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Oxidative stress responses in gills of goldfish, Carassius auratus, exposed to the metribuzin-containing herbicide Sencor

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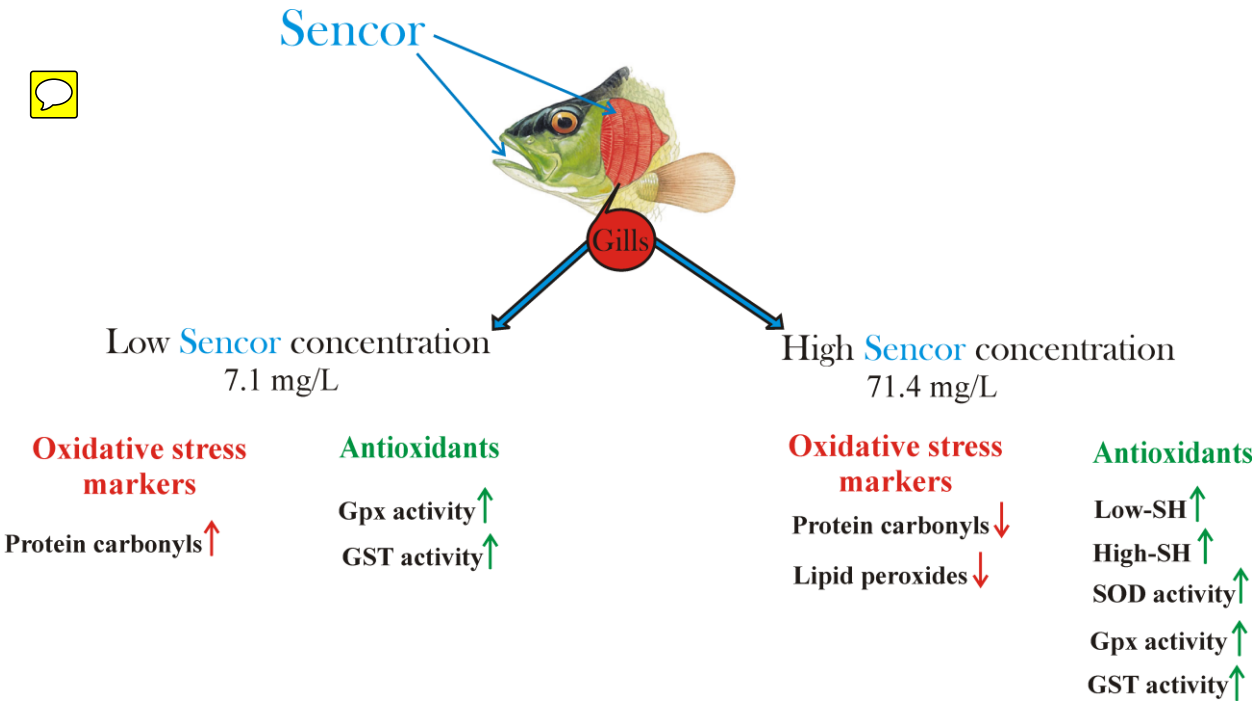
Graphical Abstract



Highlights

- Acute toxicity of Sencor was studied on goldfish gills.
- Increase of the activities of glutathione-related antioxidant enzymes was observed.
- Sencor increased level of low and high molecular mass thiols.
- Sencor changed intensity of protein and lipid oxidation.

Abstract

Metribuzin belongs to the family of asymmetrical triazine compounds and is an active ingredient in many commercial herbicides including Sencor. Effects on goldfish (*Carassius auratus* L.) of exposure for 96 h to 7.14, 35.7 or 71.4 mg L^{-1} Sencor 70 WG (corresponding to 5, 25 and 50 mg L^{-1} of metribuzin) were examined by evaluating oxidative stress markers and activities of antioxidant and associated enzymes in gills. Fish exposed to the lowest Sencor concentration (7.14 mg L^{-1}) showed a 94% increase in levels of protein carbonyls in gills as well as 45% and 144% increases in the activities of glutathione peroxidase and glutathione-S-transferase. Exposure to the highest Sencor concentration (71.4 mg L^{-1}) resulted in reduced levels of protein carbonyls by 56% and lipid peroxides by 40%, as compared with controls, but enhanced levels of low and high molecular mass thiols by 71% and 36%, respectively. The activities of superoxide dismutase, glutathione peroxidase and glutathione-S-transferase were increased in gills of goldfish exposed to 71.4 mg L^{-1} Sencor. At any concentration tested, Sencor did not affect the activities of glutathione reductase, glucose-6-phosphate dehydrogenase, lactate dehydrogenase or acetylcholine esterase in gills. The results of this study indicate that acute exposure of goldfish to Sencor had effect on free radical processes in gills and glutathione-dependent antioxidants effectively protect proteins and lipids from oxidation.

Keywords: Antioxidant enzymes; Stress markers; Toxicity; Triazine herbicides; Vertebrates.

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.

1. Introduction

Metribuzin [4-amino-6-*tert*-butyl-3-(methythio)-1,2,4-triazin-5-one] belongs to the asymmetrical triazine herbicides and is widely used for the pre- and post-emergence treatment of annual grasses and broad-leaved weeds in variety of crops (Stevens et al., 2001). It is an active ingredient of many herbicides that are used worldwide such as Lexone 2, Artist, Sencor, Sencorex, and Shotput. The herbicidal activity of triazines is based on the inhibition of photosynthesis by blocking electron transport in photosystem-II (Das et al., 2000; Pauli et al., 1990).

