Toxicology and Industrial Health
2015, Vol. 31(11) 1051–1060

DOI: 10.1177/0748233713486958
tih.sagepub.com

Pirimicarb-based formulation-induced genotoxicity and cytotoxicity in the freshwater fish *Cnesterodon decemmaculatus* (Jenyns, 1842) (Pisces, Poeciliidae)

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Abstract

We analyzed the aspects of lethality, genotoxicity, and cytotoxicity in the ten spotted live-bearer exposed under laboratory conditions to the pirimicarb-based formulation Patton Flow® (50% active ingredient (a.i.)). Acute effects were evaluated using different end points for lethality, genotoxicity, and cytotoxicity. Median lethal concentration (LC50) estimation was employed as a bioassay for lethality, whereas micronucleus (MN) induction and alterations in erythrocyte/erythroblast frequency were used as end points for genotoxicity and cytotoxicity, respectively. Results demonstrated an $LC50_{96h}$ value of 88 mg/L. Patton Flow® increased the MN frequency in fish erythrocytes after 48 h of exposure at a concentration of 66 mg/L, whereas a concentration range of 22–66 mg/L was able to exert the same genotoxic effect at 96 h of treatment. Furthermore, cytotoxicity was also observed by alterations in erythrocyte/erythroblast frequencies within the concentration range of 22–66 mg/L, regardless of the exposure time. Our current observations provide evidence that Patton Flow® (50% a.i.) should be considered a clear lethal, cytotoxic, and genotoxic agent on *Cnesterodon decemmaculatus*. Thus, repeated applications of this carbamic insecticide can enter the aquatic environment and exert deleterious effects on aquatic organisms other than the evaluated species *C. decemmaculatus*.

Keywords

Patton Flow®, mortality, micronucleus, erythrocyte:erythroblast ratio, poeciliidae

Introduction

Modern agriculture characterized by large-scale culture, monoculture, and intensive use of inputs generated a great demand for agrochemicals, including pesticides (Conway, 1997). In spite of their economic benefits, pesticides may represent potential hazards to human health and the environment. The indiscriminate use of pesticides can generate pest resistance, the emergence of new pest species, environmental pollution, toxic effects, genetic alterations on nontarget organisms including humans, and biodiversity loss, among other side effects (CASAFE, 2009; El Sebae, 1993; Joy et al., 2005; Mohanty et al., 2011).

Pesticides may be introduced into the aquatic environment since they are applied directly on surface water to control aquatic weeds or via air onto crop fields (USEPA, 2009). Indirect entrance into the freshwater environment is associated with runoff, erosion, and lixiviation events resulting from terrestrial application (USEPA, 2006, 2009). Furthermore, they may provoke harmful effects on the fish population and other aquatic organisms, contributing to long-term effects in the environment (Nwani et al., 2011).

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Pirimicarb (2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate) is an *N*-methyl carbamate-specific acaricide insecticide (WHO, 1976) used in cereal, horticultural, fruit, and ornamental crops (CASAFE, 2009). Pirimicarb acts by contact, ingestion, and inhalation, and its mode of action is to inhibit cholinesterase activity (WHO-FAO, 2004). According to the USEPA, this insecticide belongs to the group of compounds with possible carcinogenic potential in humans (Classes II–III) (USEPA, 2005). Overall, pirimicarb has been classified as a moderately hazardous compound (Class II) by the WHO (2009).

So far, available data indicate that only 23 pirimicarb-based insecticides have been registered worldwide (www.environmentalchemistry.com). The formulation PirimorG (50% active ingredient, a.i.) has been found to induce toxic effects on human lung carcinoma cell cultures (Skandrani et al., 2006). Several other formulations containing pirimicarb as the active ingredient have been found to produce adverse effects on the microbial communities of freshwater sediment (Widenfalk et al., 2004, 2008), on crustaceans *Ceriodaphnia quadrangula* (Mansour and Hassan, 1993) and *Daphnia magna* (Andersen et al., 2006; Syberg et al., 2008), on mosquito *Culex quinquefasciatus* (Magnin et al., 1988), and on amphibians *Pelophylax perezi* (Alvarez et al., 1995; Honrubia et al., 1993) and *Rhinella arenarum* (Vera-Candioti et al., 2010a). Among fish, toxic effects on *Poecilia reticulata* (OPP-EEDB, 2000) and *Cyprinus carpio* (OPP-EEDB, 2000; Svobodova, 1980) have been reported.

