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Effects of the commercial formulation containing fipronil on the non-target organism *Cyprinus carpio*: Implications for rice–fish cultivation

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ABSTRACT

The aim of this research was to evaluate possible toxic effects of commercial formulation containing fipronil on *Cyprinus carpio* tissues under rice field conditions. Antioxidant profile (SOD, catalase, glutathione *S*-transferase), oxidative stress parameters (thiobarbituric acid-reactive substances, protein carbonyl), and growth were investigated in carp exposed to fipronil under rice field conditions for 7, 30, and 90 days. Waterborne insecticide concentrations were measured and the detectable concentration of fipronil was observed up to 45 day after application. Common carp survival and growth was not affected by fipronil. Liver superoxide dismutase activity was enhanced while liver catalase activity was inhibited at 7, 30, and 90 days. Alterations were not observed in the glutathione *S*-transferase activity in any experimental periods. Protein carbonyl increased only after 30 and 90 days of exposure. The thiobarbituric acid-reactive substances levels were enhanced in all analyzed tissues (liver, muscle, and brain) and periods of exposure. This study demonstrates that fipronil insecticides cause alterations in the biochemical parameters in different tissues of carp without affecting the growth or the survival of the fish.

1. Introduction

Rice–fish culture has long been recognized as an option to improve the productivity of the country's rice based agriculture (Dewan, 1992; Frei et al., 2007). Increased adoption of rice–fish farming, with fish as natural control agent of pest organisms, provides a promising alternative for the development of ecological management strategies to minimize the use of pesticide in rice field environment (Halwart, 1998). Rice–fish cultivation is an alternative cost reduction of rice crop because fish prepare the soil for the next crop of rice, recycle organic matter, and consume weed seeds contained in soil, such as red rice, rice grass, sedges, and other aquatic plants. Fish also consume insect larvae, snails, and screwworm from the root of rice (Berg, 2001). The cost of rice production decreases, since the use of chemicals is reduced because fish take charge of eating insects, worms, and weeds (Boll et al., 1999). The pre-germinated system of rice has been expanding in Brazil, especially in Rio Grande do Sul. In this system, it is recommended to seed rice on water. The permanence of the water in the field throughout the crop cycle is responsible for weed control. Thus, the fish remain in refuges located in the rice field (Lima et al., 2006; Marchezan et al., 2006) (Fig. 1). In a number of studies an increase in the production of both rice and fish has been demonstrated when rice–fish culture is used (Lightfoot et al., 1992; Gupta et al., 1998; Uddin et al., 2001; Frei et al., 2007).

In Southern Brazil, most farmers use at least one pesticide in rice fields. Among the pesticides commonly used, fipronil is an insecticide, which was introduced in the market in 1996 showing to be less toxic compared to the insecticides used in rice culture. Fipronil is a broad-spectrum insecticide that belongs to the phenylpyrazole class of insecticides. These insecticides were recently introduced in the consumer market and are considered more selective and less damaging to ecosystems if compared with organophosphate insecticides (Ecobichon, 1996; Stark and Vargas, 2005). Fipronil is an insecticide of increasing use as it is identified by the US Environmental Protection Agency as an alternative to organophosphate compounds (US EPA, 2002b; Chiovarou and Siewicki, 2008). Since its introduction in consumer market to use in agricultural practices fipronil has been used in rice, corn,

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Fig. 1. Experimental tanks of rice crop from the Federal University of Santa Maria, RS. Arrow on A indicates the position of the cage used to expose the fish, arrow B indicates the refuge, and arrow C indicates the rice culture.