The extensive use of metribuzin-containing herbicides in agriculture, as well as their high water solubility (1.22 mg L^{-1}) and half-life of 30 days has resulted in the presence of significant quantities of these herbicides in surface and ground waters (Pauli et al., 1990; Wauchope et al., 1992). Indeed, metribuzin was found in surface waters in midwestern Brazil and in midwestern surface waters in the United States in concentrations up to 0.400 µg L^{-1} (Battaglin et al., 2001; Dores et al., 2006). As a consequence, the herbicide can affect non-target aquatic organisms, such as freshwater macrophytes, algae, invertebrates or vertebrates (Fairchild and Sappington, 2002; Quednow and Puttmann, 2007).

In fish, gills are the first site of direct contact with water contaminants and previous research proved that uptake of triazines via fish gills was a main route of its entry (Gunkel, 1981). The gill is a metabolically active tissue in the fish and it can perform biotransformation and elimination of many xenobiotics (van der Oost et al., 2003; Gomez et al., 2011). Biotransformation typically occurs in three phases: phase I metabolism, phase II conjugation, and phase III – release from the cell and organism. Phase I metabolism often involves oxidation, reduction or hydrolysis converting a compound into a slightly more polar one (Goeptar et al., 1995). Phase II involves the conjugation of the intermediate metabolite with a polar functional group to increase its water solubility (Parkinson and Ogilvie, 2007). Finally, in phase III modified xenobiotics are released from the organism. However, reactive intermediates of xenobiotics metabolism which are not neutralised efficiently can damage gill cellular components, in particular via increased generation of reactive oxygen species (ROS) and development of oxidative stress (Livingstone, 2003; Slaninova et al., 2009; Lushchak, 2011). Therefore, specific damage to gills occurring as a result of fish exposure to toxic substances under laboratory conditions may provide some clues to reveal the effects of pollutants on living organisms in nature.

Previous studies have shown that different triazine herbicides caused perturbation of free radical processes in gills of bluegill sunfish (Elia et al., 2002) and common carp (Velisek et al., 2011; Stara et al., 2013). Nevertheless, little is known about the effects of commercial

formulations of triazine herbicides on fish. The commercial formulations contain surfactants and other substances that improve effects of the principal compound. Importantly, the results of recent studies suggest that formulations of glyphosate-based herbicides may be even more toxic than the active ingredient alone (Lushchak et al., 2009; Ortiz-Ordoñez et al., 2011). Therefore, the present study was undertaken to investigate the effects of the industrial metribuzin-containing herbicide Sencor 70 WG on free radical processes in gills of goldfish (*Carassius auratus*).

2. Material and methods

2.1. Reagents

Phenylmethylsulfonyl fluoride (PMSF), 1-chloro-2,4-dinitrobenzene (CDNB), reduced glutathione (GSH), oxidized glutathione (GSSG), 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), KH_2PO_4, Tris(hydroxymethylaminomethane) and N,N,N′,N′-tetramethylethylenediamine (TEMED) were purchased from Sigma-Aldrich Corporation (St. Louis, USA). Hydrogen peroxide (H_2O_2) was from Merck (Darmstadt, Germany), $NADP^+$ was obtained from Reanal (Budapest, Hungary), acetylthiocholine iodide and NADPH were from Carl Roth (Germany), and Sencor was purchased from Bayer Crop Science (Germany). All other reagents were of analytical grade.

2.2. Animals and experimental conditions

Goldfish (*C. auratus* L.), with body mass of 80-100 g, were obtained from a local fish farm (Halych district, Ivano Frankivsk region) in November 2012. Fish were acclimated to laboratory conditions for four weeks in a 1000 L tank under natural photoperiod in aerated and dechlorinated tap water. Water parameters were 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) of 38-40 mg L^{-1}. Fish were fed *ad libitum* with commercial pellets for cyprinids (Koi Grower, Kremenchug, Ukraine), containing 36% protein, 7% fat and 3.6% cellulose, 8.7% ash, 1% phosphorus, and vitamins C, A, D_3, and E. Fish were fed during the acclimation period (4 weeks), but were fasted for one day prior and during experimentation.

Experiments were carried out in 120 L glass aquaria (containing 100 L of water), in a static mode, under the same conditions but with the addition of different concentrations of the commercial herbicide Sencor (Bayer Crop Science, Germany) that contains metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5-one] in concentration 700 g L^{-1}. Groups of seven fish were placed in aquaria with different nominal concentrations of Sencor

herbicide: 7.1, 35.7 or 71.4 mg L^{-1}. This corresponded to 5, 25 or 50 mg L^{-1} metribuzin, respectively. Fish were exposed to these conditions for 96 h (no mortality occurred during exposures). Fish in a control group were maintained in the same manner, but without addition of Sencor to the water. Aquarium water was not changed over the 96 h course in order to avoid stressing the animals. Concentrations of the pesticide were chosen on the basis of a known approximate metribuzin LC_{50} for goldfish (more than 10 mg L^{-1}) and Sencor LC_{50} for common carp (250.2 mg L^{-1} of Sencor WG 70), that corresponds to 175.1 mg L^{-1} of metribuzin (Kamrin, 1997; Velisek et al., 2009). Therefore, we chose concentrations of Sencor for our tests that were lower than the LC_{50} for carp and two of the concentrations were higher than 10 mg L^{-1}. Levels of dissolved oxygen, temperature and pH were monitored every 24 h. The experiments were carried out in two independent experimental replicates with a total number of at least five biological replicates for every measured parameter.