Studies on genotoxic effects exerted by this insecticide are scarce (IARC, 1976; WHO-FAO, 2004). Briefly, pirimicarb is recognized as nongenotoxic in bacteria, yeasts, fungi, and mammalian cells (USEPA, 1974, 2002a). It has been reported to be nonmutagenic in *Salmonella typhimurium* with S9 metabolic activation (USEPA, 1974). No chromosomal abnormalities have been found in human lymphocytes *in vitro*, with or without metabolic activation (USEPA, 1974), or in bone marrow cells of exposed rats after oral administration of pirimicarb (Anderson et al., 1980). Furthermore, mutagenic effects on L5178Y mouse lymphoma cells (WHO-FAO, 2004) and on the *Drosophila* Oregon-K strain using the white/white$^+$ somatic mutation and recombination test (Aguirrezabalaga et al., 1994) were observed. Pirimicarb significantly increased DNA damage in human lymphocytes *in vitro* (Ündeger and Basaran, 2005) and the frequency of chromosomal aberrations in circulating lymphocytes of exposed workers (Pilinskaia, 1982). We demonstrated that pirimicarb and its commercial formulation Aficida® (50% a.i.) induced an increase in both chromosomal aberrations and sister chromatid exchange frequencies, altered cell cycle progression, and inhibited mitotic index in CHO-K1 cells (Soloneski and Larramendy, 2010). Aficida® has been found to exert alterations in erythrocyte/erythroblast frequency and increase the frequency of micronuclei (MN) in circulating erythrocytes of *R. arenarum* tadpoles and *Cnesterodon decemmaculatus* exposed under laboratory conditions (Vera-Candioti et al., 2010a, 2010b).

The MN test, due to its sensitivity, reliability, and the simplicity of use, has been used widely in piscine erythrocytes to assess the genotoxicity of many xenobiotics (Bolognesi et al., 2006; Buschini et al., 2004; Caliani et al., 2008; Cavaş, 2008; Cavaş and Ergene-Gözükara, 2003, 2005b; Cavaş and Könen, 2008; Grisolia and Starling, 2001; Jerbi et al., 2011; Vera-Candioti et al., 2010b). Genotoxicity in fish associated with pesticide exposure analyzed using the MN test in fish erythrocytes is well documented (Ali et al., 2008b, 2009; Campana et al., 1999; Cavalcante et al., 2008; Cavaş, 2011; Cavaş and Könen, 2007; Farah et al., 2003; Grisolia, 2002; Jorge et al., 2006; Vera-Candioti et al., 2010b).

Pirimicarb is a highly selective compound, and in our country it is considered the first insecticide to control aphids, without affecting the beneficial insects (CASAFE, 2009). So far, only two commercial formulations containing pirimicarb have been registered in Argentina, namely Aficida® (50% a.i.) and Patton Flow® (50% a.i.) (CASAFE, 2009). The aim of this study was to assess the lethal and sublethal toxicity of one of these formulations, Patton Flow® (50% a.i.), in members of the fish family Poeciliidae *C. decemmaculatus* exposed under laboratory conditions using mortality, MN induction, and alterations in blood cell populations as end points.

Materials and methods

Chemicals

Pirimicarb (commercial grade; trade name Patton Flow®; CAS 23103-98-2; 50% pirimicarb, excipients *quantum satis*) was purchased from Gleba S.A. (Buenos Aires, Argentina). Cyclophosphamide (CAS 6055-19-2) was obtained from Sigma Chemical Co. (St. Louis, Missouri, USA), whereas potassium dichromate (Cr(VI)) (CAS 7778-50-9) was obtained from Merck KGaA (Darmstadt, Germany).

