Ecotoxicology and Environmental Safety 148 (2018) 1–10

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Are the damaging effects induced by the imazethapyr formulation Pivot® H in *Boana pulchella* (Anura) reversible upon ceasing exposure?

Juan Manuel Pérez-Iglesias[a,b,c], Guillermo S. Natale[b,c], Sonia Soloneski[a,c], Marcelo L. Larramendy[a,c,*]

[a] Cátedra de Citología, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Calle 64 N° 3, B1904AMA La Plata, Argentina
[b] Centro de Investigaciones del Medio Ambiente (CIMA), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina
[c] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

ARTICLE INFO

Keywords:
Imazethapyr
Pivot® H
Recovery capabilities
Comet assay
Micronuclei
Morphological abnormalities

ABSTRACT

In the present study, the damage recovery capabilities of *Boana pulchella* tadpoles after acute exposure (96 h) to 0.39 mg/L concentration of the imazethapyr (IMZT)-based herbicide formulation Pivot® H (25% IMZT LC_{50} value) were assessed during a period of 7 to −21 days. To appraise the recovery capabilities, frequency of micronuclei (MNs), other nuclear abnormalities and DNA single-strand breaks evaluated by single cell gel electrophoresis assay on circulating blood cells were employed as endpoints for genotoxicity. Growth, development, body mass, and morphological abnormalities were also employed as individual endpoints in the recovery assay. Results demonstrated that IMZT induced sublethal effects at both the individual (i.e., loss of keratodonts) and cytogenetic levels (e.g., increase of MN frequency, other nuclear abnormalities and DNA single-strand breaks). At 11 days of the exposure phase, tadpoles recovered their basal levels of frequency of MNs, other nuclear abnormalities, and comets. However, loss of keratodonts, observed at the end of the exposure period, was present up to 21 days thereafter. Finally, axial abnormalities and delay in development stage were observed only during the postexposure phase in IMZT-exposed tadpoles at 18 and 25 days, respectively and were observed until the end of the experiment. This is the first evidence of use the comet assay as cytogenetic biomarker of genotoxicity in evaluating the recovery capabilities of amphibians in general and also those of *B. pulchella* after exposure to IMZT.

1. Introduction

In the last decades, the expansion of agricultural activities together with the exhaustive use of agrochemicals has led to the widespread presence of pesticides in every area of the environment and thus represents one of the major anthropogenic factors leading to increased contaminants and environmental stressors in aquatic ecosystems (Cooper, 1993; Schwarzenbach et al., 2006). Among these agrochemicals are included herbicides, known to exert changes in the biochemical and metabolic parameters of wild nontarget species that inhabit aquatic systems (Köhler and Triebskorn, 2013; Salbego et al., 2010). It is well known that while a part of the amount of herbicides employed in agronomical practices reach the target, the remainder flows into the ground, through air or water, contaminating several microhabitats, including ponds and wetlands in the proximity of crops where amphibians live and breed, then affecting all amphibian life stages (Greulich and Pflugmacher, 2003; Harris et al., 1998; Rohr and Crumrine, 2005). The accumulation of such environmental stressors in aquatic environments has been proposed as a causative agent of amphibian decline, because they can have adverse effects on anuran health (Beebee and Griffiths, 2005; Blaustein and Wake, 1990; Houlahan et al., 2000; Kiesecker et al., 2001). However, it is imperative to highlight that due to the seasonality of crops concomitantly with the subsequent periodic/seasonal application of these agrochemicals, their input into aquatic systems become then, typically intermittent. Accordingly, nontarget species exposure under these types of situations to these types of environmental stressors can be short (time scale of days) and followed by periods of permanence in noncontaminated areas. Moreover, there is little information concerning the reversibility or irreversibility of biochemical, physiological, or genetic effects in aquatic organisms after the decrease or disappearance of contamination (Guilherme et al., 2014). Assessing the responses generated by pesticide exposure and the persistence of disturbances after contamination ends would provide a better understanding of environmental stressor depuration and

* Correspondence to: Facultad de Ciencias Naturales y Museo, Calle 64 Nro. 3 (esq. 120), B1904AMA La Plata, Argentina.
E-mail address: marcelo.larramendy@gmail.com (M.L. Larramendy).

http://dx.doi.org/10.1016/j.ecoenv.2017.10.009
Received 14 July 2017; Received in revised form 27 September 2017; Accepted 3 October 2017

recovery processes in aquatic organisms. In addition, the dynamics of pesticide-induced toxicity is an important step to improve the knowledge of the actual magnitude of risk posed by these compounds to aquatic biota (Guilherme et al., 2014; Mouchet et al., 2015). Summarizing, it has been recommended that to achieve a complete analysis of pesticide impact, the recovery mechanisms immediately following chemical exposure and in the period after exposure should be also considered (Bernabò et al., 2013).