After fish exposure, the animals were sacrificed and gill filaments were dissected from gill arches, rinsed in cold (4°C) 0.9% NaCl solution, blotted on filter paper, and put into cryovials. These were immediately dropped into liquid nitrogen and stored there until use.

All experiments were conducted in accordance with the Ethics Committee of Precarpathian National University.

2.3. Determination of products of ROS interaction with cellular components

Lipid peroxide (LOOH) content was assayed by the FOX (ferrous-xylenol orange) method (Hermes-Lima et al., 1995). Gill 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). Aliquots (40 μL) 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.

Levels of carbonyl groups on gill proteins 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). 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). Protein carbonyl (CP) levels were measured in the resulting pellets by reaction with 2,4-dinitrophenylhydrazine (DNPH), leading to formation of dinitrophenylhydrazones (Lenz et al., 1989). Values are expressed as nanomoles of carbonyl groups per milligram protein (nmol/mg protein).

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 (the sum of low and high molecular mass thiols) was measured as described previously (Lushchak and Bagnyukova, 2006). 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 (16000×*g*, 5 min) to remove pelleted protein and the final supernatants were used for the assay. Thiol concentrations were expressed as micromoles of SH-groups per gram tissue wet mass. 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 concentration

Tissue supernatants were prepared as described above for the CP/thiol assays. The activities of antioxidant enzymes including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) as well as activities of antioxidant-associated enzymes glutathione-S-transferase (GST), glutathione reductase (GR), and glucose-6-phosphate dehydrogenase (G6PDH) were measured as described previously (Lushchak et al., 2005). The activity of lactate dehydrogenase (LDH) was assayed spectrophometrically by monitoring the change in NADH absorbance at 340 nm (Lushchak et al., 2001).

Acetylcholinesterase (AChE) activity was determined by the method of Ellman et al. (1961) with modifications and adaptation of the procedure to microplate determination (Kubrak et al., 2013). 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 13600 $M^{-1}cm^{-1}$.

One unit of SOD activity was defined as the amount of enzyme (per mg protein) that inhibited a 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, G6PDH, LDH or AChE activity is defined as the amount of enzyme that consumed 1 μmol of substrate or generated 1 μmol of product per minute. Activities were expressed as international units (or milliunits) per milligram soluble protein (U mg $protein^{-1}$ or mU mg $protein^{-1}$).

Soluble protein concentrations were measured by the Coomassie blue method (Bradford, 1976) using bovine serum albumin as a standard.

2.5. Statistics

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

3. Results and Discussion

Xenobiotics traditionally enter the fish body via passive diffusion through semi-permeable membranes of the gills, mouth and gastrointestinal tract (Spacie and Hamelink, 1985). Gills are metabolically active tissues in fish and have been shown to contain many enzymes capable of biotransformation and elimination of xenobiotics. Presently, there are no data on metribuzin metabolism in fish tissues. However, a study on mammals showed that the principal urinary metabolites of metribuzin in mice and rats were mercapturic acids, the products of metribuzin sulfoxide or deaminometribuzin sulfoxide reaction with GSH (Bleeke et al., 1985). In mice the possible acute and subchronic metribuzin toxicity was caused by activation of metribuzin to an electrophilic metabolite which, in the absence of GSH, could bind to tissue proteins (Bleeke and Casida, 1984; Bleeke et al., 1985). Fig. 1 demonstrates the possible routes of metribuzin biotransformation in fish.

Previously we showed that induction of oxidative stress might be responsible for damage to liver and kidney in Sencor treated goldfish (Husak et al., 2014; Maksymiv et al., 2015). In the present study we investigated oxidative stress parameters in gills of goldfish exposed to 7.1, 35.7 and 71.4 mg L^{-1} of Sencor for 96 h.

3.1. Levels of ROS-modified cellular components

Increased levels of protein carbonyl groups (CP) and lipid peroxides (LOOH) are widely accepted hallmarks of oxidative stress (Lushchak, 2014). In the present study the CP content was 5.65 ± 0.64 nmol mg protein^{-1} in gills of control fish (Fig. 2A). Fish exposure to 7.1 mg L^{-1} Sencor for 96 h resulted in a 94% higher CP level in gills, whereas at the highest concentration of herbicide, 71.4 mg L^{-1}, the CP level of was 56% lower as compared with control value (Fig. 2A). The concentration of LOOH was 46.6 ± 2.6 nmol gwm^{-1} in gills of control fish. This parameter was not affected at low pesticide concentrations, but diminished by ~40% in fish exposed to the maximum concentration tested 71.4 mg L^{-1} of Sencor (Fig. 2B).

