poisoning. Clin. Toxicol (Phila.) 46, 270–273.
Caquet, T., Roucaute, M., Mazzella, N., Delmas, F., Madigou, C., Farcy, E., Burgeot, Th., Allenou, J.-P., Gabellec, R., 2013. Risk assessment of herbicides and booster biocides along estuarine continuums in the Bay of Vilaine area (Brittany, France). Environ. Sci. Pollut. Res. 20, 651–666.
Dikić, D., 2014. Prometryn. In: Wexler, P. (Ed.), Encyclopedia of Toxicology, vol 3, 3rd ed. Elsevier Inc., Academic Press, pp. 1077–1081.
Dikić, D., Benković, V., Horvat-Knežević, A., Brozović, G., Oršolić, N., Springer, O.P., 2009a. Subchronic oral exposure to prometryne changes relations of blood biochemistry indicators in mice. Acta Vet. Brno 78, 243–251.
Dikić, D., Sajli, L., Benković, V., Knezević, A.H., Brozović, G., Lisicić, D., Mojsović, A., Orsolić, N., 2010. Brain toxicokinetics of prometryne in mice. Arh. Hig. Rada. Toksikol. 61, 19–27.
Dikić, D., Zidovec-Lepej, S., Remenar, A., Bendelja, K., Benković, V., Horvat-Knežević, A., Brozović, G., Oršolić, N., 2009b. Effects of prometryne on apoptosis and necrosis in thymus, lymph node and spleen in mice. Environ. Toxicol. Pharmacol. 27, 182–186.
Elia, A.C., Waller, W.T., Norton, S.J., 2002. Biochemical responses of bluegill sunfish (*Lepomis macrochirus*, Rafinesque) to atrazine induced oxidative stress. Bull. Environ. Contam. Toxicol. 68, 809–816.
Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82, 70–77.
Ellman, G.L., Courtney, K.D., Andres Jr., V., Feather-Stone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7, 88–90.
Erickson, W., Turner, L., 2002. Prometryn analysis of risks to endangered and threatened Salmon and Steelhead, Environmental Field Branch, Office of Pesticide Programs, p. 71.
Fenoll, J., Vela, N., Navarro, G., Pérez-Lucas, G., Navarro, S., 2014. Assessment of agro-industrial and composted organic wastes for reducing the potential leaching of triazine herbicide residues through the soil. Sci. Total Environ. 493, 124–132.
Gaetani, G.F., Galiano, S., Canepa, L., Ferraris, A.M., Kirkman, H.N., 1989. Catalase and glutathione peroxidase are equally active in detoxification of hydrogen peroxide in human erythrocytes. Blood 73, 334–339.
Gunkel, G., 1981. Bioaccumulation of a herbicide (atrazine, *s*-triazine) in the whitefish (*Coregonus fera J*): uptake and distribution of the residue in fish. Arch. Hydrobiol. 59, 252–287.
Hermes-Lima, M., 2004. Oxygen in biology and biochemistry: role of free radicals. In: Storey, K.B. (Ed.), Functional Metabolism: Regulation and Adaptation. John Wiley & Sons, Inc., Hoboken, pp. 319–368, http://dx.doi.org/10.1002/047167558X.ch12
Hermes-Lima, M., Willmore, W.G., Storey, K.B., 1995. Quantification of lipid peroxidation in tissue extracts based on Fe(III) xylenol orange complex formation. Free Radic. Biol. Med. 19, 271–280.
Husak, V.V., Mosiichuk, N.M., Maksymiv, I.V., Sluchyk, I.Y., Storey, J.M., Storey, K.B., Lushchak, V.I., 2014. Histopathological and biochemical changes in goldfish kidney due to exposure to the herbicide Sencor may be related to induction of oxidative stress. Aquat. Toxicol. 155, 181–189.
Jiang, L., Yang, H., 2009. Prometryne-induced oxidative stress and impact on antioxidant enzymes in wheat. Ecotoxicol. Environ. Saf. 72, 1687–1693.
Jin, Y., Zhang, X., Shu, L., Chen, L., Sun, L., Qian, H., Liu, W., Fu, Z., 2010. Oxidative stress response and gene expression with atrazine exposure in adult female zebrafish (*Danio rerio*). Chemosphere 78, 846–852.
Kirkman, H.N., Rolfo, M., Ferraris, A.M., Gaetani, G.F., 1999. Mechanisms of protection of catalase by NADPH. Kinetics and stoichiometry. J. Biol. Chem. 274, 13908–13914.
