Chemosphere 175 (2017) 186–191

Contents lists available at ScienceDirect

Chemosphere

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

# Neurotoxic responses in brain tissues of rainbow trout exposed to imidacloprid pesticide: Assessment of 8-hydroxy-2-deoxyguanosine activity, oxidative stress and acetylcholinesterase activity

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## HIGHLIGHTS

- We investigated neurotoxic responses by observing 8-OHdG activity, oxidative stress and AChE activity in fish brain exposed to imidacloprid.
- Imidacloprid exposure caused an increase in 8-OHdG activity and immunopositiv reaction for 8-OHdG was detected in brain tissues.
- Imidacloprid exposure induces AChE inhibition in brain tissues.

## ARTICLE INFO

*Article history:*
Received 29 December 2016
Received in revised form
6 February 2017
Accepted 7 February 2017
Available online 11 February 2017

Handling Editor: David Volz

*Keywords:*
Imidacloprid
Pesticide
Oxidative stress
8-OHdG
Acetylcholinesterase
Fish
Brain
Toxicity

## ABSTRACT

The extensive use of imidacloprid, a neonicotinoid insecticide, causes undesirable toxicity in non-targeted organisms including fish in aquatic environments. We investigated neurotoxic responses by observing 8-hydroxy-2-deoxyguanosine (8-OHdG) activity, oxidative stress and acetylcholinesterase (AChE) activity in rainbow trout brain tissue after 21 days of imidacloprid exposure at levels of (5 mg/L, 10 mg/L, 20 mg/L). The obtained results indicated that 8-OHdG activity did not change in fish exposed to 5 mg/L of imidacloprid, but 10 mg/L and 20 mg/L of imidacloprid significantly increased 8-OHdG activity compared to the control ($p < 0.05$). An immunopositiv reaction to 8-OHdG was detected in brain tissues. The brain tissues indicated a significant increase in antioxidant enzyme activities (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)) compared to the control and there was a significant increase in malondialdehyde (MDA) levels ($p < 0.05$). High concentrations of imidacloprid caused a significant decrease in AChE enzyme activity ($p < 0.05$). These results suggested that imidacloprid can be neurotoxic to fish by promoting AChE inhibition, an increase in 8-OHdG activity and changes in oxidative stress parameters. Therefore, these data may reflect one of the molecular pathways that play a role in imidacloprid toxicity.



## 1. Introduction

The extensive use of pesticides in agriculture is emerging as a threat to the ecological balance in aquatic environments (Husak et al., 2014; Soydan et al., 2017). These chemicals cause adverse effects by affecting the physiological and behavioral systems in non-targeted organisms such as fish, throughout the world (Erdoğan et al., 2011; Karmakar et al., 2016). Therefore, the presence of these chemicals in the environment is a global issue which may pose a threat to fish (Khan and Law, 2005; Ceyhun et al., 2010). Neonicotinoids, systemic neuro-active pesticides similar to nicotine are the most widely used insecticides to protect household plants from sucking insects found in agriculture (Tomizawa and Casida, 2003; Karahan et al., 2015). Imidacloprid is one of the neonicotinoid family, and it works by binding to, and activating, nicotinic acetylcholine receptors in the central nervous system (Bai et al.,

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http://dx.doi.org/10.1016/j.chemosphere.2017.02.047
0045-6535/

1991; Song et al., 1997). Imidacloprid is a potential surface-water contaminant, and it may enter bodies of water from crops, soil, accidental spills or spray drift, which result in local point-source pollution (Jemec et al., 2007). Few studies have been performed on the effects of imidacloprid in non-targeted organisms despite its increasing use (Jemec et al., 2007; Tisler et al., 2009; Sillapawattana and Schäffer, 2016; Wang et al., 2016). For example, it has been reported that imidacloprid would present a potential chronic risk to Daphnia magna (Jemec et al., 2007). In another study, imidacloprid was tested on Folsomia candida and it caused a decrease in the total amount of GSH, and an increase in GST activity (Sillapawattana and Schäffer, 2016). In addition, the sub-chronic treatment with imidacloprid caused lipid peroxidation and DNA damage in earthworms, E. fetida (Wang et al., 2016). Toxicity data for imidacloprid in fish is still scarce. Therefore, more research is needed to understand the potential toxicity and adverse effects of imidacloprid in non-targeted organisms, especially fish.

Environmental contaminants can cause oxidative stress by producing reactive oxygen species (ROS), and these contaminants are important sources of ROS (Ferreira et al., 2005). When oxidative stress occurs as a consequence of an imbalance between antioxidant and pro-oxidant systems, ROS can lead to oxidative damage in nucleic acids, lipids and proteins (Wu et al., 2004; Anjana Vaman et al., 2013; Topal et al., 2017a). ROS generation can be prevented by antioxidant defense systems, that include catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) dependent enzymes (e.g., GR, GST, and GSH-Px) (Figueiredo-Fernandes et al., 2006). 8-OHdG is known to be a sensitive indicator of oxidative DNA damage that can be induced by ROS, and it is a relevant marker of cellular oxidative stress (Kasai et al., 1986; Thompson et al., 1999). 8-OHdG occurs by enzymatic cleavage after 8-hydroxylation of the guanine base in DNA following an attack by hydroxyl radicals under oxidative stress (Kasai et al., 1986; Xu et al., 2004). One of the parameters indicating oxidative damage is malondialdehyde (MDA), which is the final product of lipid peroxidation, leading to loss of cell function under oxidative stress (Ge et al., 2015; Zheng et al., 2016). MDA measurement has been used as a biomarker to determine the effects of different contaminants in aquatic environments (Liu et al., 2016).

