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Carbofuran promotes biochemical changes in carp exposed to rice field and laboratory conditions

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ABSTRACT

Effects of carbofuran commercial formulation on oxidative stress parameters were studied in carps (*Cyprinus carpio*) exposed to 50 μg/L for 7 and 30 days under rice field and laboratory conditions. Thiobarbituric acid reactive substance (TBARS) levels were increased in the brain of fish after 7 and 30 days under rice field and laboratory conditions. In the liver and muscle, TBARS levels increased after 7 and 30 days under laboratory conditions, whereas in rice field the levels increased only after 30 days. Protein carbonyl content in the liver increased after 7 and 30 days under both experimental conditions. Acetylcholinesterase (AChE) activity was decreased in the brain and muscle after 7 and 30 days under both experimental conditions evaluated. The superoxide dismutase (SOD) activity increased in the liver after 7 and 30 days under rice field condition, whereas under laboratory condition this enzyme increased only after 30 days. The catalase (CAT) activity in the liver decreased after 30 days under rice field condition, whereas no changes were observed under laboratory conditions. In rice field, glutathione S-transferase (GST) decreased after 7 days but increased after 30 days, whereas no change was observed in fish exposed to carbofuran under laboratory conditions. These results suggest that environmental relevant carbofuran concentrations may cause oxidative stress, affecting biochemical and enzymatic parameters on carps. Some parameters could be used as biomarkers to carbofuran exposure.

1. Introduction

Dependence on pesticides has been increasing especially in tropical areas such as Brazil where agriculture has increased dramatically over the last decades (Sucahyo et al., 2008). Irrigated rice fields present enormous potential to expand the aquaculture production in rice-producing countries (Frei et al., 2007). In this system, seeding rice in water is recommended allowing fish to be cultivated in refuges located in the rice field. Concurrent rice–fish culture is an integrated system which allows the use of scarce resources such as water and land in a complementary way. Most pesticides used in rice fields may produce serious detrimental effects in the ecosystems, considering their toxic effects in non-target organisms including fish. In southern Brazil, most farmers use at least one pesticide in rice fields (Adhikari et al., 2004; Oruç and Usta, 2007; Clasen et al., 2012).

Carbofuran is a broad spectrum systemic carbamate insecticide, nematicide, and acaricide that banned in the United States and Europe because of unwanted toxic effects in birds, fish, mammals, insects and aquatic invertebrates (USEPA, 2006). It is also used to control coleopteran that damages irrigated rice crops in south-eastern Brazil (Plese, 2005; Pessoa et al., 2011). Contamination of water bodies adjacent to rice fields by carbofuran, mainly through runoff, is quite possible as a result of its widespread use in agriculture and relatively good solubility in water (320 mg/L at 20 °C). The concentration used in this study is based on the recommended dose of carbofuran commercial formulations to rice fields in Brazil, which is 4.0 kg/ha (Chelinho et al., 2012). The concentration was chosen to compare effects on carps exposed to different experimental conditions: rice field and laboratory. It has been shown that carbofuran concentration in irrigated rice fields in southeastern Brazil can reach maximum concentrations of 233 μg/L in lamina of water according to studies (Plese, 2005).

Several types of environmental pollutants may cause oxidative stress in fish. Studies have shown that carbofuran induces oxidative stress leading to the generation of free radicals with an increase of

