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

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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.

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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