Quality control

Determination of the analyte concentration in the test solutions (0.0, 10.0, 50.0, 100.0, 150.0, 200.0, and 250.0 mg/L) was performed immediately prior to use (0 h) and after a 24-h period at room temperature. Analysis was performed using high-performance liquid chromatography (HPLC; Agilent 1100 LC system, Agilent Technologies, California, USA)) with ultraviolet diode array detection at a wavelength of 230 nm. The C_{18} column was 15 cm long with a 4.6 mm inner diameter, and the mobile phase was acetonitrile in 10 mM monopotassium phosphate buffer, pH 4 (60:40). The flow rate was 0.8 mL/min. Pirimicarb was identified by comparison of chromatographic peaks corresponding to solutions from the initial time and 24 h after treatment, as reported by Vera-Candioti et al. (2010b).

Test organisms

The ten spotted live-bearer fish *C. decemmaculatus* (Jenyns, 1842) (Pisces: Poeciliidae), an endemic organism and widely distributed in Neotropic America (Gómez et al., 1998), was selected as the target organism for the bioassays. This small poeciliid inhabits a variety of basins of South America, including the system of the Rio de La Plata (Menni et al., 1996). It is a representative species of the fish fauna of the Pampas region, Argentina, accessible throughout the year and relatively easy to maintain under laboratory conditions (Menni et al., 1996). Adults were collected from a permanent pond, free from pluvial runoff from agricultural areas, in the vicinity of La Plata, Buenos Aires, Argentina. Fish were transported to the laboratory and then acclimatized for at least 20 days before pirimicarb exposure in 40 L tanks with dechlorinated tap water and permanent aeration. The physical and chemical parameters of water were (mean $\pm$ SE) as follows: temperature, 21.0 $\pm$ 1°C; pH 7.55 $\pm$ 0.1; dissolved oxygen, 6.3 $\pm$ 0.3 mg/L; conductivity, 994 $\pm$ 8.5 µS/cm; and hardness, 143 $\pm$ 23.5 mg calcium carbonate ($CaCO_3$/L). During this period, the fish were fed *ad libitum* daily with fish food (TetraMin®, TetraWerke, Germany) and maintained on a 16-/8-h light–dark photoperiod.

Exposure conditions

Short-term semistatic toxicity tests were conducted with dechlorinated tap water in 5 L polypropylene containers without aeration. Water quality during the experiments was (mean $\pm$ SE) as follows: pH 7.55 $\pm$ 0.1; dissolved oxygen, 6.3 $\pm$ 0.3 mg/L; ammonium < 0.2 mg/L; conductivity, 994 $\pm$ 8.5 µS/cm; and hardness, 143 $\pm$ 23.5 mg $CaCO_3$/L. Other parameters were a temperature of 21.0 $\pm$ 1°C and a 16-/8-h light–dark photoperiod. All test solutions were replaced every 24 h and prepared daily immediately before the renewal. Fish were not fed throughout treatments. The average body weight of the specimens was 0.26 $\pm$ 0.1 g, and the mean body length was 29.5 $\pm$ 2.7 mm.

Determination of LC50

Concentrations assessed throughout the study represent the nominal concentrations of active ingredient present within the Patton Flow® pirimicarb-based formulation expressed as milligrams pirimicarb per liter. Experiments were performed following recommendations proposed by the USEPA standardized methods for acute toxicity testing (1975, 2002b). Briefly, for each experimental point, 10 specimens were exposed in a final 1 L volume to 10 different concentrations of Patton Flow® (0.1, 1.0, 10.0, 50.0, 75.0, 100.0, 125.0, 150.0, 200.0, and 250.0 mg/L) for 96 h. While the negative control group consisted of 10 organisms kept in dechlorinated tap water, the positive control group consisted of 10 fish treated with 21.4 mg/L Cr(VI), as reported previously (Vera-Candioti et al., 2011). All treatments were carried out in triplicate. Fish were not fed throughout the experiment. Fish were visually examined daily and considered dead when no respiratory movements were observed or a lack of sudden swimming in response to gentle touching was observed in regard to control organisms.