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reactive oxygen species (ROS) and alteration in the antioxidant profile in different species of fish (Hernández-Moreno et al., 2010; Ensibi et al., 2012). ROS are involved in energy production, phagocytosis, cell growth regulation and cell signaling. The production of ROS associated with the presence of pollutants has been imputed as a possible mechanism of toxicity in aquatic organisms exposed to pesticides (Masella et al., 2005; Oropesa et al., 2009). Lipid peroxidation in fish has been suggested as one of the oxidative damage involved in pesticide-induced toxicity (Almroth et al., 2005). Parvez and Raisuddin (2005) suggested that protein carbonyl may serve as a general biomarker of oxidative stress. However, ROS also convert the amino groups of proteins and alter the structure or function of the proteins (Almroth et al., 2005). In line with oxidative damage caused by pesticide exposure the Acetylcholinesterase (AChE) is a key enzyme in the nervous system terminating nerve impulses by catalyzing hydrolysis of the neurotransmitter acetylcholine in acetate and choline. AChE is reported to be a specific biomarker of exposure to some pesticides including organophosphorus and carbamates, such as carbofuran (Lionetto et al., 2003). Representing antioxidant system the enzymes have a crucial role. Superoxide dismutase (SOD) is responsible for catalyzing the conversion of the superoxide anion into hydrogen peroxide. Hydrogen peroxide degrades into water and molecular oxygen via catalase (CAT), a family of enzymes which is present mainly in peroxisomes. Another important enzyme is glutathione S-transferase (GST), which acts in the process of biotransformation. It catalyzes the conjugation of a variety of metabolites, including pesticide metabolites and lipoperoxidation products, transforming the toxic compound into a more easily excretable metabolite (Parvez and Raisuddin, 2005; Clasen et al., 2012). Studies showed that several biochemical parameters are altered when exposed to carbofuran, such as metabolic parameters, AChE and TBARS indicating the toxicity of this pesticide for different fish species (Begum, 2004; Hernández-Moreno et al., 2010).

Considering the contamination potential of pesticides used in agriculture practices and possible contamination of fish, this study aimed at examining the effects of carbofuran at environmental relevant concentrations on the oxidative stress parameters in organs of *Cyprinus carpio* in rice fields and laboratory. Furthermore, we assessed the usefulness of these parameters as biomarkers of exposure to carbofuran due to the economic importance of the association rice–fish.

2. Materials and methods

2.1. Chemicals

A commercial formulation of the insecticide carbofuran (Furadan® 100 g) obtained from the FMC Química do Brasil Ltda (CAS 1563-66-2), containing ten percent carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl metylcarbamate) was used in the experiment. Bovine serum albumin, Triton X-100, hydrogen peroxide (H_2O_2), malondialdehyde (MDA), 2-thiobarbituric acid (TBA) and sodium dodecyl sulfate (SDS) were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Animals

Carps (weight, 15.0 ± 2.0 g; length, 10.0 ± 3.0 cm) were obtained from a commercial fish farm (RS, Brazil). Fish were acclimated in laboratory conditions for 10 days in tanks (250 L) containing clean water (free from insecticides) prior to experiments. They were kept in continuously aerated water with a static system and with a natural photoperiod (12 h light/12 h dark). After the acclimation period, 90 fish were transferred to points located in the rice field and other 30 fish were transferred to laboratory tanks. Both rice field and laboratory experiments were conducted for 7 and 30 days. In the period of acclimation, as in the period of exposure, the fish were fed twice a day with commercial fish pellets (42 percent crude protein, Supra, Brazil).

2.3. Experimental design

2.3.1. Rice field experiment

Fish were allocated to two groups, the control group (without insecticide) and exposure group (with insecticide). Each group was composed of 45 animals distributed in three tanks (triplicate) with 15 fish per tank. The fish were exposed to initial measured concentration of 50 μg/L of the insecticide for 7 and 30 days. The insecticide concentration used in this experiment corresponds to the concentration recommended in Brazil for use in rice culture. The control fish were placed in tanks with separate water supply from the exposure tanks, but conditions and placing of tanks were similar for both groups. During the experiment in the rice field, the fish were placed in submerged tanks, measuring 1.00 m (diameter) × 1.05 m (length). Fine-mesh plastic screens were used at the entrances and exits of water to avoid the presence of predators. Other conditions, such as climate changes, were not avoided in order to make the field experimental condition as real as possible. The following parameters were monitored during the experiments: temperature (24 ± 2.0 °C), pH (6.5 ± 0.2), dissolved oxygen (4.21 ± 2.0 mg/L), nonionized ammonia (0.8 ± 0.01 μg/L) and nitrite (0.06 ± 0.01 mg/L) of the water in the rice field. The insecticide concentration in water was monitored from the first day until it was not detected in either experimental condition (rice field and laboratory). The insecticide was analyzed by high-pressure liquid chromatography (HPLC) using the method described by Sabin et al. (2009). After each exposure period (7 and 30 days), the fish were killed by punching the spinal cord (behind the opercula), and a sample of five individuals was taken from the tanks and submitted to organs (brain, liver, and muscle) collected.