It can be suggested that defense systems could not prevent modifications of gill proteins, but protect lipids from oxidation at the lower concentration of Sencor. However, Sencor at the highest concentration used lead to activation of some mechanisms for degradation of oxidized

proteins or lipids and in such way exceeded the possible intensification of protein and lipid oxidation. It is known that multicatalytic protease degrades oxidatively modified proteins and oxidative stress activates this enzyme (Bagnyukova et al., 2007). Lipid peroxides can be degraded by phospholipid hydroperoxide glutathione peroxidase and/or GST (Lushchak, 2012; 2014). Also, different low molecular mass antioxidants can detoxify LOOH (Lushchak, 2014). On the other hand, lipid hydroperoxides and protein carbonyls are not only markers of oxidative damage – they are also possibly involved in up-regulation of activities of antioxidant enzymes. As a result, an enhanced antioxidant response could suppress the intensity of lipid peroxidation and protein carbonylation (Lushchak and Bagnyukova, 2006). In our previous experiments we observed decreased level of CP in liver and LOOH in kidney of goldfish exposed to Sencor as compared to tissues of control fish (Husak et al., 2014; Maksymiv et al., 2015). Previously, Velisek and coauthors (2011) reported elevated CP levels in gills of common carp exposed to 0.2 and 2.0 μg L^{-1} of terbutryn. Paulino et al. (2012) reported no change in the level of oxidized lipids in the neotropical freshwater fish, *Prochilodus lineatus*, after acute exposure to atrazine. However, long-term exposure (14 days) to 10 μg L^{-1} atrazine significantly increased LOOH concentrations in the gills of this species (Paulino et al., 2012). Similarly, Blahová and colleagues (2013) showed enhanced level of oxidized lipids in 30-day old zebrafish exposed to 30 and 90 μg L^{-1} atrazine (subchronic toxicity). Levels of LOOH were not affected in gills of common carp after long-term exposure to terbutryn (Velisek et al., 2011).

Thiol-containing compounds are frequently used as markers of oxidative stress (Lushchak and Bagnyukova, 2006). The low molecular mass thiols (L-SH) are mainly represented by the tripeptide glutathione (GSH), whereas protein thiols represent the high molecular mass thiols (H-SH) (Lushchak, 2012). Glutathione plays a central role in the antioxidant system and is the major cytosolic low molecular mass sulfhydryl compound that acts as a cellular reducing and protective reagent against ROS (Maher et al., 2005; Lushchak, 2012). It is involved in detoxification of pesticides via conjugating them with GSH either nonenzymatically or with participation of GSH-transferases (Lushchak, 2012). In the present study, the levels of both low and high molecular mass thiols were affected in gills of fish exposed to Sencor (Fig. 3). The concentrations of L-SH were enhanced by 74% and 71% in gills of fish exposed to 35.7 and 71.4 mg L^{-1} of Sencor, respectively, as compared with the control value (0.42 ± 0.04 μmol gwm^{-1})(Fig. 3A). Enhanced levels of L-SH in gills of fish exposed to Sencor could suggest involvement of glutathione in herbicide detoxification. The concentration of H-SH was 4.88 ± 0.15 μmol gwm^{-1} in gills of control fish and was elevated by 36% only in animals exposed to Sencor at the highest concentration (Fig. 3B). Previously, we also found increases in L-SH concentrations in gills of goldfish treated with the mancozeb-containing carbamate fungicide Tattoo (Kubrak et al., 2012)

or the triazine herbicide Gesagard 500 FW (Mosiichuk et al., 2015). Also, Sencor had no effects on L-SH or H-SH in kidney of goldfish, but L-SH content was decreased in liver of goldfish exposed to Sencor at the highest concentration (Husak et al., 2014; Maksymiv et al., 2015).

3.2. Activities of antioxidant and related enzymes

Fish possess multiple antioxidant and associated enzymes, which protect organs from ROS-mediated damage. These include 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 (Lushchak, 2014; Sies, 2015).

In the present study SOD activity in gills of control goldfish was 27.0 ± 1.5 U mg protein^{-1}. Fish exposure to Sencor at the highest concentration (71.4 mg L^{-1}) resulted in 41% higher SOD activity in gills, whereas at lower concentrations no significant pesticide effects were found (although an upwards trend was seen) (Fig. 4A). One may suggested that SOD activity was increased in order to protect proteins and lipids from attack by superoxide radicals (or other radicals generated from superoxide) under fish exposure to the highest concentration of Sencor where oxidative stress was developed. Previous studies reported either increase or decrease in the SOD activity in tissues of fish exposed to different triazine herbicides (Stara et al., 2012; Velisek et al., 2011; Xing et al., 2012). For example, Paulino and coauthors (2012) reported increased SOD activity in gills of *P. lineatus* at subchronic exposure to atrazine at concentration 10 μg L^{-1}. Other studies showed no change of SOD activity in gills of common carp exposed to simazine for 14, 28 or 60 days (Stara et al., 2012), as well as in gills of bluegill sunfish after acute treatment with atrazine (Elia et al., 2002). Velisek and colleagues (2011) observed no significant changes in SOD activity in gills of common carp exposed to terbutryn at different concentrations. Goldfish exposure to the triazine herbicide Gezagard did not affect SOD activity in gills (Mosiichuk et al., 2015).

There are several enzymes which scavenge the hydrogen peroxide produced via SOD or other superoxide-producing reactions. Catalase and GPx are the main ones. In gills of control fish, catalase activity was 3.7 ± 0.3 U mg protein^{-1} and goldfish exposure to Sencor at any of the concentrations used did not significantly affect it (Fig. 4B). Previously, we found similar effects in gills of goldfish exposed to Gezagard (Mosiichuk et al., 2015). Furthermore, we found that catalase activity was lower in liver and kidney of goldfish exposed to Sencor than in tissues of control fish (Husak et al., 2014; Maksymiv et al., 2015). In addition, some scientists observed no changes in catalase activity in gills of *Lepomis macrochirus* (Elia et al., 2002) and *P. lineatus* (Paulino et al., 2012), respectively, after acute exposure to atrazine at different concentrations.