Determination of MN frequency

MN assay was performed on peripheral circulating blood erythrocytes according to the procedures of Ali et al. (2008a, 2009), with some modifications as described previously (Vera-Candioti et al., 2010b). Each experiment was conducted following the experimental design described in the determination of median lethal concentration (LC50) section, with specimens exposed to 22, 44, and 66 mg/L pirimicarb. These concentrations correspond to 25, 50, and 75% of the $LC50_{96h}$ of this insecticide for *C. decemmaculatus*. Negative (dechlorinated tap water) and positive controls (5 mg/L cyclophosphamide) were conducted and run simultaneously with Patton Flow®-exposed fish as reported previously (Vera-Candioti et al.,

2010b). Frequency of MN was recorded at 48 and 96 h. Tests were conducted in triplicate.

Fish specimens were killed by severing the spinal column below the opercula. Two drops of peripheral blood from each specimen were smeared on clean slides, air-dried, fixed with 100% (v/v) cold methanol (4°C) for 20 min, and stained with 5% Giemsa solution. Slides were coded and blind scored at 1000× magnification. The frequency of MN was determined by analyzing 1500 mature erythrocytes from each fish as suggested previously (Cavaş, 2008; Cavaş and Könen, 2007, 2008; Vera-Candioti et al., 2010b) and expressed per 1000 cells (%). MN were determined following the previously reported examination criteria (Cavaş and Könen, 2007; Vera-Candioti et al., 2010b). The criteria employed for MN identification in intact erythrocytes were as follows: a diameter smaller than 1/3 of the main nuclei diameter; nonrefractability; staining intensity similar to or lighter than that of the main nuclei; no connection or link with the main nuclei; no overlapping with the main nuclei; an MN boundary distinguishable from the main nuclei boundary (Fenech et al., 2003).

Analysis of erythrocyte–erythroblast frequency

The circulating erythrocyte–erythroblast frequencies were determined as described previously for aquatic organisms (Vera-Candioti et al., 2010a, 2010b). Briefly, frequencies of mature erythrocytes and erythroblasts were blind scored at 1000× magnification by analyzing a total of 1500 erythrocyte/erythroblast cells from each fish specimen in those slides employed for MN analysis and expressed as the total number of erythrocytes and erythroblasts per 1000 cells. In brief, the criteria employed for erythrocyte and erythroblast identification in peripheral circulating blood were as follows: an erythrocyte has a cell nucleus oval to rounded oval (Claver and Quaglia, 2009) and compact chromatin that stains purple in preparations stained with aqueous Giemsa (Rey Vázquez and Guerrero, 2007). Moreover, an erythroblast cell has a circular or slightly ovoid basophilic cytoplasm and round to slightly ovoid nucleus containing diffuse chromatin of variable density that represents 40–60% of cell volume (Peters and Schwarzer, 1985).

Statistical analyses

Data of lethality tests were analyzed using the Probit Analysis Program, version 1.5 (www.epa.gov). Statgraphics Centurion XV (StatPoint, Inc., Virginia, USA) software was used for statistical analyses. After assessing the normality of the distribution of the data, even after logarithmic transformation, nonparametric tests were used to detect differences. The Kruskal–Wallis test and the one-tailed Mann–Whitney U test for independent samples were applied to assess differences between treated and control groups. The relationship among mortality, MN frequency, and circulating erythrocyte/erythroblast frequencies and insecticide concentrations was evaluated by simple linear regression analyses. The level of significance was $\alpha = 0.05$, unless indicated otherwise.

Results

Chemical analyses

HPLC results of chemical analyses showed no changes in the concentration of the toxicant in treatments during the 24-h interval renewals of the testing solutions.

