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Assessment of the mutagenic, recombinogenic and carcinogenic potential of fipronil insecticide in somatic cells of *Drosophila melanogaster*

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HIGHLIGHTS

- The mutagenic, recombinogenic and carcinogenic potential of fipronil was studied *in vivo*.
- Fipronil has mutagenic and recombinogenic action in standard and high-bioactivation crosses.
- Fipronil has carcinogenic action.
- Increasing the metabolic capacity increases the toxic effect of fipronil.

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ABSTRACT

Fipronil (FP) is an insecticide that belongs to the phenylpyrazole chemical family and is used to control pests by blocking GABA receptor at the entrance channel of the chlorine neurons. The aim of this study was to evaluate the mutagenic, recombinogenic and carcinogenic potential of FP. The mutagenic and recombinogenic effects were evaluated using the somatic mutation and recombination test (SMART) on wing cells of *Drosophila melanogaster*. Third instar larvae from standard (ST) and high bioactivation (HB) crosses were treated with different concentrations of FP (0.3, 0.7, 1.5 or 3.0 $\times$ 10^{-5} mM). The results showed mutagenic effects at all concentrations tested in the HB cross; and all concentrations tested in the ST cross, except at concentration of 0.7 $\times$ 10^{-5} mM. The carcinogenic effect of FP was assayed through the test for detection of epithelial tumor (*warts*) in *D. melanogaster*. Third instar larvae from *wts*/TM3 virgin females mated to *mwh*/*mwh* males were treated with different concentrations of FP (0.3, 0.7, 1.5 or 3.0 $\times$ 10^{-5} mM). All these concentrations induced a statistically significant increase in tumor frequency. In conclusion, FP proved to be mutagenic, recombinogenic and carcinogenic in somatic cells of *D. melanogaster*.

1. Introduction

Pesticides are single compounds or complex mixtures used to kill, repel or control organisms (microorganisms, plants or animals) considered pests and that, depending on the physicochemical characteristics, mechanism of action, dose or persistence in the environment can cause an impact on non-target species (Tsutsui et al., 1984; Farooqui and Farooqui, 2012). Pesticides were developed to act in a scenario where the effective pest control is critical to ensure global food demand, making agriculture dependent on chemical pest control (Ecobichon, 2000).

The harmful effects presented by conventional insecticides such as organophosphates, organochlorines, pyrethroids and carbamates have raised concerns about the use of technology because of the damages caused to the environment. Due to occupational and environmental damages that pesticides can cause, a good agrochemical must comply the expectations of pest control management and present selective toxicity, as well as low persistence in the environment and does not present bioaccumulation feature in

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trophic levels (Çelik et al., 2014).

The long-term exposure to pesticides can affect human health due to chronic exposure to its active ingredients and metabolites present in the environment and foods (Houk, 1992), which can generate various negative effects such as dysfunction in biochemical pathways or genetic instability (Çelik et al., 2014).

Fipronil (5 - amino - 1 - [2,6 - dichloro - 4 - (triflurometh yl) phenyl] - 4 - [(trifluoromethyl) sulfinyl] - 1H - pyrazole) (FP) is a broad-spectrum insecticide from the phenyl pyrazole group. Reported as a second generation pesticide, FP was discovered by Rhone-Poulene Agro in 1987, commercially introduced in 1993 and registered in the United States in 1996 (Aajoud et al., 2003).

Fipronil acts in the insect nervous system. The active ingredient binds to the chloride channel receptors, acting to damage and/or block Cl^- ions, preventing the influx in cellular pathway and nullifying the effect of neurorregulator receptors (Connelly, 2001; Aajoud et al., 2003; Stenersen, 2004). As a consequence of the blockade of chloride channels, the nerve impulse is impaired, resulting in an excessive neuronal activity (Cole et al., 1993). When exposed to the insecticide, insects start to present hyper-arousal problems in the muscles and nervous system, leading to severe paralysis and death (Bobé et al., 1998; Gunasekara and Troung, 2007).

In Brazil, according to the Agência Nacional de Vigilância Sanitária (ANVISA), FP is considered highly toxic to the environment and due to this fact it is classified as belonging to Class II insecticides. Several studies have shown harmful effects on non-target organisms such as bees (Hassani et al., 2005; Le Faouder et al., 2007; Lourenço et al., 2012), fish, birds and mammals (Ohi et al., 2004; Stehr et al., 2006; Das et al., 2006; Leghait et al., 2009). According to the Environmental Protection Agency (EPA) FP is classified as a possible carcinogen (U.S. EPA, 1996).