As it was mentioned above, enzymes associated with glutathione (GSH) play important roles in defense against ROS. In the present study we found that the activities of another H_2O_2-detoxifying enzyme, glutathione peroxidase (GPx), responded to goldfish exposure to Sencor. The activity of GPx was 67.0 ± 2.7 mU mg protein^{-1} in gills of control fish and was enhanced by 45%, 33%, or 30% after 96 h fish exposure to 7.1, 35.7 or 71.4 mg L^{-1} of Sencor, respectively (Fig. 5A). In this study, higher GPx activities in gills of Sencor-exposed fish could be responsible for the lower levels of LOOH detected in gills of fish treated with 71.4 mg L^{-1} Sencor because it is known that GPx may also reduce lipid peroxides (Lushchak, 2012). No changes were found in the activity of GPx in gills of *P. lineatus* (Paulino et al., 2012) and *L. macrochirus* (Elia et al., 2002) after acute exposure to atrazine, or in gills of common carp exposed to simazine (Stara et al., 2012). A reduction in GPx activity was observed in kidney and liver of goldfish exposed to Sencor (Husak et al., 2014; Maksymiv et al., 2015).

Glutathione-S-transferase (GST) is a very important enzyme involved in the detoxification of many xenobiotics, including pesticides (Lushchak, 2012). It has been demonstrated that GST activity can be altered in fish from polluted locations, and that the presence of organic contaminants may increase GST activity (Machala et al., 1997). In the present study, GST activity in gills of goldfish in the control group was 180 ± 30 U mg protein^{-1} and fish exposure to Sencor at concentrations 7.1, 35.7 and 71.4 mg L^{-1} resulted in higher activities by 144, 206 and 67%, respectively (Fig. 5B). These results may reflect the importance of one of the possible biotransformation pathways for Sencor detoxification in gills. As shown in Fig. 1, this involves metribuzin (an active ingredient of Sencor) catalysis by GST leading to its conjugation with glutathione. Previously, we observed enhanced GST activity in gills of fish exposed to Gezagard as well as in liver of Sencor-treated goldfish (Maksymiv et al., 2015; Mosiichuk et al., 2015). Elia and colleagues (2002) also reported increased GST activity in gills of bluegill sunfish exposed to 9 mg L^{-1} of atrazine. Paulino et al. (2012) reported no changes in GST activity in gills of *P. lineatus* after acute exposure to different atrazine concentrations but in a later study these authors reported increased GST activity in gills of *P. lineatus* when exposed subchronically to atrazine (Paulino et al., 2012). Hostovsky and coauthors (2012) evaluated GST activity in early developmental stages of common carp after subchronic exposure to terbuthylazine and metribuzin and found no significant changes in GST activity at any of the pesticide concentrations tested.

Glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PDH) are auxiliary enzymes in antioxidant defense: GR reduces oxidized glutathione (GSSG) to reduced glutathione (GSH), whereas G6PDH provides the reducing power (NADPH) needed for the GR reaction (Lushchak, 2014). In the present study, Sencor exposure had no effect on the activities

GR and G6PDH (Table 1). Also, the activity of lactate dehydrogenase (LDH) in gill did not change on fish treatment with Sencor (Table 1). Lactate dehydrogenase is generally associated with cellular intermediary metabolism, which could be inhibited or elevated under oxidative stress. Similarly, we found no changes in G6PDH activity and only minor changes in GR activity in gills of goldfish exposed to Gezagard (Mosiichuk et al., 2015). Other authors reported no changes in GR activity in gills of common carp after long-term exposure to terbutryn or simazine (Stara et al., 2012; Velisek et al. 2011) and in our previous study we observed no changes of G6PDH activity and decreased GR and LDH activities in liver of goldfish exposed to Sencor (Maksymiv et al., 2015). However, Sencor treatment resulted in reduced GR activity but increased G6PDH and LDH activities in goldfish kidney (Husak et al., 2014).

The triazine herbicide atrazine is a known inhibitor of AChE and has been shown to operate in a dose-dependent manner in freshwater fish species *Oreochromis niloticus* and *Chrysichthyes auratus* (Hussein et al., 1996). However, in our work on goldfish exposure to Sencor did not influence AChE activity in gills at any of the concentrations used (Table 1). Similarly, AchE was not affected in liver of goldfish exposed to Sencor (Maksymiv et al., 2015). In contrast, the activity of this enzyme was enhanced in gills of goldfish exposed to Gesagard (Mosiichuk et al., 2015).

Conclusions

In conclusion, the results of this study indicate that Sencor exposure may result in oxidative stress development in goldfish gills. The glutathione-related antioxidants responded to Sencor treatment in gills by increased of L-SH concentration and the activities of GPx and GST and such effect was not observed in liver and kidney in our earlier studies (Husak et al., 2014; Maksymiv et al., 2015). Increased activities of GPx and GST seem were enough to prevent lipid peroxidation in gill even at low concentration of Sencor, but it could not protect proteins from ROS-promoted oxidation. However, additional involvement of L-SH, H-SH, and SOD was sufficiently to protect proteins and lipids from oxidation at higher pesticide concentrations.