Mortality assays

Probit analysis of the mortality experiments allowed determination of the LC50 values of Patton Flow® after 24, 48, 72, and 96 h of exposure, with mean values of 119 (range, 109–130), 114 (range, 104–125), 88 (range, 24–142), and 88 mg/L (range, 24–142) pirimicarb, respectively. As revealed by regression analysis, mortality showed a concentration-dependent increase following treatment with Patton Flow® ($r = 0.98$, $p < 0.001$). On the other hand, LC50 values were not affected by the exposure time ($r = -0.92$, $p > 0.05$).

Genotoxicity and cytotoxicity assays

No mortality was registered during the experiments. The frequency of MN in cyclophosphamide-exposed specimens of *C. decemmaculatus* was significantly increased compared with the negative control values when the analysis was performed both at 48 ($p < 0.05$) and 96 h ($p < 0.05$) (Table 1).

The fish specimens exposed to Patton Flow® during 48 h of treatment exhibited an enhancement in the MN frequency only at the highest concentration of 66 mg/L pirimicarb ($p < 0.05$) (Table 1). However, after 96 h of treatment, a significant increase in micronucleated erythrocyte frequency was observed in specimens exposed to 22 ($p < 0.01$), 44 ($p < 0.001$), and 66 mg/L pirimicarb ($p < 0.001$) (Table 1).

Overall, a regression analysis revealed that the frequency of MN increased as a function of the pirimicarb

Table 1. Incidence of MN and erythrocyte/erythroblast frequencies in peripheral blood cells of *Cnesterodon decemmaculatus* exposed to different concentrations of Patton Flow®.

			MN induction		Erythrocyte–erythroblast frequencies		
Compound exposure time (h)	Dosage (mg/L)	No. of fish analyzed	No. of cells analyzed	Frequency of MN (% ± SE)	No. of erythrocytes analyzed	No. of erythroblasts analyzed	Frequency of erythroblasts (% ± SE)
48	Control	15	22,500	0.27 ± 0.11	22,444	56	2.49 ± 1.02
	Positive control[a]	15	22,500	0.89 ± 0.17[b]	21,307	1193	53.02 ± 18.89[c]
	22	15	22,500	0.53 ± 0.13	22,334	166	7.38 ± 1.93[d]
	44	15	22,500	0.76 ± 0.20	22,344	156	6.93 ± 3.72[b]
	66	15	22,500	0.89 ± 0.23[b]	22,232	268	11.91 ± 3.52[c]
96	Control	15	22,500	0.22 ± 0.08	22,415	85	3.78 ± 2.46
	Positive control[a]	15	22,500	0.89 ± 0.23[b]	20,123	2377	105.64 ± 26.40[d]
	22	14	21,000	1.00 ± 0.21[c]	20,872	128	6.10 ± 1.66[c]
	44	17	25,500	1.33 ± 0.28[d]	25,341	159	6.24 ± 2.60[c]
	66	16	24,000	1.58 ± 0.22[d]	23,753	247	10.29 ± 2.37[d]

MN: micronucleus.
[a] Cyclophosphamide, 5 mg/L.
[b] $p < 0.05$.
[c] $p < 0.01$.
[d] $p < 0.001$; significant differences with respect to control values.

concentrations after 96 h of treatment ($r = 0.44$, $p < 0.001$) but not during the first 48 h of exposure ($r = 0.19$, $p > 0.05$). Furthermore, a time-dependent increase in MN frequency was observed within 0–96 h of pirimicarb treatment, regardless of the concentrations assayed ($r = 0.20$, $p < 0.01$).

Analysis of the alterations in the erythrocyte–erythroblast frequency in exposed fish is shown in Table 1. Cyclophosphamide treatment significantly decreased the frequency of erythrocytes and increased the frequency of erythroblasts in fish exposed for 48 ($p < 0.01$) and 96 h ($p < 0.001$) when compared with negative controls.