Kirkman, N.H., Gaetani, G.F., 1984. Catalase: a tetrameric enzyme with four tightly bound molecules of NADPH. Proc. Natl. Acad. Sci. U. S. A. 81, 4343–4347.
Lenz, A.G., Costabel, U., Shaltiel, S., Levine, R.L., 1989. Determination of carbonyl groups in oxidatively modified proteins by reduction with tritiated sodium borohydride. Anal. Biochem. 177, 419–425.
Livingstone, D.R., 2003. Oxidative stress in aquatic organisms in relation to pollution and aquaculture. Rev. Med. Vet. 154, 427–430.
Luna, G.L., 1968. Manual of Histopathological Staining Methods of the Armed Force Institute of Pathology, third ed. McGraw-Hill Co, New York, NY.
Lushchak, V.I., 2011. Environmentally induced oxidative stress in aquatic animals. Aquat. Toxicol. 101, 13–30.
Lushchak, V.I., 2012. Glutathione homeostasis and functions: potential targets for medical interventions. J. Amino Acids 2012, 736837.
Lushchak, V.I., 2014. Free radicals, reactive oxygen species, oxidative stress and its classification. Chem. Biol. Interact. 224C, 164–175.
Lushchak, V.I., Bagnyukova, T.V., 2006a. Effects of different environmental oxygen levels on free radical processes in fish. Comp. Biochem. Physiol. B Biochem. Mol. Biol 144, 283–289.
Lushchak, V.I., Bagnyukova, T.V., 2006b. Temperature increase results in oxidative stress in goldfish tissues: 1. Indices of oxidative stress. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 143, 30–35.
Lushchak, V.I., Bagnyukova, T.V., Husak, V.V., Luzhna, L.I., Lushchak, O.V., Storey, K.B., 2005. Hyperoxia results in transient oxidative stress and an adaptive response by antioxidant enzymes in goldfish tissues. Int. J. Biochem. Cell Biol. 37, 1670–1680.
Maksymiv, I.V., Husak, V.V., Mosiichuk, N.M., Matviishyn, T.M., Sluchyk, I.Y., Storey, J.M., Storey, K.B., Lushchak, V.I., 2015. Hepatotoxicity of herbicide Sencor in goldfish may result from induction of mild oxidative stress. Pest. Biochem. Physiol., http://dx.doi.org/10.1016/j.pestbp.2014.12.020
Maynard, M.S., Brumback, D., Itterly, W., Capps, T., Rose, R., 1999. Metabolism of [^{14}C] prometryn in rats. J. Agric. Food Chem. 47, 3858–3865.
Monteiro, D.A., De Almeida, J.A., Rantin, F.T., Kalinin, A.L., 2006. Oxidative stress biomarkers in the freshwater characid fish, Brycon cephalus, exposed to organophosphorus insecticide Folisuper 600 (methyl parathion). Comp. Biochem. Physiol. C: Comp. Pharmacol. Toxicol. 143, 141–149.
Nwani, C.D., Lakra, W.S., Nagpure, N.S., Kumar, R., Kushwaha, B., Srivastava, S.K., 2010. Toxicity of the herbicide atrazine: effects on lipid peroxidation and activities of antioxidant enzymes in the freshwater fish *Channa punctatus* (Bloch). Int. J. Environ. Res. Public Health 7, 3298–3312.
Oropesa, A.L., García-Cambero, J.P., Soler, F., 2009. Glutathione and malondialdehyde levels in common carp after exposure to simazine. Environ. Toxicol. Pharmacol. 27, 30–38.
Paulino, M.G., Souza, N.E.S., Fernandes, M.N., 2012. Subchronic exposure to atrazine induces biochemical and histopathological changes in the gills of a Neotropical freshwater fish, *Prochilodus lineatus*. Ecotoxicol. Environ. Saf. 80, 6–13.
Pfeuffer, R.J., 2014, Pesticide surface water and sediment quality report. March 2014 sampling event. West Palm Beach, FL: South Florida Water Management District MSC 4441 3301 Gun Club Road West Palm Beach FL, 33406, p.17.
Qi, W., Singer, H., Berg, M., Müller, B., Pernet-Coudrier, B., Liu, H., Qu, J., 2015. Elimination of polar micropollutants and anthropogenic markers by wastewater treatment in Beijing, China. Chemosphere 119, 1054–1061.
