Ecotoxicology (2018) 27:360–368
https://doi.org/10.1007/s10646-018-1900-1

CrossMark

Acute and chronic toxicity of pesticides on tadpoles of *Physalaemus cuvieri* (Anura, Leptodactylidae)

Juliana Wrubleswski[1] · Francisco Wilson Reichert Jr[2] · Leandro Galon[3] · Paulo Afonso Hartmann[3] · Marilia Teresinha Hartmann[3]

Accepted: 12 January 2018 / Published online: 3 February 2018

Abstract

Brazil is the largest consumer of pesticides in the world. However, knowledge on how these pesticides affect wildlife is scarce. Among the vertebrates, amphibians are particularly important in research to assess the impact of pesticides because of the correlation between pesticide and the decline of these species. This study aimed to evaluate the acute and chronic toxicity of commercial formulations of pesticides, i.e., atrazine (herbicide), cypermethrin (insecticide), and tebuconazole (fungicide) in *Physalaemus cuvieri* tadpoles. Eggs were collected in nature and cultivated under controlled conditions in the laboratory. Toxicity tests were carried out under standard conditions to determine the lethal concentration (LC_{50}) after 96 h of exposure and to determine the effect of sublethal concentrations after 7 days. In addition, we performed swimming activity tests on tadpoles exposed to sublethal concentrations. The lethal concentration (LC_{50}) was 19.69 mg/L for atrazine, 0.24 mg/L for cypermethrin and 0.98 mg/L for tebuconazole. In the acute test, atrazine showed lower toxicity than cypermethrin and tebuconazole for *P. cuvieri*. Swimming activity was affected at sublethal doses of atrazine and cypermethrin, but was not after exposure to tebuconazole. Cypermethrin was the insecticide that most altered the swimming activity of the individuals tested. The risk evaluation analysis indicated risks for tadpoles exposed to three tested pesticides, specially cypermethrin.

Keywords Ecotoxicity · Atrazine · Cypermethrin · Tebuconazole · Ecological risk evaluation

Introduction

Currently, the main method to control diseases, insects, and weeds that infest crops is the use of pesticides (Jardim and Caldas 2012). These products can be hazardous to the ecosystem and health, and persist in the environment or even bioaccumulate (EPA 2017). According to Carneiro et al. 2015, Brazil has become one of the largest consumers of agrochemicals in the world, a fact that has been related to the contamination of water (Albuquerque et al. 2016), soil and food. The contamination of the environment by pesticide derivatives is related, mainly, to mistaken or excessive application, allowing these chemicals to reach surface water or ground water (Hayes et al. 2003).

The pesticides used on crops for the control of diseases, weeds, and insects present active and inert ingredients in their formulations. In many cases, the inserts present in a formulation may be even more harmful than the active ingredients. However, both can cause damage to non-target organisms continuously exposed to the product (Candioti et al. 2010; Vieira et al. 2014). In this sense, several studies have been carried out using commercial formulations of agrochemicals to test their effects on wild animals, such as amphibians (e.g., Silva et al. 2013; Svartz and Pérez-Coll 2013), since producers apply commercial formulations and not the isolated active or inert components.

In Brazil, agrochemicals containing the active ingredients atrazine, cypermethrin and tebuconazole in their formulation are widely used for the control of crop pests (e.g. Pignati et al. 2014). According IBAMA (2014), atrazine is the third most commercialized herbicide in Brazil (the first is glyphosate). Cypermethrin is the most commonly used insecticide and tebuconazole is a commercially available fungicide (IBAMA 2010, 2014). Atrazine is

✉ Marilia Teresinha Hartmann
marilia.hartmann@gmail.com

[1] Prefeitura Municipal de Erechim, Erechim, RS, Brazil

[2] Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil

[3] Universidade Federal da Fronteira Sul, Campus Erechim, Brazil. ERS 135 - Km 72, nº 200, Erechim, RS, Brazil

Table 1 Pesticides used in the study

Active Ingredient (a.i)	Half-life (days)	Application rate (g-a.i/ha)	Octanol-water partition coefficient (log Kow)	Adsorption Coefficient (Koc in g/ml)	Vapor pressure (mmHg)
Atrazine	60–100	1,500–8,000	2.61	100	2.89×10^{-7} (25 °C)
Cypermethrin	8–30	10.0–62.5	6.60	160,000	1.7×10^{-9} (20 °C)
Tebuconazole	>365	0.60–1.0	3.70	803–1251	1.3×10^{-8} (20 °C)

classified as Class III—moderately toxic, cypermethrin as Class II—highly toxic and Tebuconazole as Class IV—slightly toxic. However, regarding environmental classifications, atrazine, cypermethrin, and tebuconazole are classified as Class II, i.e., they are very dangerous to the environment (IBAMA 2010).

