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Evaluation of genotoxicity of profenofos to freshwater fish *Channa punctatus* (Bloch) using the micronucleus assay

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The present study investigates the genotoxicity of profenofos (PFF), an organophosphate pesticide in erythrocyte cells of freshwater fish, *Channa punctata* (Bloch) using micronucleus assay. The 96 h LC_{50} value of PFF (50% EC) was estimated for the fish species in a semistatic system and then 50% of LC_{50} was determined as 1.15 ppb. The fish specimens were exposed to 1.15 ppb sublethal concentration of the pesticide and samplings were done on 24, 48, 72 and 96 h post exposure for assessment of DNA damage by micronucleus assay. The study confirms that PFF is toxic for aquatic organism and the micronucleus assay is a useful tool in determining the potential genotoxicity and mutagenicity of xenobiotic compounds and can be considered as sensitive parameter for toxicity monitoring program.

Key words: Genotoxicity, profenofos, *Channa punctatus,* DNA damage, micronucleus assay.

INTRODUCTION

The pesticides and related chemicals originating from human activities or agricultural farming are discharged directly or indirectly into waterbodies. The presence of these chemicals in the environment has become a global problem. The studies have shown that the reproduction, growth and development of organisms, including invertebrates, amphibians, reptiles, fish, birds and mammals may have interacted with these chemicals and interfere with the endocrine system and other hormonal processes (Khan and Law, 2005; Kayhan et al., 2007).

Profenofos (O-4-bromo-2-chlorophenyl-O-ethyl S-propyl phosphorothioate), a broad-spectrum organophosphate pesticide is widely used for agricultural and household purposes in India and also in developing and developed countries. Profenofos (PFF) had been investigated to be highly toxic to different organisms including mammals, insects and fish. It has also been classified as moderately hazardous (toxicity class II) pesticide by WHO and it has a moderate order of acute toxicity following oral and dermal administration (Pandey

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Table 1. Characteristics/ properties/ specification of the test chemical.

Parameter	Characteristics/ properties/ specification of pesticide
Common Name	Profenofos
Chemical/ Product Name	(*RS*)-*O*-4-bromo-2-chlorophenyl *O*-ethyl S-propyl phosphorothioate
Chemical/ Product Name	CELCRON
Grade	EXCEL crop care Ltd., Mumbai
WHO and EPA toxicity class	II, Moderately toxic
CAS No.	41198-08-7
Alkyl groups	*S*. propyl-*O*. ethyl

et al., 2011a).

Fishes are considered as sensitive organism in toxicity studies and can play a significant role in assessing potential risks associated with contamination in aquatic environment by environmental contaminants. Fishes can respond to mutagens at low concentrations of toxicants in a manner similar to higher vertebrates (Pandey et al., 2011b).

The micronucleus (MN) test is an *in vivo* and *in vitro* short-time screening test, developed by Schmid (1975), and is widely used to detect genotoxic assessment (Saleh and Alshehri, 2011). It is the most frequently used bio-markers for genotocixity testing in aquatic environments. The MN test has gained high relevance in bio-monitoring of aquatic environments due to nucleated nature of fish erythrocytes. The MN is a chromatin mass in the form of small nuclei which appear within the cytoplasm and close to the main nucleus in interphase cells. They are originated spontaneously or as consequence of clastogenic and/or aneugenic effects, which ultimately generate acentric chromosomal fragments and/or lagging chromosomes during the mitotic anaphase (Betancur et al., 2009). Several researcher showed that the micronucleus (MN) test is one of the simple, sensitive, reliable, least expensive and rapid screening system for both clastogenic (chromosome breakage, formation of a centric fragments), eugenic (chromosome lagging and effects on spindle) and genotoxic effects of xenobiotic chemicals under field and laboratory conditions (Pandey et al., 2009; Chaudhary et al., 2006; Ali et al., 2008).

The information regarding the mutagenic and genotoxic nature of profenofos in aquatic organism is rare, especially the data pertaining to its effects on fishes. The present study investigates the genotoxic effects of profenofos using MN assay in erythrocytes cells of *C. punctatus* exposed *in vivo*.