Sies, H., 2015. Oxidative stress: a concept in redox biology and medicine. Redox Biol. 4C, 180–183.
Slaninova, A., Smutna, M., Modra, H., Svobodova, Z., 2009. A review: oxidative stress in fish induced by pesticides. Neuro. Endocrinol. Lett. 30, 2–12.
Smalling, K.L., Orlando, J.L., 2011. Occurrence of pesticides in surface water and sediments from three central California coastal watersheds, 2008–09: U.S. Geological Survey Data Series 600. 70 p.
Stará, A., Kouba, A., Velíšek, J., 2014. Effect of chronic exposure to prometryne on oxidative stress and antioxidant response in red swamp crayfish (*Procambarus clarkii*). Biomed. Res. Int. 2014, 6, ID 680131.
Stará, A., Kristan, J., Zuskova, E., Velíšek, J., 2013. Effect of chronic exposure to prometryne on oxidative stress and antioxidant response in common carp (*Cyprinus carpio* L.). Pest. Biochem. Physiol. 105, 18–23.
Stará, A., Machova, J., Velíšek, J., 2012a. Effect of chronic exposure to prometryne on oxidative stress and antioxidant response on early life stages of common carp (*Cyprinus carpio* L.). Neuro Endocrinol. Lett. 33, 130–135.
Stará, A., Machova, J., Velíšek, J., 2012b. Effect of chronic exposure to simazine on oxidative stress and antioxidant response in common carp (*Cyprinus carpio* L.). Environ. Toxicol. Pharmacol. 33, 334–343.
U.S. Environmental protection agency. 1996. Registration Elibility Decision (RED)–Prometryn, p. 171.
Valavanidis, A., Vlahogianni, T., Dassenakis, M., Scoullos, M., 2006. Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. Ecotoxicol. Environ. Saf. 64, 178–189.
Van der Oost, R., Beyer, J., Vermeulen, N.P., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57–149.
Velíšek, J., Stará, A., Kolarova, J., Svobodova, Z., 2011a. Biochemical, physiological and morphological responses in common carp (*Cyprinus carpio* L.) after long-term exposure to terbutryn in real environmental concentration. Pest. Biochem. Physiol. 100, 305–313.
Velíšek, J., Stará, A., Svobodova, Z., 2011. The effects of pyrethroid and triazine pesticides on fish physiology. In: Stoytcheva, M. (Ed.), Pesticides in the Modern World – Pests Control and Pesticides Exposure and Toxicity Assessment. ISBN: 978–953-307–457-3, InTech, 10.5772/17289.
Velisek, J., Stara, A., Machova, J., Svobodova, Z., 2012. Effects of long-term exposure to simazine in real concentrations on common carp (*Cyprinus carpio* L.). Ecotoxicol. Environ. Saf. 76, 79–86.
Velisek, J., Stara, A., Zuskova, E., Svobodova, Z., 2013. Use of biometric, hematologic, and plasma biochemical variables, and histopathology to assess the chronic effects of the herbicide prometryn on Common carp. Vet. Clin. Path. 42, 508–515.
Vryzas, Z., Alexoudisa, C., Vassilioua, G., Galanisa, K., Papadopoulou-Mourkidoub, E., 2011. Determination and aquatic risk assessment of pesticide residues in riparian drainage canals in northeastern Greece. Ecotoxicol. Environ. Saf. 74, 174–181.
Wakabayashi, K., Böger, P., 2004. Phytotoxic sites of action for molecular design of modern herbicides (Part 1): the photosynthetic electron transport system. Weed Biol. Manage. 4, 8–18.
Winzer, K., Van Noorden, C.J., Kohler, A., 2002. Glucose-6-phosphate dehydrogenase: the key to sex-related xenobiotic toxicity in hepatocytes of European flounder (*Platichthys flesus* L.). Aquat. Toxicol. 56, 275–288.
Xing, H., Li, S., Wang, Z., Gao, X., Xu, S., Wang, X., 2012. Histopathological changes and antioxidant response in brain and kidney of common carp exposed to atrazine and chlorpyrifos. Chemosphere 88, 377–383.
Zhu, L.S., Shao, B., Song, Y., Xie, H., Wang, J., Wang, J.H., Liu, W., Hou, X.X., 2011. DNA damage and effects on antioxidative enzymes in zebra fish (*Danio rerio*) induced by atrazine. Toxicol. Mech. Methods 21, 31–36.