The herbicide atrazine is a photosystem II inhibitor and belongs to the group of triazines (Silva et al. 2007). It has been banned in the European Union since 2004 because it is considered toxic (Sass and Colangelo 2006). In Brazil, it is used for the control of weeds of the main crops of agronomic interest (Silva et al. 2007). Herbicides of the triazine group have a high environmental contamination potential due to their high leaching and runoff capacity, prolonged soil persistence, slow hydrolysis, low-water solubility and moderate organic matter absorption (Grizolia 2005). The literature estimates the half-life of atrazine at between 16 and 100 days, with an average of 60 days. In rivers, the half-life is 1.5–13.3 days (Chung and Gu 2003) and in lakes the half-life can be between 124 and 365 days (Schottler and Eisenreich 1997).

Cypermethrin is a synthetic pyrethroid insecticide with a wide spectrum delivered via foliar application (Andrei 2017). It is highly toxic to aquatic animals, as it causes the disruption of endocrine system in fish (Singh and Singh 2008). Cypermethrin is categorized as restricted use by the United States Environmental Protection Agency (USEPA) due to its high toxicity to fish (Saha and Kaviraj 2009).

The fungicide tebuconazole belongs to the chemical group of triazoles and is used in agriculture to control fungi (Andrei 2017). Tebuconazole formulated fungicides are persistent in the environment (Herrero-Hernández et al. 2011), and may cause adverse effects on aquatic organisms (Sancho et al. 2016). Tebuconazole's adsorption coefficient is 2500 mL/g, the half-life is 403 days in the soil, and it has the potential to be transported in water associated with sediment because of its high affinity with organic matter. Thus, this fungicide presents high mobility in the environment (Ferracini et al. 2001).

The presence of agrochemicals in the ecosystem has been related to the decline of amphibian populations (Bishop et al. 1999; Allran and Karasov 2000; Boone et al. 2001; Sparling and Fellers 2009; Sparling et al. 2010). Amphibians are highly sensitive to different chemicals found in the aquatic ecosystem (Silvano and Segalla 2005). There have been several studies on atrazine (e.g., Ezemonye and Tongo 2009; Hayes et al. 2002; Brodeur et al. 2009; Svartz et al. 2012) and cypermethrin (e.g., Izaguirre et al. 2000; 2006a, b; Svartz and Pérez-Coll 2013; Svartz et al. 2016; Macagnan et al. 2017), but few on tebuconazole (e.g., Bernabò et al. 2016) and their effects on amphibians.

Physalaemus cuvieri is a very common species found throughout Brazil, and widely distributed in South America (Frost 2017). It is a nocturnal species of amphibian, and occurs in many habitats, including open grassland, flooded savannahs, and pastureland (Abraham et al. 2010). It is locally threatened in Argentina by the destruction of habitat for agriculture and wood extraction, land, and water pollution caused by agrochemical runoff (Abraham et al. 2010).

The objective of this study was to evaluate the acute and chronic toxicity of the pesticides atrazine, cypermethrin, and tebuconazole in *P.cuvieri*, a non-target organism to pesticides, which breeds in agricultural ecosystems in South America. Because, it is widely distributed and can be found in lands being used for agriculture, *P. cuvieri* is a good choice for evaluating the effects these pesticides have on non-target organisms—particularly amphibians.

Materials and methods

Pesticides

The pesticides studied (Table 1) were atrazine (commercial herbicide product Atrazine 500 SC), cypermethrin (commercial insecticide product Cypermethrin 250 EC) and tebuconazole (commercial fungicidal product 200 EC). All of these agrochemicals are registered with the Ministry of Agriculture and Livestock (Brasil 2017) to be used in the management of weeds, insects, and diseases, respectively.

To generate pesticide solutions, we dissolved commercial grade solution of pesticides in distilled water to create a stock solution of 500 mg/L as needed. The stock solution was added with a micropipette at the beginning of the experiment to each of the glass containers to obtain the desired concentrations for each pesticide. The pesticides were added in begin the test.

For acute exposure, concentrations of 0, 3, 12, 24, 35, 45, and 55 mg/L of atrazine, 0, 0.14, 0.4, 1.5, and 6 mg/L of cypermethrin, and 0, 0.4, 0.7, 1, 2, 3, 4, and 8 mg/L of tebuconazole were used in the tests. These concentrations were based on bibliographical references, taking into account the recommendations of the package insert for field applications. For cypermethrin, concentrations were used close to the LC_{50} recorded for *P. biligonigerus* (Izaguirre et al. 2006a). For tebuconazole, no studies were found in the literature with species similar to *P. cuvieri*. In this way, several concentrations were tested to find the LC_{50} based on the work of Toni et al. 2011. For atrazine, the reference of Moutinho (2013) was used for *P. cuvieri*.