MATERIALS AND METHODS

Experimental fish specimens

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The fish *C. punctatus* (Bloch) belongs to family: Channidae and order: Perciformes. It was obtained from the local market and acclimatized in the laboratory condition for 10 days before experimentation. They were kept in a large holding tank of 1000 L in capacity during acclimatization. Length and weight of the fish ranged from 12.0 ± 3.0 cm and 23 ± 2.0 g, respectively. A set of 10 acclimatized fish specimens was randomly selected for experiment. Fishes were fed on boiled chicken, eggs or poultry waste material daily at the rate of approximately 4% of fish body weight.

Test chemical

The pesticide used for this study, technical-grade profenofos (EC50) with product name CELCRON (manufactured by EXCEL Crop Care Ltd., Mumbai) was purchased from local market. The test chemical specifications are summarized in Table 1.

In vivo exposure experiment

The fishes were exposed to profenofos 1.15 ppb (50% of LC 50); aforementioned test concentrations of PFF in a semi-static system for 96 h, Keeping 10 fish in each test concentration in 20 L of water in a 50-L plastic tub as an aquarium without change of water. No crowding stress was observed during experimentation. The exposure was continued up to 96 h and tissue sampling was done at intervals of 24, 48, 72 and 96 h on each sampling day. The erythrocytes were collected and immediately processed for MN. Feeding was stopped 24 h before exposure, and fish were not fed during the experimentation period (Pandey et al., 2011a). The physico-chemical properties of test water, namely temperature, pH, dissolved oxygen, electrical conductivity and total hardness were analysed by using a digital analyzer.

Micronucleus test

Peripheral blood samples obtained from the caudal vein were smeared on clean microscopic slide. The slides were then fixed by dipping in methanol for 10 min and left to air-dry at room temperature and finally stained with 6% Giemsa in 0.04 M phosphate buffer (pH = 6.8) for 30 min. After dehydration through graded alcohol and clearing in xylene, slides were mounted in DPX which is a mixture of distyrene (Polystyrene), plasticizer (Tricresyl phosphate) and xylene. The slides were observed under a light microscope (Leitz Wetzlar Germany; Type 307-083.103; oil immersion lens, 100/1.25) and 1000 cells from each specimen were examined for the presence of MN. The MN frequency was calculated as:

$$\text{MN \%} = \frac{\text{Number of cells containing micronucleus}}{\text{Total number of cells counted}} \times 100$$

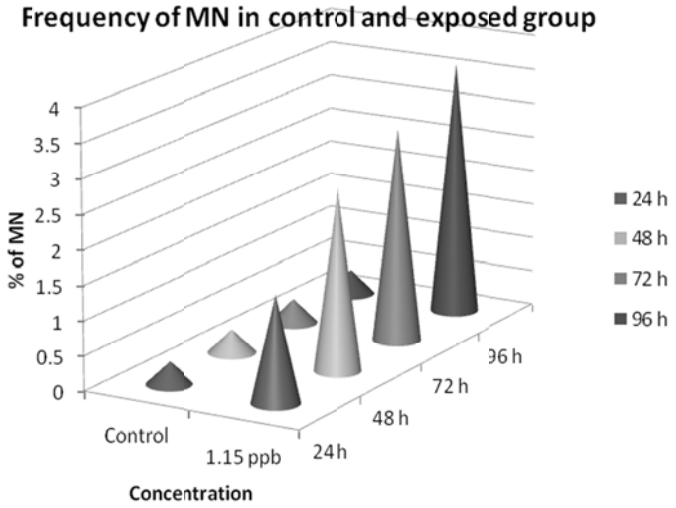

Figure 1. Profenofos concentration response relationship of MN frequency in the erythrocytes of *C. punctatus* for multiple sampling times.

