Environmental Pollution 234 (2018) 830–837

Contents lists available at ScienceDirect

Environmental Pollution

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

Aberrations of the peripheral erythrocytes and its recovery patterns in a freshwater teleost, silver barb exposed to profenofos☆

Mst. Mansura Khan [a], Md. Moniruzzaman [a], Golam Mohammod Mostakim [b], Mohammad Sadequr Rahman Khan [c], Md. Khalilur Rahman [d], M. Sadiqul Islam [a, *]

[a] Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[b] Department of Fisheries Biology & Aquatic Environment, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh
[c] Department of Marine Bioresources Science, Chittagong Veterinary and Animal Sciences University, Chittagong 4225, Bangladesh
[d] Freshwater Station, Bangladesh Fisheries Research Institute (BFRI), Mymensingh 2201, Bangladesh

ARTICLE INFO

Article history:
Received 4 September 2017
Received in revised form
8 December 2017
Accepted 9 December 2017

Keywords:
Biomarker
Genotoxicity
Peripheral erythrocytes
Profenofos
Silver barb

ABSTRACT

The present experiment was conducted to explicate the genotoxic effects of profenofos, an organophosphate insecticide, on the erythrocytes of silver barb (*Barbonymus gonionotus*). Silver barb were exposed to a solution of 10% and 50% of lethal concentrations (LC_{50}) of profenofos as sub-lethal concentrations at different days (1, 7, 15, and 30 d), along with a control (0% profenofos). Subsequent recovery patterns were assessed allowing the fish exposed to profenofos free water for the same period that they were exposed to profenofos. Our results revealed that with the progression of time and concentration, fish exposed to profenofos showed significantly ($p < .05$) higher level of erythrocytic nuclear abnormalities (ENA) such as micronuclei, bi-nuclei, degenerated nuclei, notched nuclei, nuclear bridge and nuclear buds, as well as erythrocytic cellular abnormalities (ECA) such as echinocytic, elongated, fusion, spindle, tear-drop and twin shaped cells. After exposure, the silver barb recovered spontaneously, and the abnormal erythrocytic parameters were normalized with a concentration- and duration-dependent fashion. Therefore, these abnormalities and their recovery can be used to assess the toxic levels of pesticides on aquatic organisms. There is great potential to use this technique as *in vivo* to predict susceptibility of aquatic animals to environmental pollution.

1. Introduction

Pesticides are organic and inorganic compounds that are frequently used to boost crop productivity and to control pests from agriculture (Hussain et al., 2012). The expansion of urbanization, industrialization and technological development has led to the vigorous application of synthetic chemicals like pesticides responsible for the contamination and degradation of many ecosystems (Hussain et al., 2014; Witeska et al., 2014). Pesticides can inadvertently contaminate land and aquatic systems when they are aerially sprayed on agricultural land to protect crops, or when they leak out of the production sites through rain water or when they are discarded (Fang et al., 2015). Pesticides may have no immediate effects when they are present at low concentrations, but at high exposure levels they can be extremely lethal to non-target aquatic organisms, especially fish (Hussain et al., 2013). Organophosphorus is a group of pesticides that have the capacity to bioaccumulate, biomagnify and bioconcentrate along the food web until they have the capacity to potentially harm humans, as we are often at the apex trophic level (Kohler and Triebskorn, 2013).

Profenofos(4-bromo-2-chloro-1-[ethoxy(propylsulfanyl)phosphoryl]oxy-benzene) is an organophosphorus insecticide that is used on agricultural crops; it can leak into the aquatic bodies through percolation through the soil, as almost all agricultural land is located beside water bodies. There was very little literature on the fate of profenofos in the environment especially in tropical climates. However, based on the high water solubility (20 mg/L) and short half-life (3–8 days) of profenofos (Wan Abdullah et al., 1999), it should dissipate faster and leach easily. Acute exposure of profenofos in fish and humans cause neurotoxicity through inhibiting the acetylecholine- and butylcholine-esterase activity as it is a powerful nerve agent (Rusha et al., 2013). Moreover,

☆ This paper has been recommended for acceptance by Dr. Chen Da.

* Corresponding author.
E-mail address: sadiqul1973@yahoo.com (M.S. Islam).

https://doi.org/10.1016/j.envpol.2017.12.033
0269-7491/

profenofos can also kill beneficial aquatic microorganisms and algae killing a high quantity of fish due to a shortage of oxygen and the production of excessive amounts of ammonia through the decomposition of rotting vegetation (Miller, 2004).

The physical and chemical change within an organism due to the exposure of pesticides is reflected in the organism's blood (Ambali et al., 2011). For example, exposures to various organophosphorus pesticides alter erythrocytes morphology, blood concentration, serum biochemical composition in different animals (Sharaf et al., 2010; Hussain et al., 2012; Ghaffar et al., 2017). The changes in morphology and abnormalities in erythrocytes are considered important biomarkers of oxidative stress (Celik et al., 2005; Hussain et al., 2012). Micronuclei, which are extra nuclear bodies of chromosome fragments, also reflect the physical and chemical alterations due to pesticides. Therefore, micronuclei, are considered as the best biomarker for DNA damage or genetic alteration (Hussain et al., 2014; Sadiqul et al., 2016).