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Figure captions

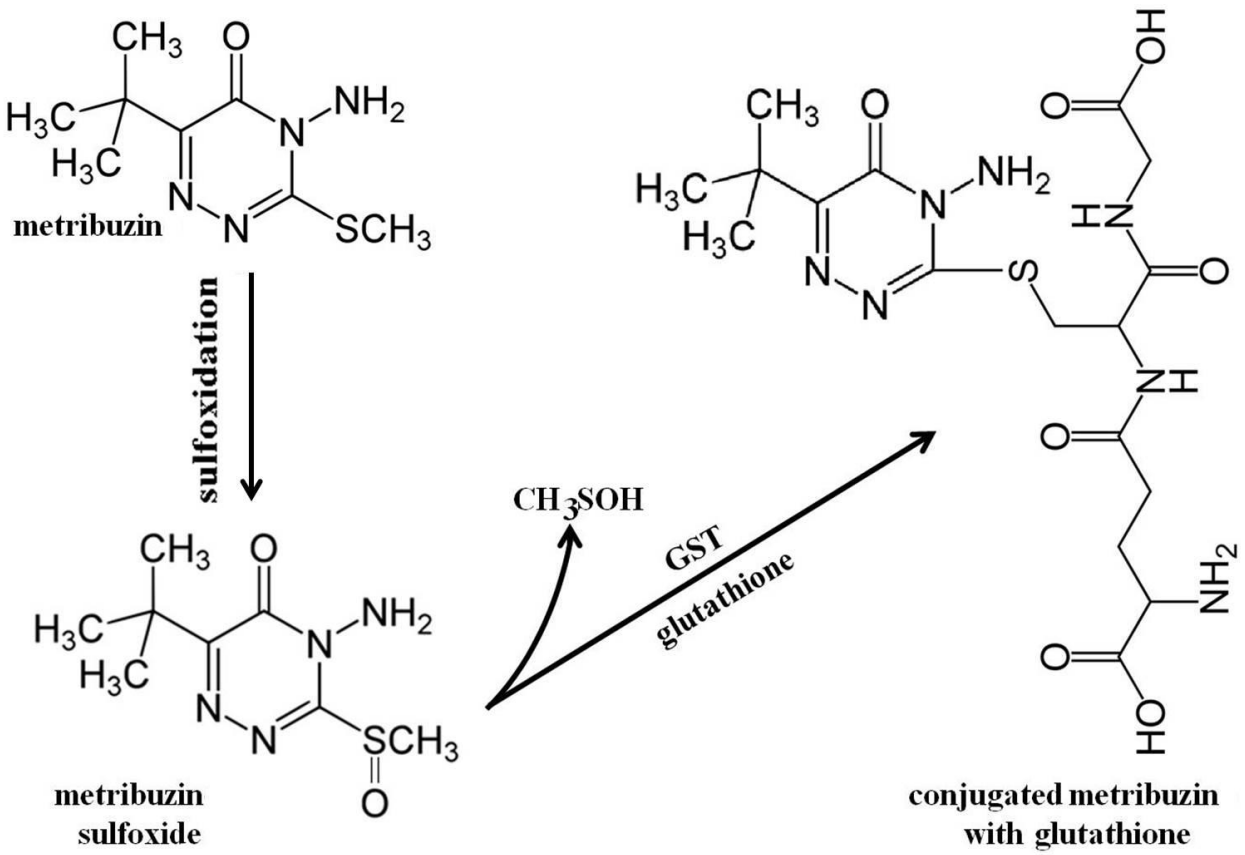
Fig. 1. The tentative scheme of metabolic transformation of metribuzin in fish.

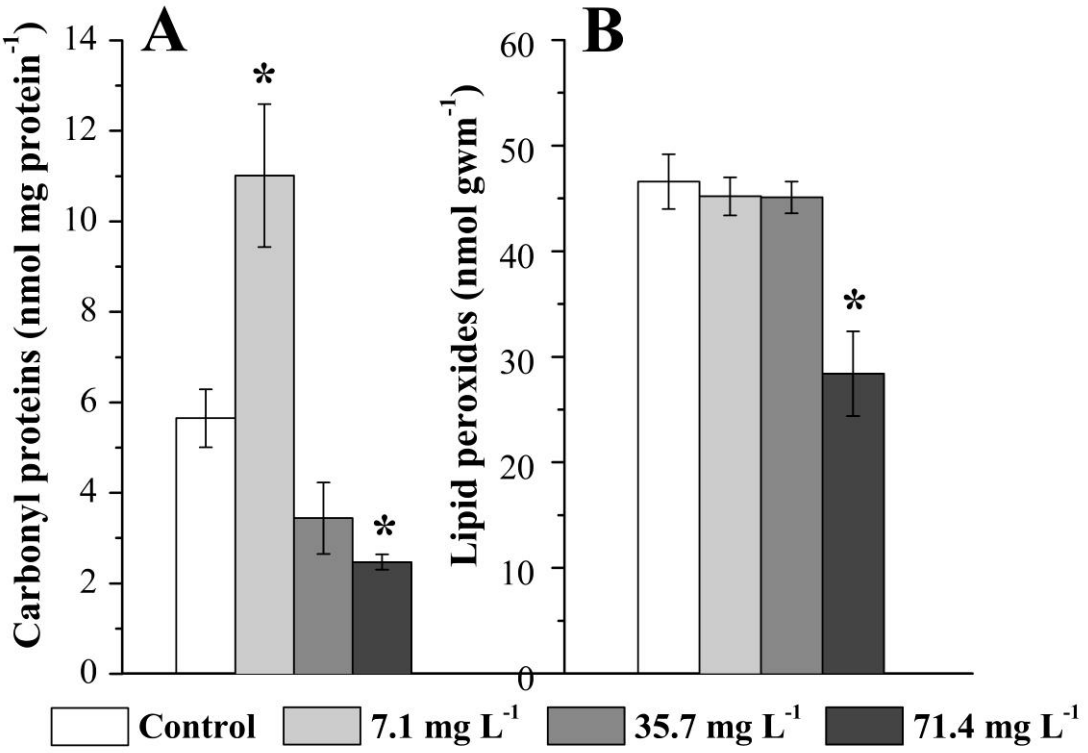
Fig. 2. The levels of oxidative stress indices, carbonyl proteins (A) and lipid peroxides (B), in gills of goldfish exposed to control conditions or to 7.1, 35.7 or 71.4 mg L^{-1} of Sencor for 96 h. Data are presented as means ± S.E.M, n = 5–7. *Significantly different from the control group with $P < 0.05$.