Several authors have studied the recovery capabilities of aquatic biota after exposure to environmental stressors, including fish (Bearr et al., 2010; Corcoran et al., 2014; de Menezes et al., 2011; Dû-Lacoste et al., 2013; Ferrari et al., 2004; Guilherme et al., 2014; Guzmán-Guillén et al., 2014; Michel and Vincent-Hubert, 2015; Mohanty et al., 2011; Stankevičiūtė et al., 2016; Wessel et al., 2010) and amphibians (Bernabò et al., 2013; Bulaeva et al., 2015; Ferrari et al., 2004; García-Muñóz et al., 2009; Lajmanovich et al., 2009; Marques et al., 2014; Mouchet et al., 2015; Vogiatzis and Loumbourdis, 1997). In particular, studies on anurans tadpoles have focused on assessing recovery capabilities after exposure to environmental stressors at ecotoxicological, individual (e.g., behavior, growth, and morphological abnormalities) (Bernabò et al., 2013; Bulaeva et al., 2015; García-Muñóz et al., 2009), biochemical (e.g., enzymatic responses) (Ferrari et al., 2004; Lajmanovich et al., 2009; Vogiatzis and Loumbourdis, 1997), immune/histological (Bernabò et al., 2013), teratogenic (Svartz et al., 2012), and cytotoxic and genotoxic levels (Bulaeva et al., 2015; Mouchet et al., 2015). Nevertheless, information about these recovery capabilities on Neotropical species is scarce (Ferrari et al., 2004; Lajmanovich et al., 2009; Svartz et al., 2012), and there is no record about recovery capabilities after induction of genetic damage in Neotropical anurans reported so far.

Imidazolinones represent a group of agrochemicals widely used in more than 200 countries worldwide as selective pre- or postemergence herbicides that effectively controls a broad spectrum of weed species. This class of herbicides currently consists of six commercially available enantiomers and their methyl derivatives, namely, imazapic, imazapyr, imazethapyr (IMZT), imazamox, imazaquin, and imazamethabenz-methyl. Imidazolinone herbicides inhibit the action of the acetohydroxyacid synthase, also called acetolactate synthase (Lin et al., 2007). These agrochemicals are among the most popular herbicides because while they have proved to be potent and highly selective for plants, they are considered overall as nontoxic for vertebrates like mammals and fishes (PPDB, 2014).

IMZT [5-ethyl-2-(4-isopropyl-4-methyl-5-oxo-4,5-dihydroimidazol-1H-2-yl) nicotinic acid] is employed to control grasses and broad-leaved weeds in a variety of crop and noncrop areas (MacBean, 2012). The herbicide has a low sorption coefficient, though it possess high solubility in water (1400 mg/L), and thus has a high affinity with water (Johnson et al., 2000; Senseman, 2007). Furthermore, the California Department of Pesticide Regulation (www.pesticideinfo.org) has ranked this herbicide as a probable ground water contaminant. According to the U.S. Environmental Protection Agency (U.S. EPA), IMZT has been classify as a chemical with slight toxicity (Class III) (USEPA, 1989), whereas it has been considered as unlike hazardous chemical by the World Health Organization (www.pesticideinfo.org). The European Union has concluded that IMZT is an unsafe compound for the environment and it has been associated with human irritant effects on the eyes and skin, respiratory tract irritation (PPDB, 2014).

