Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/etap

Effects of chronic carbosulfan exposure on liver antioxidant enzyme activities in rainbow trout

Erol Capkin, Ilhan Altinok*

Karadeniz Technical University, Faculty of Marine Science, Department of Marine Sciences and Technology Engineering, 61530 Surmene, Trabzon, Turkey

ARTICLE INFO

Article history:
Received 5 February 2013
Received in revised form
22 March 2013
Accepted 23 March 2013
Available online 6 April 2013

Keywords:
Carbosulfan
Oncorhynchus mykiss
CAT
SOD
GST
Se-GPx

ABSTRACT

Catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST), and selenium-dependent glutathione peroxidase (Se-GPx) activities in liver of rainbow trout (*Oncorhynchus mykiss*; 116.88 ± 21.69 g) were evaluated after exposing fish to sublethal concentrations (25 μg/L) of carbosulfan in flow-through tanks for 60 days. During the experiment activities of CAT, SOD, GST, and Se-GPx and histopathological effects were determined once a week and once at the end of the 21 days of recovery period. All enzymes were affected by carbosulfan when compared to control fish. Fish had intracellular oedema, cell necrosis, pycnotic nucleus, and increase of sinusoidal space in the liver. After 21 days of the recovery period, all enzyme activities had returned to control levels and fish had no histological lesions in liver. Therefore all the changes observed during exposure were reversible. Results indicate that the liver CAT, SOD and GST enzymes are highly sensitive to carbosulfan as their activities altered significantly, suggesting they could be useful in predicting sublethal pesticide toxicity and useful as an indicator for assessment of pesticides in contaminated water.

1. Introduction

The increasing amount of pollutants such as organophosphorus and carbamic pesticides, heavy metals and detergents in the environment require fast and sensitive analytical techniques (Snejdarkova et al., 2004). Hence, the use of biochemical measurements in organisms as pollution indicators, gives valuable information about deleterious responses of organisms (Tortelli et al., 2006). Biochemical effects of pollutants occur more quickly; thus they provide earlier warning signalling before other toxicological endpoints become evident (Livingstone, 1998). Among biochemical markers, measurement of fish enzyme activity is a classical tool employed to monitor pollution in both marine and continental waters (Bocquené et al., 1990; Sturm et al., 1999).

Chronic exposure to low levels of pesticides may have a more significant effect on fish populations than acute poisoning (Zaheer Khan and Francis, 2005). Doses of pesticides that are not high enough to kill fish affect behavioural and physiological systems of the fish (Radhaiah et al., 1987). Biochemical changes lead to metabolic disturbances, inhibition of essential enzymes and retardation of growth. Most of the organochlorine, organophosphate and carbamate pesticides are capable of changing the enzyme activity (Radhaiah et al., 1987; Dutta and Arends, 2003; Dogan, 2006). Furthermore, changing of enzyme activities is used as a biomarker to determine the presence of pesticides in water environment (Chandrasekara and Pathiratne, 2005; Varo et al., 2007). Fish health may thus reflect, and be a reliable indication of the health status of a specific aquatic ecosystem (Burkepile et al., 2000).

* *Corresponding author*. Tel.: +90 462 7522805/103; fax: +90 462 7522158.
E-mail address: ecapkin@ktu.edu.tr (E. Capkin).
1382-6689/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.etap.2013.03.022

Carbosulfan is a benzofuranyl methylcarbamate pesticide closely related to its main metabolite carbofuran (Giri et al., 2003). Carbosulfan is available as emulsifiable concentrates, dusts and granular formulations for the control of insects, mites and nematodes, mainly on potatoes, sugar beet, rice, maize, and citrus. Carbosulfan is banned in the Europe, but it has been used widely in others countries such as Mexico and Brazil (EU, 2007; FAO, 2010). Environmental concentrations of carbosulfan range between 0.64 μg/L and 29 μg/L (Leppert et al., 1983; Sao et al., 2008). Carbosulfan, as with other carbamates, is highly toxic to fish, and its toxicity is mediated through inhibition of acetyl cholinesterase (AChE) (Chandrasekara and Pathiratne, 2007). Altinok et al. (2012) reported that carbosulfan affects AChE, d-aminolevulinic acid dehydratase (ALA-D) and paraoxonase (PON) enzyme activity in red blood cells and induces genotoxic and mutagenic effects in *Oncorhynchus mykiss*. In another study, AChE of *Carassius auratus* was inhibited following carbosulfan exposure has also been reported (Yi et al., 2006).

