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Histopathological and biochemical changes in goldfish kidney due to exposure to the herbicide Sencor may be related to induction of oxidative stress

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ABSTRACT

Molecular mechanisms of toxicity by the metribuzin-containing herbicide Sencor to living organisms, particularly fish, have not yet been extensively investigated. In the present work, we studied the effects of 96 h exposure to 7.14, 35.7, or 71.4 mg L^{-1} of Sencor (corresponding to 5, 25, or 50 mg L^{-1} of its herbicidal component metribuzin) on goldfish (*Carassius auratus* L.), examining the histology, levels of oxidative stress markers, and activities of antioxidant and related enzymes in kidney as well as hematological parameters and leukocyte profiles in blood. The treatment induced various histopathological changes in goldfish kidney, such as hypertrophy of intertubular hematopoietic tissue, small and multiple hemorrhages, glomerular shrinkage, a decrease in space between glomerulus and Bowman's capsule, degeneration and necrosis of the tubular epithelium. Sencor exposure also decreased activities of selected enzymes in kidney; activities of catalase decreased by 31–34%, glutathione peroxidase by 14–33%, glutathione reductase by 17–25%, and acetylcholinesterase by 31%. However, glucose-6-phosphate dehydrogenase and lactate dehydrogenase activities increased by 25–30% and 22% in kidney after treatment with 7.14 or 35.7 mg L^{-1} and 71.4 mg L^{-1} Sencor, respectively. Kidney levels of protein carbonyls increased by 177% after exposure to 35.7 mg L^{-1} of Sencor indicating extensive damage to proteins. Lipid peroxide concentrations also increased by 25% after exposure to 7.14 mg L^{-1} of Sencor, but levels were reduced by 42% in the 71.4 mg L^{-1} exposure group. The data indicate that induction of oxidative stress is one of the mechanisms responsible for Sencor toxicity to fish.

1. Introduction

The extensive use by modern agriculture of different agrochemicals such as pesticides is emerging as a threat to the ecological balance of aquatic ecosystems. Synthetic pesticides are recognized as serious pollutants in the aquatic environment with the potential to cause deleterious effects on the biota, especially fish occupying the upper trophic level.

Triazines (a six-membered ring containing three carbon and three nitrogen atoms) have been extensively used for weed control since the early 1950s. Triazine herbicides are categorized in two groups, the asymmetrical triazines, such as metribuzin, and the symmetrical triazines, such as simazine, atrazine, terbutryn and others (Stevens et al., 2001). The herbicidal activity of triazines is believed to be mediated by inhibition of photosynthesis (Das et al., 2000) and intensification of reactive oxygen species (ROS) production through its interference with photosystem-II (Pauli et al., 1990; Nemat and Hassan, 2006). In fish, the triazine pesticides affect hematological and histopathological parameters (Velisek et al., 2008, 2012; Oropesa et al., 2009), stimulate DNA damage (Santos and Martinez, 2012), immune response (Fatima et al., 2007), induce

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

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oxidative stress (Elia et al., 2002; Fatima et al., 2007), and reduce growth rate and reproduction (Tillitt et al., 2010).

Metribuzin [4-amino-6-*tert*-butyl-3-(methythio)-1,2,4-triazin-5-one] is an asymmetrical triazine herbicide often used for the control of grasses and broad-leaved weeds in the production of soybeans, potatoes, tomatoes, sugar cane, alfalfa, asparagus, maize and cereals. It is extensively used as an active ingredient in multiple herbicides that are used worldwide such as Artist, Lexone 2, Sencor, Sencorex WG, Shotput and others. Runoff of metribuzin, like other triazine and triazinone herbicides, can readily contaminate surface waters due to its high water solubility 1.22 mg L^{-1} and half-life of 30 days (Pauli et al., 1990; Wauchope et al., 1992). The half-life of metribuzin in pond water is approximately seven days (Hartley and Kidd, 1983). It was shown that the concentration of metribuzin in surface water of Middle West of Brazil ranged up to 0.351 μg L^{-1} (Dores et al., 2006), but was less than 25 μg L^{-1} in Midwestern United States surface water (Battaglin et al., 2001).

Today, there are some data concerning metribuzin effects on different fish species. In particular, Velisek et al. (2008, 2009) showed histopathological changes in the caudal kidneys of rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*) after acute exposure to Sencor 70 WG. Plhalova et al. (2012) found no morphological changes in the kidney, but histopathological lesions in hepatocytes of *Danio rerio* after 28-day exposure to Sencor 70 WG. Mekhed et al. (2004) observed increase in activities of basic enzymes of gluconeogenesis (glucose-6-phosphatase and fructose-1,6-bisphosphatase) and decrease in glucose levels in liver, muscle and brain of carp exposed to Sencor for 14 days. Exposure of fish *Tilapia mossambica* to sublethal concentrations of metribuzin decreased total protein, carbohydrate and cholesterol levels in liver, muscle, kidney and gills (Saradhamani and Selvarani, 2009). Unfortunately, very little is known about free radical processes in the kidneys of various fish species under acute and chronic exposure to metribuzin.