and cotton (US EPA, 1996; Stark and Vargas, 2005). Its mechanism of action involves non-competitive binding to the gamma-aminobutyric (GABA) receptor, effectively blocking the chloride channel in the nervous system, resulting in a disruption of neuron signaling and eventually the shutdown of the central nervous system (Wirth et al., 2004; Tan et al., 2008). The insecticide fipronil is adequate to advanced seed treatments applied before sowing and has an aquatic half-life of 14.5 days and in soil its half-life is of 123 days. The recommended concentration of fipronil in rice fields in Brazil is 150 mL/ha (Conelly, 2001; Grützmacher et al., 2008). In this study, we used this recommended dose that is a sublethal concentration of commercial formulation containing fipronil; thus, it does not cause death of fish. We chose this concentration of fipronil in order to observe what happens to fish when exposed in rice field conditions. However, some questions remain unclear, particularly the one linked to the effects in carps exposed to sublethal doses of fipronil. Considering that the stocking of fish in rice fields is a 2000 year old successful practice (Vromant and Chau, 2005), it is necessary to study the interaction between fish and rice to determine a minimal insecticide concentration to use in this association. At this moment, we can discuss if the use of insecticide is necessary as well as what kind of toxic effects pesticides can cause in fish. Insecticides can be responsible for causing oxidative stress in fish, since these contaminants could induce the formation of reactive species and alterations in the antioxidant system (Sayeed et al., 2003; Üner et al., 2005; Üner et al., 2006). Fish endogenous protection to oxidative stress occurs through a cellular antioxidant system, which includes enzymes, such as superoxide dismutase (SOD) and catalase (CAT). SOD catalyzes the conversion of reactive superoxide anions (O_2^-) into yield hydrogen peroxide (H_2O_2), which is an important Reactive Oxygen Species (ROS). H_2O_2 is subsequently detoxified by two types of enzymes, namely, CAT and glutathione peroxidase (GPx). SOD is an antioxidant enzyme, which plays important roles in the line of defenses against oxidative stress. The elimination of excessive ROS via antioxidant enzymes, particularly SOD, is crucial for maintaining cellular homeostasis in fish (Cho et al., 2009). CAT is one of the most efficient enzymes known and has been frequently reported in toxicity studies. The function of this enzyme is to convert hydrogen peroxide into oxygen and water (Matés, 2000; Atli and Canli, 2007). Another important enzyme for fish detoxification process is glutathione-*S*-transferase (GST). This enzyme displays defense against oxidative stress, facilitating the nucleophilic attack of pesticides by GSH and removing the compound that otherwise would lead to toxic effects (Maran et al., 2009). Another important parameter to verify insecticide toxic effects is the measurement of carbonyl protein that is a good indicative of protein damage. However, this biomarker has been commonly used in researches on oxidative stress in humans. In addition few reports are available regarding its use in fish exposed to toxic chemicals, such as insecticides (Almroth et al., 2005; Parvez and Raisuddin, 2005). Oxidative stress frequently induces lipid peroxidation (LPO). LPO in fish could be estimated by measuring thiobarbituric acid reactive substances (TBARS) and has been used as a biomarker in a large number of studies (Almroth et al., 2005; Üner et al., 2005; Oruç and Usta, 2007; Ballesteros et al., 2009). Lipid peroxidation results in the production of lipid radicals and in the subsequent formation of a complex mixture of lipid degradation products (Almroth et al., 2005).

Due to the high consumption of common carp, *Cyprinus carpio*, by humans and considering the insecticides used in agriculture practices, the possible toxic effects of these products in tissues of fish for commercial interest have become the object of our study. Common carp, a native fish of Eastern Europe and Western Asia and widely distributed in Brazil, is an omnivorous species that is fed on invertebrates, plants, algae, insect larvae, crustaceans, and small fish (Querol et al., 2005; Mabuchi et al., 2006). The integration of rice and fish is a promising alternative, which offers new opportunities to farmers. The rice–fish culture has proven to be an economically viable alternative to rice monoculture in a number of socio-economic surveys conducted in various Asian countries (Berg, 2002; Frei and Becker, 2005). Thus, considering the importance of common carp and the possible toxic effects of fipronil, the aim of this study was to examine if a sublethal concentration of fipronil under pre-germinated system of rice conditions causes oxidative stress and affect growing parameters of this species.

2. Materials and Methods

2.1. Chemicals

The fipronil insecticide (CAS 120068-37-3) used was an available commercial formulation (Standak®-BASF), containing 25% fipronil [(±)-5-amino-1-(2,6-dichloro-α-α-α–trifluoro-p-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile]. 1-Chloro-2,4- dinhitrobenzene (CDNB), bovine serum albumin (BSA), hydrogen peroxide (H_2O_2), malondialdehyde (MDA), 2-thiobarbituric acid (TBA), sodium dodecyl sulfate (SDS) and 2,4-dinitrophenylhydrazine (DNPH). All reagent-grade chemicals were purchased from Sigma (St. Louis, MO).

2.2. Fish

Common carps of both genders weighting 10.0 ± 2.0 g and measuring 12.0 ± 1.0 cm total length were obtained from a commercial fish farm (RS, Brazil) without exposure to insecticides. Fish were acclimated to laboratory conditions for 10 days in tanks (250 L) containing water free from insecticides prior to the experiments. They were kept in continuously aerated water with a static system and with a natural photoperiod (12 h light/12 h dark). After acclimation fish were divided in two groups according to the description in Section 2.3. In the period of acclimation as well as in the period of exposure, fish were fed once a day with commercial fish pellets (42% crude protein, Supra, Brazil) at the proportion of 1% of the total weight of fish in each tank or parcel. The experimental protocols were authorized by the board on experimentation on animals of the Federal University of Santa Maria, reference number: 23081.013362/2009-50.

2.3. Experimental design

Fish were allocated in two groups: the control group (without insecticide) and exposure group (with insecticide). Each group composed of 45 animals distributed in three tanks (triplicate) with 15 fish per tank. The fish were exposed to initial measured concentration 0.65 mg/L of the insecticide for 7, 30, and 90 days. The