For chronic exposure, the tested concentrations of atrazine were 0, 2.4, 0.48, and 0.24 mg/L, for cypermethrin 0, 0.14, 0.028, and 0.014 mg/L, and for tebuconazole 0, 0.11, 0.02, and 0.01 mg/L.

As indicated in the leaflet for each pesticide, the tested formulations were (a) atrazine 3–8 L/ha as the commercial product Atrazina 500 SC, according to the soil characteristics (for example, if the soil is sandy, the dose is lower; if it is clayey and rich in organic matter, the dose is higher) or depending on the crop to be applied and the weeds present in the crop; (b) cypermethrin, as the commercial product Cypermethrin 250 EC is indicated to be used from 40 to 600 μL/ha, depending on the crop and insect to be monitored, and (c) tebuconazole 200 EC, is applied from 300 mL/ha to 1 L/ha, depending on the disease and the crop.

Test species

The selected species was *P. cuvieri*, a species of frog in the Leptodactylidae family, popularly known as the dog frog, with a wide geographical distribution in Brazil, Argentina and Paraguay (Frost 2017).

Collection and maintenance of eggs and larvae

Eggs of *P.cuvieri*, with a maximum of 24 h of oviposition (until stage 14, according to Gosner's classification (1960)) were collected in a pond in the municipality of Erechim/RS (27°30′50″S; 52°16′21″ W), from November 2014 to February 2015.

The eggs were transported to the Ecology and Conservation Laboratory of the Federal University of Fronteira Sul–Erechim Campus, and kept separately in aquariums of ~50 L with artesian well water and acclimatized at 25 °C until the hatching.

The larvae were fed every 24 h with complete feed in flakes for fish (Alcon Basic) *ad libitum* every other day. The acute test was performed with the animals fasted and not fed during the test. In the chronic test, feeding was maintained daily after the start of the experiment, with three flakes of balanced fish food per glass container.

The tadpoles that were not used in the experiments were released at the same site where eggs were collected.

Experimental design

Individuals from six breedings of *P. cuvieri* were used. Individuals were selected with a body mass between 0.0803 and 0.1186 g, and between stages 24 and 27 of development, according to Gosner's classification (1960) in order to reduce the variation of the measured parameters. Only organisms considered healthy, with normal swimming movements, and with the ability to move through the aquarium without any deformity or apparent injury were used in the experiments.

Artesian well water was used for the tests and for the development of larvae. Water quality parameters were recorded for all aquaria and before the test solutions were applied. The well water used in the aquaria, controls and treatments had the following characteristics: 23 °C (±1 °C), pH 7.0 (±0.5), dissolved oxygen 5.0 (±1.0 mg/L), turbidity <5, conductivity 160 (±10) μS/cm, alkalinity 9.74 mg $CaCO_3$/L, Ca 6.76 mg/L, Na 44.1 mg/L, Mg 1.35 mg/L, Fe 0.08 mg/L, Ni <0.001 mg/L.

Acute exposure

The laboratory ecotoxicological tests of acute exposure lasted for 96 h. All tests were performed in triplicate, including the control test. The temperature and oxygen of the water were evaluated every 24 h. Dead individuals were counted and withdrawn every 12 h until the end of the tests.

First, the tadpoles were arranged randomly in glass containers, in groups of 10, to be assigned later in the tests. For the classification of compounds according to the acute toxicity (LC_{50}) for aquatic organisms, the present work used the classification of the Globally Harmonized System of Classification and Labeling of Chemicals (GHS Criteria, GHS 2011). Therefore, the compounds can be classified into three categories after exposure for 96 h: high toxicity ($LC_{50} < 1$ mg/L), moderate toxicity (LC_{50} between 1 and 10 mg/L) and low toxicity ($LC_{50} > 10$ mg/L).

The containers used in the tests had a capacity of 10 L, containing one tadpole per liter of water. The size of the vessel simulated a water pool ~10 cm deep, similar to the environment where the eggs were collected.

Chronic exposure

The static test, with daily exposure, was carried out for 7 days using larvae raised in the same manner as in the previous experiment. The laboratory conditions and the water evaluations were the same as described for the acute test. Every 24 h, the mortality of the individuals was assessed, as well as the effect of the chronic concentration on the swimming activity of surviving tadpoles.