Kidney is a major route for the excretion of xenobiotics and receives the largest portion of postbranchial blood in fish. Hence, intoxication with pesticides may potentially induce histopathological changes and modify biochemical composition of fish kidney (Ortiz et al., 2003; Atamaniuk et al., 2013). The present work aimed to disclose some molecular toxicity mechanisms of the metribuzin-based pesticide, Sencor, in goldfish with a focus on blood and kidney parameters that could potentially be developed as biomarkers of pesticide intoxication.

2. Material and methods

2.1. Reagents

Phenylmethylsulfonyl fluoride (PMSF), ethylenediaminetetraacetic acid disodium salt (Na_2EDTA), 1-chloro-2,4-dinitrobenzene (CDNB), reduced glutathione (GSH), oxidized glutathione (GSSG), glucose-6-phosphate (G6P), xylenol orange, cumene hydroperoxide, ferrous sulphate, 2,4-dinitrophenylhydrazine (DNPH), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), Tris(hydroxymethylaminomethane), NaCl, KH_2PO_4, NADH, NADP, hydrogen peroxide (H_2O_2) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) were purchased from Sigma-Aldrich Corporation (USA). Acetylthiocholine iodide and NADPH was purchased from Carl Roth (Germany). Sencor 70 WG was purchased from Bayer Crop Science (Germany). All other reagents were of analytical grade.

2.2. Animals and experimental conditions

Goldfish (*Carassius auratus* L.) with body masses of 80–100 g were obtained from a local fish farm (Halych district, Ivano-Frankivsk region, Ukraine) in November 2012. Fish were acclimated to laboratory conditions for 4 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 once a day with commercial Cyprinid Carp Co Excellent (Koi Grower, The Netherlands) pellets, containing 36% protein, 7% fat, 3.6% cellulose, 8.7% ash, 1% phosphorus and vitamins C, A, D_3 and E. Both, control and experimental fish groups were fed during the acclimation period (4 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. Groups of seven fish were placed in aquaria with different nominal concentrations of the herbicide Sencor (Bayer, Germany): 7.14; 35.7 or 71.4 mg L^{-1}, which corresponded to 5, 25 or 50 mg L^{-1} of metribuzin, respectively, and exposed to these conditions for 96 h. No mortality occurred during exposures. Fish in the control group were maintained in the same manner, but without addition of Sencor.

Aquarium water was not changed over the 96 h experimental course in order to avoid stressing the fish. Levels of dissolved oxygen, temperature and pH were monitored every 24 h and did not change over the experimental time course. After fish exposure, blood was quickly taken from caudal vessels using a syringe rinsed with 50 mM Na_2EDTA as an anticoagulant. Fish were then quickly sacrificed by transspinal transsection without anesthesia and kidneys 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 accordance with the institutional animal ethics guidelines of Precarpathian National University and were approved by the Animal Experimental Committee of Precarpathian National University.

2.3. Evaluation of hematological parameters and leukocyte formula in blood

2.3.1. Estimation of total hemoglobin concentration and hematocrit value

Total hemoglobin concentration was determined after erythrocyte hemolysis in Drabkin's solution using a commercial kit (Genesis Co, Ltd., Ukraine) following the manufacturer's instructions.

Hematocrit was determined following the procedure of Ptashynski et al. (2002). Immediately after blood sampling, small amounts of whole blood were transferred to microcapillary tubes, which were then carefully sealed on both ends and centrifuged (2000 × *g*, 20 min, 4 °C) using an OPN-8 centrifuge (USSR). Hematocrit values were calculated as the percentage of red blood cell pellet in the total blood column.

2.3.2. Examination of leukocyte content

For microscopic examination of leukocyte content, small drops of whole blood were directly smeared on slides ($n = 2$ per fish) and air-dried. Smears were fixed and stained with azure-eosin water solution as described previously (Vasylkiv et al., 2010). Cytological analysis was conducted by scoring at a 1600× magnification using a Leitz microscope (Leitz Wetzbar GmbH, Germany). Different types of leukocytes were identified according to the Fish Blood Cell Atlas (Ivanova, 1983). A total of 200 leukocyte cells were counted per smear and assigned to different leucocyte categories. Data are shown as the percentages of different leucocytes per 200 cells counted.

2.4. Histological examination of goldfish kidney

Kidney samples from control and treated fish were fixed in 10% neutral-buffered formalin, and then the samples were processed