Overall, toxic, genotoxic, and cytotoxic studies of IMZT are limited and contradictory. Very little is known about IMZT-induced toxicity in nontarget organisms. Low levels of toxicity have been reported for the algae *Raphidocelis subcapitata* and aquatic invertebrates such as *Daphnia magna* after 72 and 48 h of exposure, respectively. Contrarily, high acute toxicity was observed in *Lemna gibba* (Magdaleno et al., 2015; PPDB, 2014; Reimche et al., 2015). Among terrestrial invertebrates, honey bees and earth worms have been reported to have particularly high sensitivity and low sensitivity to IMZT, respectively (PPDB, 2014). Toxicity exerted by IMZT has been found not to be acutely toxic for fish including *Ictalurus punctatus*, *Oncorhynchus mykiss* and *Lepomis macrochirus* with reported LC_{50} 96 h values of 240, 344, and 423 mg/L IMZT, respectively (Kegley et al., 2014; PPDB, 2014). Nevertheless, Moraes et al. (2011) demonstrated in hepatic tissues of *Cyprinus carpio* alterations in oxidative stress parameters after 0.0148 mg/L exposure to both the active ingredient IMZT and to the IMZT-based commercial herbicide formulation Only®. In addition, inhibition of acetylcholinesterase in the Mozambique tilapia *Oreochromis mossambicus* was reported after exposure to commercial formulations of IMZT (Pasha, 2013; Pasha and Singh, 2005). Finally, when IMZT was administered to Sprague Dawley rats by oral route or by dermal exposure to New Zealand White rabbits, low or moderate acute toxicity was reported after 96 h of treatment (USEPA, 2002a).

In analyses of cyto- and genotoxic effects, IMZT has generally been reported to be nonmutagenic in the *Salmonella T*yphimurium and *Escherichia coli* reversion assay both in the presence and absence of S9 metabolic fraction (Magdaleno et al., 2015). When the SMART in *Drosophila melanogaster* and the CHO/HGPRT assays were employed, negative results were observed, regardless of the presence of S9 mix (Fragiorge et al., 2008; USEPA, 2002a). Whereas chromosome alterations were not detected in bone marrow cells from IMZT-treated rats, both negative and positive results have been found to be induced in CHO cells with and without metabolic activation, respectively (USEPA, 1989). Growth inhibition was reported after exposure to a IMZT commercial formulation Verosil® in the green micro alga *Pseudokirchneriella subcapitata* (Magdaleno et al., 2015). IMZT altered nontarget communities of rotifers, cladocers, copepods and chironomids (Marchiori et al., 2012; Reimche et al., 2015). In addition, IMZT induced both cytotoxicity and chromosomal anomalies in meristematic root cells of *Triticum durum* and *Vicia faba* (El-Nahas, 2000; Rad et al., 2011). Cytotoxicity estimated by both root growth and mitotic index inhibition was reported in *Allium cepa* root tip cells after treatment with pure IMZT (Liman et al., 2015). Similarly, Magdaleno et al. (2015) observed that the IMZT-based formulation Verosil® induced cytotoxicity by arresting cells at prophase in meristematic tissues. Furthermore, toxicity of IMZT using the root elongation assay, chromosomal aberrations and DNA single-strand breaks evaluated by the single cell gel electrophoresis. This bioassay, also called the comet assay, is one of the most widely used methods to detect the genotoxic capability of xenobiotics both in vivo and in vitro since it is simple, fast, specific, and sensitive. The methodology in its alkaline or neutral version, detects a variety of DNA lesions at the single-cell level, including single-DNA strand breaks as well as alkali-labile lesions and double-strand breaks, respectively (Azqueta et al., 2014; Collins et al., 2014). Applying this methodology, it has been also demonstrated that IMZT induced DNA damage in *Lactuca sativa* after 120 h of exposure (Liman et al., 2015). Recently, an increased frequency of MNs was reported in *A. cepa* after exposure to an IMZT-based preparation (Magdaleno et al., 2015). Qian et al. (2015) demonstrated that phytotoxicity following IMZT-treatment was due to inhibition of the biosynthesis of branched chain amino acids (BCAAs) valine, leucine, and isoleucine affecting, then, the pattern of growth on roots, shoots, and leaves of *Arabidopsis thaliana.* Recently, we reported that an acute in vivo exposure to the herbicide jeopardized anuran Montevideo tree frog *Boana pulchella* tadpoles (Pérez-Iglesias et al., 2015). We found in the species an increase in the frequency of MNs, other nuclear abnormalities as well as the induction of primary DNA lesions after an acute IMZT-based herbicide commercial formulation Pivot® H exposure (Pérez-Iglesias et al., 2015). Recently, using the same biotic matrix, we were able to demonstrate that the herbicide induces DNA oxidative lesions at purine but pyrimidines bases which may be at least partially responsible for IMZT-induced genotoxicity (Pérez-Iglesias et al., 2017).

However, no approach to analyze whether *B. pulchella* tadpoles possess the capacity to recover the acute IMZT-inflicted morphological and cytogenetic damage has been included in none of our previous