The liver performs many essential body functions including regulation of metabolism, synthesis of plasma proteins, energy storage and excretion of steroid and xenobiotics (Lawrence and Hemingway, 2003). Liver like kidney, brain and gills is the most vulnerable organ of the fish exposed to the medium containing any toxicant (Jana and Bandyopadhyaya, 1987). Sublethal concentrations of contaminants depending on the species and concentrations are generally considered to be safe because they do not cause death (Rodrigues and Fanta, 1998.). As the liver has many vital functions, changes in its morphology and necrosis will certainly cause secondary consequences to the organism, impairing its fitness for life, even if it survives after having been contaminated (Rodrigues and Fanta, 1998). At the same time liver is associated with metabolism and elimination of toxicants from the body and its biochemical parameters are considered as key points to elucidate toxicity of the chemicals (Leblanc, 2004; Ferrari et al., 2007). Mugachia et al. (1992) showed that DDT, lindane and aldrin residues levels higher in the liver of fish. Reports regarding carbosulfan effects on liver of fish are scanty. The present study can be useful to describe these effects in detail. The aim of this study was to evaluate the toxic effects of carbosulfan on catalase, selenium-dependent glutathione peroxidase (Se-GPx), superoxide dismutase (SOD), and glutathione S-transferase (GST) activity in liver, and elucidate the effects on liver histology and recovery in the biochemical parameters.

2. Materials and methods

2.1. Pesticide

The carbamate insecticide carbosulfan was obtained from Sigma–Aldrich (Steinheim, Germany). Solutions of the pesticide were made by diluting with double-distilled water to obtain required concentrations.

2.2. Fish

Rainbow trout *O. mykiss* (116.88 ± 21.69 g; 22.39 ± 1.40 cm; Mean ± SD) were obtained from Karadeniz Technical University, Faculty of Marine Sciences, Trabzon, Turkey. Five months old fish were held in four flow-through tanks (150 L) for at least 15 days to acclimate to laboratory conditions prior to experiments. Throughout the acclimation period and subsequent periods of carbosulfan exposure, fish were held under a photoperiod of 12 h of light and 12 h of darkness. During the acclimatization period, fish were fed 2% body weight twice a day with commercial trout pellets (ILAR, 1996). All the supplied feed was quickly consumed by fish in all treatments.

2.3. Chronic toxicity test

Based on the preliminary 96 h acute toxicity tests (Boran et al., 2007) and 14 d chronic toxicity test results (Capkin et al., 2010), 25 μg/L for carbosulfan concentration (5% LC_{50}) was selected for chronic exposure taking into account the environmental concentrations (0.64–29 μg/L). Briefly, the fish were examined and determined to be free of external parasites before chemical exposure (AFS-FHS, 2003). After acclimatization, fish were exposed to the sublethal concentration of carbosulfan in a group of 50 fish in 150 L of the test water in flow-through fibreglass tanks for 60 days. The experiments were conducted in duplicates and control fish were also maintained in two flow-through fibreglass tanks (50 fish in each 150 L tank). Water flow for each tank was 6 L/h and carbosulfan was added to test solution with infusion pump at the rate of 5.25 mL/h to ensure 25 ± 4 μg/L actual carbosulfan concentration measured daily in the water. Carbosulfan test solution in the infusion pump was replaced every 12 h. During the carbosulfan exposure, four fish from each group were sampled once a week to determine enzyme activities and histological lesions in liver. The weights of fish were measured in each sampling. During the exposure, water in each aquarium was aerated. Water temperature, pH, dissolved oxygen concentration, total hardness, alkalinity, unionized ammonia, and nitrite were measured daily. Fish were fed with commercial trout pellets daily at 2% BW.

At the end of the 60 days of sublethal toxicity tests, fish were transferred to flow-through tanks to observe further effects of carbosulfan. Activities of CAT, SOD, GST, and Se-GPx were determined daily. At the end of the 21 days of recovery period, 10 fish were sampled to determine enzyme activity and histopathological effects.

2.4. Determination of carbosulfan in water samples

Carbosulfan in water samples was determined according to Sao et al. (2008). Briefly, 1 N H_2SO_4 (0.3 mL) was added to an aliquot of a standard solution containing carbosulfan (eight points between 8 and 56 μg) in a graduated flask. It was hydrolysed in phenol by adding NaOH (0.5 mL). Then 1.5 mL of diazotised p-aminoacetophenone (DPAAP) was added and solution was kept for 5 min with occasional shaking to ensure complete coupling. 4 M NaOH (1.0 mL) was added, and volume was made up to 10 mL. Water samples were extracted twice with chloroform (5 mL) in a separating funnel. Extract was evaporated to dryness under reduced pressure. Residue was dissolved in ethanol (5 mL) and diluted up to 50 ml with double distilled water. Absorbance of water samples and standard