The effects of prolonged exposure to pesticides at low doses on human health are difficult to be assessed, since the symptoms are often not clinically evident (Hernández et al., 2005). Information about pesticide toxicity is not sufficient to predict the occupational hazard, since some compositions may have harmful compounds that act at molecular levels and can lead to genotoxic and mutagenic effects (Bolognesi, 2003). Many of these compounds are considered potential carcinogens and may be associated with the development of cancer and other chronic diseases, such as congenital malformations and degenerative diseases (Bolognesi, 2003; Bhalli et al., 2009; Bolognesi and Moretto, 2014). Few data on the genotoxic and mutagenic effects of FP are available in the literature (Ghisi et al., 2011; Çelik et al., 2014), which points to the need for further studies aimed to evaluate the effects of this molecule in the DNA level.

The somatic mutation and recombination test (SMART) in *Drosophila melanogaster* was developed to detect loss of heterozygosity in marker genes, which are phenotypically expressed on the wings of the flies. This test is an effective method to assess and quantify the mutagenic and recombinogenic potential caused by physical and chemical agents (Graf et al., 1996). In SMART two crosses are used: standard cross (ST) (Graf et al., 1984) and high bioactivation (HB) cross (Graf and van Schaik, 1992). The progeny of the ST expresses basal levels of cytochrome P450 enzyme complex (CYP6A2) that are associated with the metabolism of xenobiotics. The progeny of the HB has a high level of CYP6A2 enzymes.

The epithelial tumor detection test in *D. melanogaster* is based on a strain possessing the *warts* (*wts*) mark that when expressed in wild condition acts as a tumor suppressor gene (Xu et al., 1995). The gene deletion and subsequent expression of the recessive allele leads to the formation of clones of cells that are considered to be highly invasive, resulting in epithelial tumor manifestation in the body appendages of the flies (Nishiyama et al., 1999).

Based on the fact that the evaluation of the effects caused by xenobiotics on DNA is important with regard to preventing diseases related to genetic instability, the aim of this study was to evaluate the mutagenic, recombinogenic and carcinogenic potentials of the FP insecticide through SMART and *wts* tests in *D. melanogaster*.

2. Material and methods

2.1. Chemical agents

Fipronil (FP) Regent® 800 WG (CAS 120068-37-3) was obtained from BASF S.A. (São Paulo, Brazil). Fig. 1A shows the chemical structure of Fipronil Regent® 800 WG. Ethyl carbamate - Urethane (CAS51-79-6) (Fig. 1B) was obtained from Fluka AG (Buchs, Switzerland). Mitomycin C (CAS 07.07.50) (Fig. 1C) was manufactured by Kyowa Hakko Kirin Co. Ltd. (Shizuoka, Japan), packed by Bristol-Myers Squibb S.r.1.Sermoneta-Latin-Italy and imported by Bristol-Myers Squibb S.A.

2.2. Somatic mutation and recombination test (SMART) in *Drosophila melanogaster*

2.2.1. Strains, crosses and treatments

Three *D. melanogaster* strains were used in this study: multiple wing hairs, flare and ORR. The multiple wing hairs line is kept in recessive homozygosity for the *mwh* marker that is located on chromosome 3, in the distal position relative to the centromere (*mwh*, 3–0.3). The *mwh* gene expressed in homozygous recessive produces a multiple format phenotype on wing hairs, different from the wild phenotype that produces a single hair per cell.

The flare line possesses the flr^3 marker that is kept in homozygosity in the presence of chromosomal balancers TM3, Bd^s, located on chromosome 3, proximal to the centromere (flr^3, 3–38.9), compared to the *mwh* marker. When expressed, the flr^3 marker leads to the formation of hair with an appearance of a candle flame,

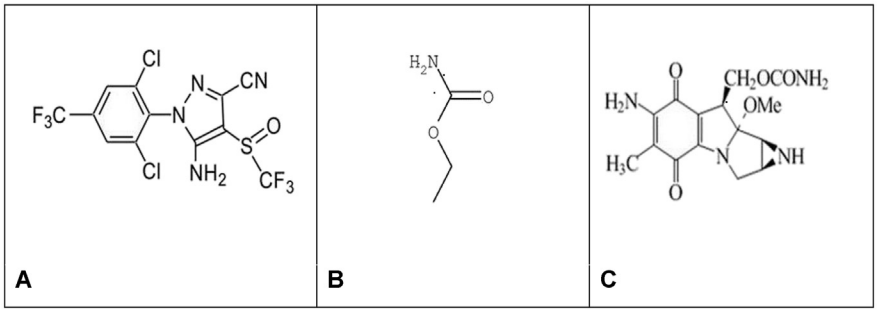

Fig. 1. Structural formula of the chemical of compounds. A. Fipronil (FP); B. Ethil carbamate - Urethane (URE); C. Mitomycin-C (MMC).