Fig. 3. The concentrations of low molecular mass (A) and high molecular mass (B) thiols (μmol gwm^{-1}) in gills of goldfish exposed to control conditions or to 7.1, 35.7 or 71.4 mg L^{-1} of Sencor for 96 h. Data are presented as means ± S.E.M, n = 5–7. *Significantly different from the control group with $P < 0.05$.

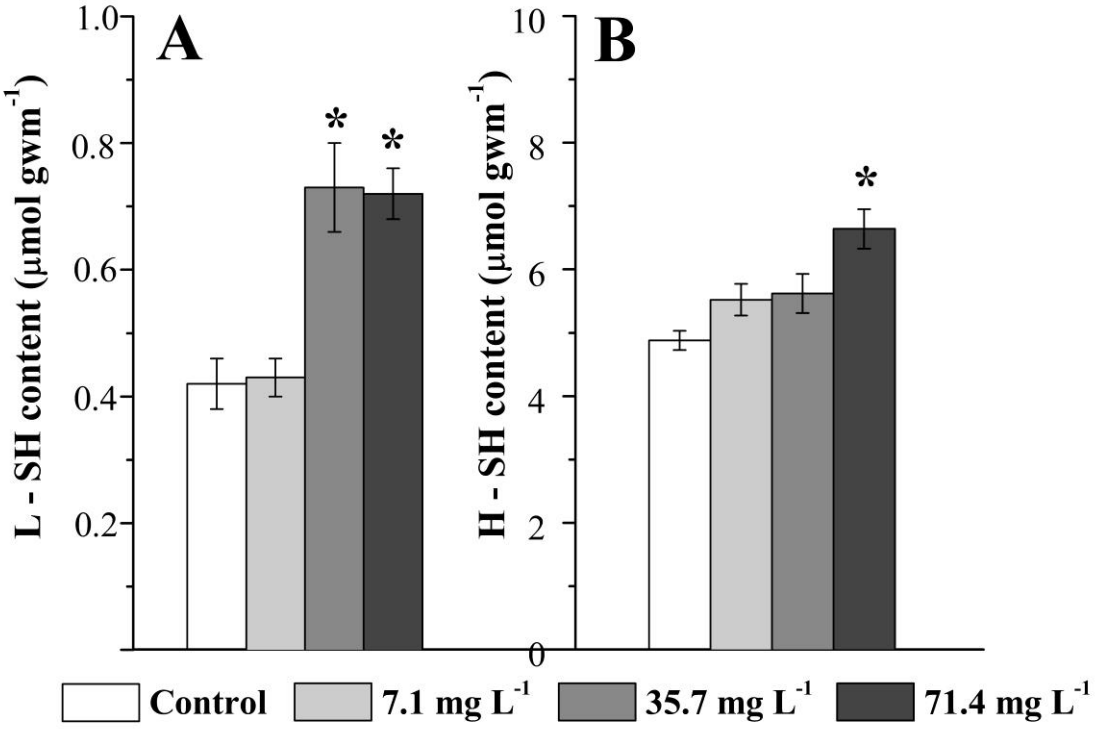
Fig. 4. The activity of (A) superoxide dismutase (SOD), (B) catalase in gills of goldfish exposed to control conditions or to 7.1, 35.7 or 71.4 mg L^{-1} of Sencor for 96 h. Data are presented as means ± S.E.M, n = 5–7. *Significantly different from the control group with $P < 0.05$.

Fig. 5. The activity of (A) glutathione peroxidase (GPx) and (B) glutathione-S-transferase (GST) in gills of goldfish exposed to control conditions or to 7.1, 35.7 or 71.4 mg L^{-1} of Sencor for 96 h. Data are presented as means ± S.E.M, n = 5–7. *Significantly different from the control group with $P < 0.05$.