Several studies are available in assortments of pesticides inside the fish's body that causes diverse modifications in blood parameters (hematological, biochemical and enzymological) and changes in the erythrocytes nuclear and cellular structure (Pandey et al., 2014; Ghaffar et al., 2015; Pamanji et al., 2016). Alterations of these parameters can be used to assess the health of a water body, and provide early warning tools while monitoring environmental quality (Pimpao et al., 2007). In addition, understanding rates of adaptation and recovery after exposure to pesticides is very important in the field of risk assessment (Du et al., 2009). Knowing how long it takes to exposed fish to recover completely can help improving and maintaining fish health, and indirectly human health (Adhikari et al., 2004). Information about the effects of pesticides on the fish body and its recovery after exposure is lacking, even though pesticides are the main cause of abnormalities in their blood and internal organs (Khattak and Hafeez, 1996). Therefore, the present study was conducted to assess the erythrocytic abnormalities (nuclear and cellular), as well as the possibility and patterns of recovery of these biomarkers after exposure to profenofos in a natural setting using *in-vivo* technique on a freshwater teleost, the silver barb (*Barbonymus gonionotus*).

2. Material and methods

Healthy silver barb (*B. gonionotus*) with a 10.11 ± 1.44 cm and body weight 5.9 ± 3.61 g were collected from local fish farms. The random allocations of three hundred eighty individuals were thru and transferred into a cemented tank retaining dechlorinated tap water oxygenated through an aeration system. Fish of both sexes were used. To dispel the assumed detrimental subjects the fish were allowed to acclimatise to the laboratory conditions (temperature ranging from 24.0 to 26.0 °C) for two weeks. Fish were fed twice a day with floating fish feed pellets (Mega Fish Feed Ltd.) with 32% protein. Feeding supplement was obsoleted 24 h prior to the initiation of the test and tank water was altered once every two days. Experimental fish were handled according to the guidelines of the Animal Care Committee of Bangladesh Agricultural University, Bangladesh. The commercial grade (50% EC with 100% purity) profenofos was obtained in original sealed containers from an authorized dealer of the pesticide from Mymensingh, Bangladesh. It was found in as liquid form and reddish brown in color.

2.1. Exposure of fish to profenofos

To test the toxicity of profenofos, a static bioassay was performed. Ten fish were released into each glass aquarium (72×43 cm^2) containing 25 L of water and sequential concentrations (0.2, 0.1, 0.05, 0.06, 0.07, 0.08 mg/L) of profenofos with a control (0 mg/L) group. Liquid profenofos was added to dechlorinated tap water, which was then gently stirred with a glass rod to ensure complete mixing. To determine mortality and other behavioral changes of fish each aquarium was monitored twice a day. Mortality was recorded at the start of the test and then every 12 h (i.e. 0, 12, 24, 48, 60, 72, 84, 96 and 120 h) and dead fish were removed immediately. Lethal concentration (LC_{50}) for profenofos was recorded at 0.1 mg/L through a probit analysis.

We then tested the effects of sub-lethal concentrations of profenofos on silver barb, using a semi-static bioassay. 180 healthy fish were introduced into the aquaria (72×43 cm^2) with 10% (0.01 mg/L) or 50% (0.05 mg/L) of the LC_{50} of profenofos for 30 days, while the third group served as control (0% profenofos) and each with three replications. Exceeding aeration was applied to the aquarium for 2 h in order to obtain a homogenous concentration of the toxic compound and randomly selected thirty fish were transferred to each test aquarium. During the experimentation, feeding and adding of fresh dose of profenofos was done after changing water on alternate day. At the end of day 1, 7, 15 and 30 at least 7 fish from each aquarium were collected for the assays.

2.2. Recovery assessment

After exposures to 10% and 50% of LC_{50} of profenofos for 30 days, fish were transferred to profenofos free freshwater tanks for recovery observations. The water was changed daily, and the fish were fed every day also. At the end of day 1, 7, 15 and 30 post exposures, at least 7 fish from each aquarium were collected and their recovery was assessed.

2.3. Estimation of erythrocytic abnormalities

Silver barb were taken from their aquarium on each sampling day, and then anesthetized with clove oil in a concentration with 5.5 mg/L. Water and slime present on the body surface of the fish were removed by using blotting paper. The peripheral blood samples were collected using a heparinized plastic syringe from the caudal vein and smeared onto pre-threshed slides and air dried for 10 min. The smear was fixed with methanol for 10 min and stained

Table 1
Mortality percentages of the silver barb exposed to different concentrations of profenofos at different time intervals.

Concentration (mg/L)	Initial count of fish	Number of dead fish at different exposure times (h)						% Mortality
		0	12	24	36	72	120	
0.00 (Control)	10	–	–	–	–	–	–	00
0.08	10	–	–	–	–	–	–	00
0.07	10	–	–	–	–	–	–	00
0.06	10	–	–	–	–	–	1	10
0.05	10	–	–	–	1	–	1	20
0.10	10	–	2	1	1	–	1	50
0.20	10	–	4	2	–	–	2	80