Fish exposed to Patton Flow® showed a cytotoxic effect evidenced by a decrease in the frequency of erythrocytes and a concomitant increase in the frequency of erythroblasts after 48 and 96 h of treatment for all concentrations assayed ($p < 0.05$ and $p < 0.001$, respectively). A regression analysis revealed that the cytotoxic effect exerted by Patton Flow® was neither concentration dependent ($r = 0.15$, $p > 0.05$ and $r = 0.18$, $p > 0.05$ for 48 and 96 h, respectively) nor time dependent ($r = -0.09$, $p > 0.05$).

Discussion

In the current study, the pirimicarb-based herbicide Patton Flow® induced acute lethal toxicity, genotoxicity, and cytotoxicity in *C. decemmaculatus* (Pisces, Poeciliidae) exposed under laboratory conditions. According to the $LC50_{96h}$ value obtained in the present study (88 mg/L, range 24–142 mg/L), Patton Flow® can be classified as a harmful Category III compound based on the substance classification criteria proposed by the United Nations (2011), or a compound that may cause long-term adverse effects in the aquatic environment following the European Union directives (Mazzatorta et al., 2002), at least for the evaluated species. Furthermore, pirimicarb increased MN frequency in fish erythrocytes after 48 h of exposure at a concentration of 66 mg/L, whereas a concentration range of 22–66 mg/L was able to exert the same genotoxic effect at 96 h of treatment.

Comparing the toxicity of Patton Flow® with that of Aficida®, the other pirimicarb-based insecticide (50% a.i.) registered for Argentina, the former is more toxic than the latter, considering the $LC50_{96h}$ for *C. decemmaculatus*. In a previous study, for *C. decemmaculatus*, we observed an $LC50_{96h}$ for Aficida® as high as 113 mg/L pirimicarb (Vera-Candioti et al., 2010b), 1.3 times higher than the $LC50_{96h}$ found in the current study for Patton Flow®, namely 88 mg/L pirimicarb. In other words, regarding the lethal effects, *C. decemmaculatus* was more sensitive to Patton Flow® than to Aficida®. This could not be ruled out by the presence

of xenobiotics in the formulation Patton Flow® that are exerting a toxic effect on themselves or have an additive and/or synergistic effect with the active ingredient, increasing their toxicity with regard to the formulated Aficida®. Several reports have shown that the toxicity of several commercial formulations is higher than that of their active ingredients. Hence, additional genotoxic and cytotoxic effects exerted by inert ingredients must be taken into consideration for risk assessment, as suggested previously (Lin and Garry, 2000; Mann and Bidwell, 1999; Rayburn et al., 2005; Soloneski and Larramendy, 2010; Soloneski et al., 2008; Zeljezic et al., 2006). Unfortunately, the identities of the additive compound(s) present in the commercial formulations Patton Flow® and Aficida® were not made available to us by the manufacturers.

Taking into account the $LC50_{96h}$ value obtained for pirimicarb for *C. decemmaculatus* exposed to Patton Flow® with those $LC50_{96h}$ values registered for other fish species exposed to the same active ingredient, it could be noted that the commercial formulation exerts a lethal effect within the range of lethality cause on rainbow trout (*Oncorhynchus mykiss*; 29–129 mg/L pirimicarb) (OPP-EEDB, 2000) but higher than that exerted on the common carp (*C. carpio*; 410 mg/L pirimicarb) (Svobodova, 1980).

Previous reports have demonstrated the ability of pirimicarb and several pirimicarb-based products to induce MN both *in vitro* and *in vivo* (Soloneski and Larramendy, 2010; Vera-Candioti et al., 2010a, 2010b). Among *in vivo* studies, Aficida® induced an increase in MN frequency in *R. arenarum* (Vera-Candioti et al., 2010a) as well as in fish *C. decemmaculatus* (Vera-Candioti et al., 2010b). So far, no studies of genotoxic and cytotoxic effects have been carried out on *C. decemmaculatus* exposed to a pirimicarb-based formulation other than Patton Flow®.