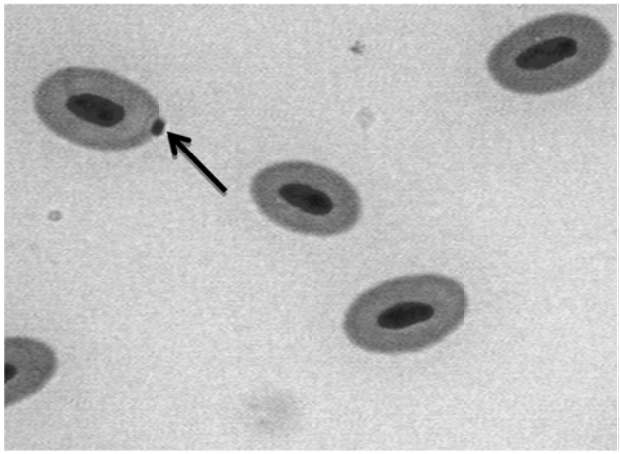

Figure 2. Micronuclei formation in the erythrocytes after exposure to profenofos.

Statistical analysis

The percentage of MN frequency among different exposure intervals and concentrations were compared using the Mann-Whitney test.

RESULTS AND DISCUSSION

The temperature of test water varied from 18.2 to 24.6°C and pH values ranged from 7.1 to 8.3. The dissolved oxygen (DO) ranged from 6.0 to 8.4 mgl^{-1}. The electrical conductivity of the water ranged from 250 to 304 µM cm^{-1} while total hardness ranged from 166 to 185 mg l^{-1} during experiment period.

The fish specimens were exposed *in vivo* to the aforementioned test concentrations of PFF than the control groups at different time periods and the result of MN analysis in erythrocytes of *C. punctatus* for the control and exposed group concentrations of PPF was significant in the fish specimens. Higher induction of MN with the highest MN frequency was recorded at 50% of LC_{50} at 96 h (3.653%), whereas the lowest induced MN frequency was recorded at 50% of LC_{50} at 24 h (1.538%) in blood erythrocyte with 96 h exposure. The observed MN varied from cell to cell. In some cells MN were found attached to the cell wall or boundary while others were located near the main nucleus. The results are summarized in Figures 1 and 2.

Several researchers reported a dose-dependent increase in the induction of MN in peripheral blood of Fish (Chaudhary et al., 2006; Ali et al., 2008; Normann, et al., 2008; Ali et al., 2009; Betancur et al., 2009; Nwani et al., 2010; Saleh and Alshehri, 2011) in response to pesticides, heavy metals and other toxicants. In fishes, the damages are very significant, making it possible to monitor the environmental health state through this parameter (Normann et al., 2008). This study emphasizes the importance of the peripheral blood MN assay and suggests its broader application as an early biological marker of exposure of fish to clastogenic pollutants in the aquatic environment.

Erythrocyte MN test in fish was also widely applied for genotoxicity assessment of aquatic organism *in situ* using native or caged animals following different periods of exposure. Genotoxicity biomarkers must be an integral part of the suite of biomarkers considered as exposure to genotoxic agents which may exert damage beyond that of the individual and may be active through several generations (Magni et al. 2006).

The fish respond to toxic agents similar to higher vertebrates and can allow the assessment of substances that are potentially hazardous to humans. However, the low amount of DNA per cell, the large numbers of small chromosomes and the low mitotic activity in many fish species impaired the metaphase analysis of chromosomal damage and sister chromatid exchanges (Bolognesi and Hayashi, 2011). The MN test is one of the suitable methods for assessing DNA damage at the chromosome level. It permits to measure chromosome loss and chromosome breakage (Fenech, 2000; Hovhannisyan, 2010). The MN assay was developed as a simpler short-term screening test and now accepted as valid alternative to the chromosome aberration assay. In this method, chromosome aberrations are detected indirectly *via* chromatin loss from the nucleus leading to MN in the cytoplasm of the cell (Kirsch-Volders et al., 2003; Hovhannisyan, 2010). It is crucial to assess genotoxicity and cytotoxicity of the environmental pollutants on aquatic organisms. In fish, there are several

types of nuclear lesions whose origin is not still understood (Ayllon and Garcia-Vazquez, 2000; Guner and Muranli, 2011). Toxic chemicals produced nuclear abnormalities thus could help to know the potential risks of water quality as well as the health of fish species.

The results demonstrate that the technical-grade PFF was found to be genotoxic to fishes, which indicates that there is serious apprehension about the potential danger of this pesticide to aquatic organisms. Thus, this is encouraging for judicious and careful use of pesticide in agricultural and non-agricultural practices and also ensures that adverse effects on aquatic organism, human health and the environment are prevented.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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