2.3.2. Laboratory experiment

Fish were distributed into 40 L tanks and allocated to two experimental groups as follows: the first was considered as a control group with 15 fish distributed into three tanks (5 fish per tank) containing insecticide-free water. The second with 15 fish distributed into three tanks (5 fish per tank) was exposed to initial measured concentration of 50 μg/L of the insecticide. Each group remained with the same experimental conditions for a period of 7 and 30 days. The insecticide concentration used in this experiment corresponds to the recommended concentration for growing rice. Moreover, these concentrations are likely to occur in the natural environment, close to agricultural areas. During the experimental period in the laboratory, the average water parameters were as follows: temperature 22.3 ± 2.0 °C, pH 6.6 ± 0.2 units, dissolved oxygen 6.4 ± 1.0 mg/L, nonionized ammonia 0.5 ± 0.01 μg/L, nitrite 0.06 ± 0.01 mg/L. After each exposure period (7 and 30 days) the fish were killed by punching the spinal cord (behind the opercula) and then the organs (brain, liver, and muscle) were collected.

2.4. Biochemical parameters

2.4.1. Tbars AsSAY

Lipid peroxidation was estimated by the TBARS assay, performed by a MDA reaction with TBA, which was spectrophotometrically measured according to Buege and Aust (1978). The liver, brain, and muscle were homogenized in 10 volumes (w/v) of potassium phosphate buffer (20 mM) and then TCA ten percent and TBA 0.67 percentwere added to adjust to a final volume of 1.0 mL. The reaction mixture was placed in a micro-centrifuge tube and incubated for 30 min at 95 °C. After cooling, it was centrifuged at 5000*g* for 15 min and optical density was measured by a spectrophotometer at 532 nm. The TBARS levels were expressed as nmol MDA/mg protein.

2.4.2. Protein carbonyl assay

The liver was homogenized in 10 volumes (w/v) of 10 mM Tris–HCl buffer pH 7.4 using a glass homogenizer. Protein carbonyl content was assayed by the method described by Yan et al. (1995), with some modifications. Soluble protein (1.0 mL) was reacted with 10 mM DNPH in 2 N hydrochloric acid. After incubation at room temperature for 1 h in the dark, 0.5 mL of denaturing buffer (150 mM sodium phosphate, pH 6.8, containing SDS 3.0 percent), 2.0 mL of heptane (99.5 percent) and 2.0 mL of ethanol (99.8 percent) were added sequentially, vortexed for 40 s and centrifuged at 10,000*g* for 15 min. The protein extracted from the interface was washed twice by resuspension in ethanol/ethyl acetate (1:1), and suspended in 1 mL of denaturing buffer. The carbonyl content was then measured spectrophotometrically at 370 nm. The total carbonylation was calculated using a molar extinction coefficient of 22,000 M/cm. The protein carbonyl content was expressed as nmol carbonyl/mg protein.

2.4.3. Acetylcholinesterase assay

AChE activity was measured as described by Ellman et al. (1961). Brain and muscle were weighed and homogenized in a Potter-Elvejhem glass/Teflon homogenizer with 50 mM sodium phosphate buffer, pH 7.2 (with one percent Triton X-100). The homogenates were centrifuged for 15 min at 3000*g* and 5 °C, and the supernatant was used as the enzyme source. Aliquots of the supernatant (50–100 μL) (brain and muscle, respectively) were incubated at 30 °C for 2 min with 0.1 M phosphate buffer, pH 7.5, 10 mM DTNB as chromogen. After 2 min, the reaction was initiated by the addition of acetylthiocholine (AcSCh; 0.5 mM) as the