Acetylcholinesterase (AChE) plays an important role in the cholinergic system including nerve impulse transmission in synapses (Modesto and Marinez, 2010a), and it cleaves acetylcholine into choline and acetate. The AChE enzyme in the brain can be a target for toxic chemicals (Schmidel et al., 2014), and these chemicals cause disruption of nerve function and excessive ACh accumulation by inhibiting the AChE enzyme (Bhattacharya, 1993). Therefore, AChE can be a biomarker in the evaluation of neurotoxic changes (Topal et al., 2015). The brain has important physiological and regulatory roles in fish, and it has become the most important organ in fish toxicology, especially in the nervous systems of fish exposed to pesticides (Mishra and Devi, 2014). There is little information related to the effects of imidacloprid on rainbow trout. To our knowledge, there is no study associated with 8-OHdG activity in fish exposed to imidacloprid. Thus, this study was designed to evaluate 8-OHdG activity, acetylcholinesterase enzyme activity and antioxidant defense parameters including SOD, CAT, GPX and MDA in the brain tissues of rainbow trout after 21 days of imidacloprid exposure.

## 2. Materials and methods

### 2.1. Fish and experimental design

The rainbow trout, *Oncorhynchus mykiss*, (weighing 160 ± 15 g, 25 ± 0.5 cm) used in this study were obtained from the Inland Water Fish Breeding and Research Center, the Faculty of Fisheries, Ataturk University (Erzurum, Turkey). Fiberglass tanks containing 400 L of de-chlorinated tap water (temperature: 10–12° C, dissolved oxygen: 7.3 mg/L, water hardness: 177.2 mg/L, pH: 7.4), and a flow through system (water flow rate: 1 L/min) were used. Fifteen fish were placed in each fiberglass tank and then acclimatized to their environment for 15 days. The fish were fed daily with commercial trout pellets equal to 2.5% of their body weight during the acclimatization periods and the trials.

A commercial formulation of imidacloprid (600 g/L imidacloprid, (1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylide neamine) was purchased from a distribution company (Turkey). A stock solution of imidacloprid was prepared by dissolving it in distilled water. Three treatment groups (using 5 mg/L, 10 mg/L, 20 mg/L, respectively) and the control group each comprised 15 fish. The fish were exposed to concentrations of imidacloprid by adding them to the water every day for 21 days under controlled laboratory conditions. At the end of the trial period, the fish were euthanized by cervical section and brain tissues were collected from the control and treatment groups. One part of the tissue was stored at −80 °C for later analysis of antioxidant parameters, 8-OHdG and AChE activities. The other part of the tissue was fixed in a 10% neutral buffered formalin solution for 8-OHdG immunohistochemical staining. Samples were repeatedly studied for each group.

### 2.2. Determination of antioxidant enzyme activities and MDA levels

Brain hemolysates were prepared from each sample according to Alak et al. (2013) with modifications. The samples were homogenized (1% v/v) in 0.1 M phosphate buffer (pH 7.4) containing Triton-X 100, using a homogenizer. The brain homogenates were centrifuged at 13,000 rpm for 60 min (at 4 °C), and the supernatants were used as the enzyme source for the determination of the enzyme activities and MDA levels. Catalase (CAT) activity was measured according to Aebi method (1974) with a decrease in absorbance at 240 nm due to $H_2O_2$ consumption. The reaction mixture contained potassium phosphate buffer and $H_2O_2$. Superoxide dismutase (SOD) activity was assayed according to Sun et al. (1988). The reduction of NBT by superoxide anions to blue formazan was measured at 560 nm. The measurement of GPx activity was initiated by adding phosphate buffer (pH 6.0), 0.1 M of reduced glutathione (GSH), 10 U/Ml of glutathione reductase (GR), 2 mM of NADPH, and 0.1 mL of the enzyme fraction to the reaction tube. Before the measurement, the mixture was incubated for 10 min at 37 °C and the activity measurement was performed at 340 nm spectrophotometrically (Beutler, 1984). The malondialdehyde (MDA) secondary product is formed as a result of malondialdehyde (MDA) lipid peroxidation. The measurement was based upon the absorbance measurement of the pink complex at 532 nm, formed as a result of the incubation of MDA with thiobarbituric acid (TBA) at 95 °C (Gülçin et al., 2009). The protein levels of each sample were determined spectrophotometrically at a wavelength of 595 nm and recorded by using bovine serum albumin (BSA) as a protein concentration standard (Bradford, 1976).

### 2.3. Determination of 8-OHdG activity

Brain hemolysates were prepared from each individual according to a Fish (8-OHdG) ELISA kit ((Catalogue No:201-00-0041) (SunRed)) with modifications (Yin et al., 1995; Chen and Tang, 2011). For the detection of brain 8-hydroxy-2′-deoxyguanosine (8-OHdG) activity, a competitive ELISA analysis kit for 8-OHdG was used according to the manufacturer's protocol (Fish (8-OHdG) ELISA kit (Catalogue No:201-00-0041) (SunRed)).