Husak et al., Fig. 2



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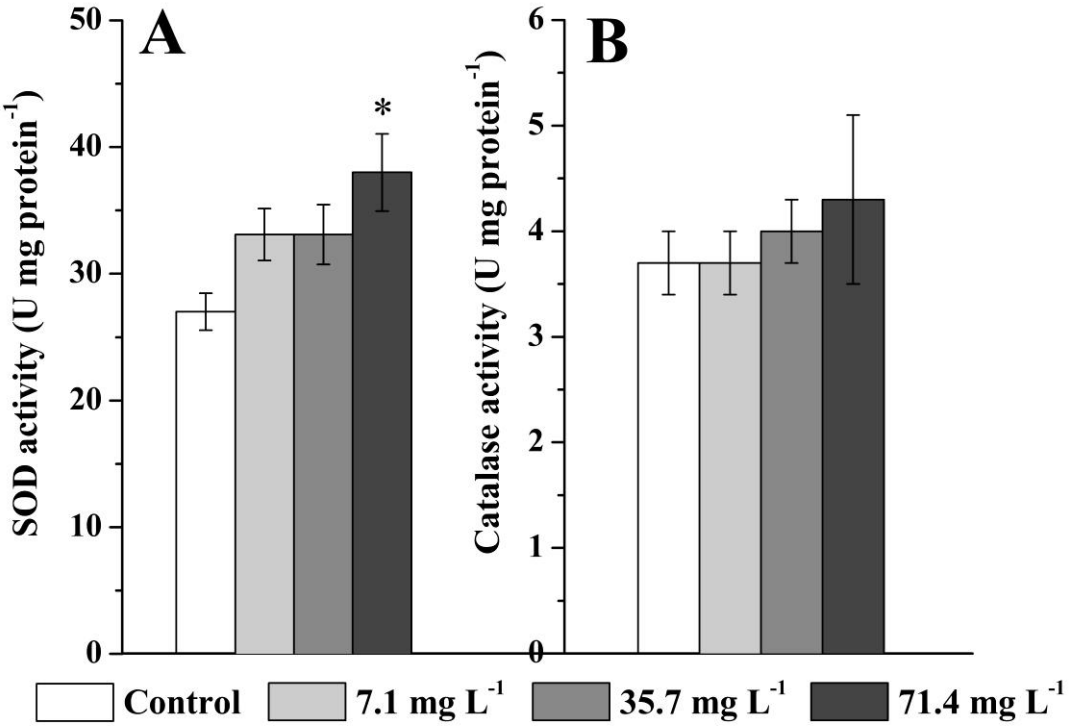

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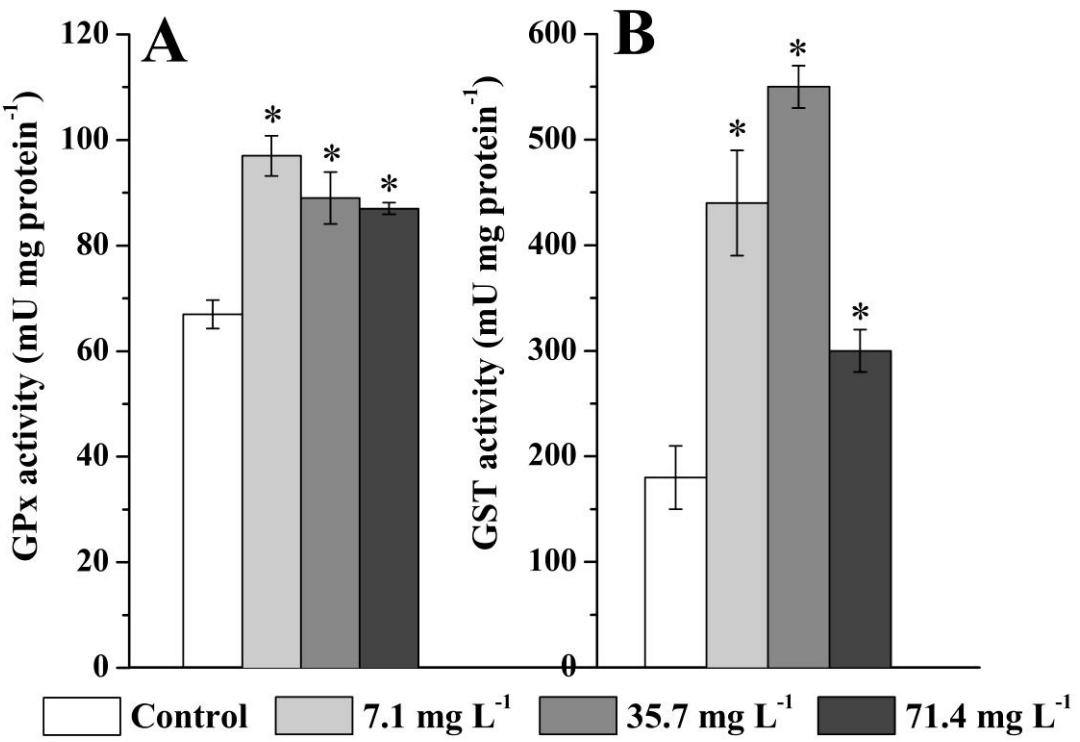

Husak et al., Fig. 5

Table

Table 1 The activity of glutathione reductase (GR), glucose-6-phosphate dehydrogenase (G6PDH), lactate dehydrogenase (LDH), and acetylcholinesterase (AChE) in gills of goldfish, exposed to control conditions of 7.1, 35.7 or 71.4 mg L^{-1} of Sencor for 96 h.

Parameter	Fish group			
	Control	7.1 mg L^{-1}	35.7 mg L^{-1}	71.4 mg L^{-1}
GR activity (mU/mg protein)	18.6 ± 0.7	16.7 ± 0.3	16.9 ± 1.5	19.7 ± 0.9
G6PDH activity, (mU/mg protein)	32.3 ± 2.1	33.3 ± 2.0	27.9 ± 0.9	36.9 ± 2.8
LDH activity, (mU/mg protein)	760 ± 30	680 ± 30	720 ± 50	750 ± 20
AChE activity, (mU mg $protein^{-1}$)	12.4 ± 0.5	13.7 ± 0.6	13.3 ± 0.6	12.5 ± 1.0

Data are presented as means ± S.E.M, n = 5-7. *Significantly different ($P < 0.05$) from the control group as assessed by ANOVA followed by Dunnett's test.