The results demonstrated that pirimicarb increased MN frequency in fish erythrocytes after 48 h of exposure at a concentration of 66 mg/L; whereas with a concentration range of 22–66 mg/L, the same genotoxic effect was exerted at 96 h of treatment. It is worth mentioning that the concentration range tested in the present study should not be considered environmentally realistic. Although the *in vivo* pirimicarb treatments in this study covered a wide range of concentrations, the concentration range represents a relatively high end of the threshold value of 0.004 mg/L pirimicarb found in surface water bodies, or even 0.0004 mg/L pirimicarb as reported in rainfall samples (TOXNET, 2010). Unfortunately, there is no available information on the insecticide concentration found in pampasic Argentinean water streams, where *C. decemmaculatus* is commonly found near crop fields. Thus, the concentrations of pirimicarb employed in this investigation would be expected to be rare in the environment, perhaps only observed when specific events occurred, for example, a direct application adjacent to surface waters in creeks, ponds, and drainage ditches by accidental discharge or spills. We cannot rule out that fish populations and also occupationally exposed human workers could be exposed accidentally to this range of concentrations.

Available data in the literature indicate that the maximum piscine MN frequency in peripheral erythrocytes occurs between 1 and 5 days of treatment with a xenobiotic (Al-Sabti and Metcalfe, 1995). Previous evidence reported by Udroiu (2006) demonstrated that the maximal induction of MN in fish usually occurs after 2–3 days of exposure. Experiments with the green snake head *Channa punctatus* (Perciformes, Channidae) exposed to Tricel® (20% chlorpyrifos-based formulation) revealed that the increase in MN frequency in circulating erythrocytes could require 96 h of treatment to reach the maximum damage level (Ali et al., 2008b). In agreement with this observation, it was reported recently for *C. decemmaculatus* that a 96-h exposure time is a period long enough to observe the maximum induction of MN after exposure to Credit® and Panzer®, two glyphosate-based herbicides (Vera-Candioti et al., 2012) as well as Aficida®, another pirimicarb-based formulation (Vera-Candioti et al., 2010b). Our results agree well with these observations.

As suggested previously (Vera-Candioti et al., 2010b), to achieve a better understating of Patton Flow®-induced cytotoxicity, the frequency of circulating erythrocytes and erythroblasts in those insecticide-exposed fish was analyzed. The results showed a cytotoxic effect evidenced by a decrease in the frequency of erythrocytes and a concomitant increase in the frequency of erythroblasts after both harvesting times and for all treatments. The cytotoxic effect induced by Patton Flow® on *C. decemmaculatus* could be due to either an alteration in the erythropoietic function with an increase in erythroblasts and a poor replenishment of erythrocytes on circulation or a decrease in the removal of damaged cells such as erythrocytes by the spleen, with the consequent accumulation of erythroblasts in the bloodstream (Udroiu, 2006). These results are in accord with the previous observations reported for other xenobiotics including lead and mercury on *Carassius auratus auratus* (Cavaş, 2008),

benzo[*a*]pyrene and dehydroabietic acid on *Anguilla anguilla* (Pacheco and Santos, 1997), metronidazole on *Oreochromis niloticus* (Cavaş and Ergene-Gözükara, 2005a), formaldehyde and oxytetracycline on the European sea bass *Dicentrarchus labrax* (Jerbi et al., 2011), and pyrethroid lambda-cyhalothrin on *Gambusia affinis* (Gökalp Muranli and Güner, 2011) as well as Aficida® on *C. decemmaculatus* specimens (Vera-Candioti et al., 2010b).

Our study indicates that the pirimicarb-based insecticide Patton Flow® (50% a.i.) is able to reveal in *C. decemmaculatus* the genotoxic properties by increasing the MN in peripheral erythrocytes and the cytotoxic ability by altering the erythrocyte–erythroblast ratio. Considering the scarce knowledge of pirimicarb toxicity and its use for agricultural and nonagricultural purposes, the present results are complementary to those reported by other research groups, highlighting the environmental risk of this insecticide.

Conflict of interest

The authors declared no conflicts of interest.

Funding

This research was supported by grants from the National University of La Plata (Grants 11/N619 and 11/N699) and the National Council for Scientific and Technological Research (CONICET, PIP N° 0106) from Argentina.

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