To test the swimming activity of the tadpoles during the chronic test, every 24 h, movements were made in the water of the aquarium with the aid of a glass rod, three times in a circle. This movement stimulated the movement of tadpoles in the aquarium without causing stress. As a way of comparison, the standard of swimming activity established was: (3) swimming movements equal to control, that is, without changes in tadpole swimming, when compared to the control group, (2) swimming movements different from control, i.e., in circles or half the speed of the control group, (1) swimming movements only after a touch stimulus to some part of the body and (0) static, but alive. In the latter case, the tadpoles did not present any movement with the stimulus or touch of the rod. However, when they were removed from the aquarium with a sieve, if they moved they were then returned to the aquarium and classified as zero (0).

Data analysis

Median lethal concentration (LC_{50}) values and their respective 95% confidence intervals were statistically estimated by Trimmed Spearman-Karber method. We used a one-way analysis of variance (ANOVA) to evaluated acute and chronic effects, and Tukey o Dunnet post hoc test when $p < 0.05$. For chronic test, we calculated the no observable-effect concentration (NOEC) and the lowest observable-effect concentration (LOEC) by analysis of variance with mean comparison made by Dunnett's test, when $p < 0.05$. The maximum acceptable toxicant concentration (MATC) was calculated from NOEC and LOEC and expressed mathematically as the geometric mean of the NOEC and LOEC. This analysis was performed with the use of the Statistica software 8.0.

Ecological risk evaluation

The ecological risk was assessed using the hazard quotient (HQ) approach, which was calculated as EEC/LC_{50} of lethality for acute hazard quotient (AHQ) and EEC/NOEC for chronic risk (CHQ). The EEC is an estimated (or maximum) environmental contaminant concentration next to geographic range of the specie. The maximum level of atrazine reported in Brazil in literature was 0.075 mg/L (Moreira et al. 2012), the maximum level cypermethrin reported in Argentina in literature was 0.194 mg/L (Marino and Ronco 2005) and maximum level of tebuconazole was 0.026 mg/L (Toni et al. 2011). After the HQ was calculated, it was compared to the USEPA (United States Environmental Protection Agency) level of concern (LOC), where the risk presumption for aquatic animals is LOC = 0.5 (acute high risk) and LOC = 1 (chronic risk). If the HQ >1, harmful effects are likely due to the contaminant in question.

For comparison, we also use toxicity exposure ratio (TER; Damalas and Eleftherohorinos 2011). TER is the inverse of HQ and that is calculated by dividing the indicated toxic dose with the predicted environmental concentration or EEC (LC_{50}/EEC and NOEC/EEC). When TER is below 100 for acute risk, is needed a detailed higher tier risk assessment, whereas TER <10 for chronic risk, assessment is required.

Results

The commercial formulations of cypermethrin and tebuconazole were more toxic than atrazine regarding the mortality of *P. cuvieri* tadpoles. The LC_{50} of the acute test was 0.24 mg/L for cypermethrin, 0.98 mg/L for tebuconazole and 19.69 mg/L for atrazine (Table 2).

The chronic test showed gradual mortality during 7 days of exposure to the three pesticides evaluated. At the end of the test, survival was from 63 to 100% for atrazine and tebuconazole and between 23 and 53% for cypermethrin. In the chronic test, mortality started at 0.11 mg/L of the LC_{50} of tebuconazole in the first 24 h. For cypermethrin and atrazine, mortality started within 48 h of testing. Cypermethrin had the highest number of dead tadpoles at the end of the test (Table 3).

The different concentrations of atrazine ($F_{2,18} = 0.16$, $p = 0.84$), cypermethrin ($F_{2,18} = 0.76$, $p = 0.48$) and tebuconazole ($F_{2,18} = 1.90$, $p = 0.17$) did not significant for the mortality of the individuals analyzed. In other words, mortality occur in all concentrations of the three pesticides.

The swimming activity of tadpoles was affected by chronic (sublethal) doses of atrazine ($F_{3,80} = 4.71$, $p = 0.004$, significant for 0.24 and 0.48 mg/L, Dunnet test, $p < 0.05$) and cypermethrin ($F_{3,83} = 50.81$, $p = 0.00$, significant for all concentrations, Dunnet test, $p < 0.05$), but not of tebuconazole ($F_{3,80} = 1$, $p = 0{,}41$). For atrazine, the NOEC was swimming activity was 0.24 mg/L and LOEC was 0.48 mg/L, and MATC was 0.36 mg/L. For cypermetrhrin, the NOEC was 0.014 mg/L and LOEC was 0.028 mg/L, and MATC was 0.021 mg/L.

In the presence of atrazine in the water, the tadpoles swam in pattern 3, equal to the control from the first to the fourth day. On the fifth, sixth, and seventh days of the test, swimming activity was standard 2 (different from the control) at the three concentrations of atrazine tested. With exposure to cypermethrin, tadpoles demonstrated swimming activity 2, different and smaller than the control since

Table 2 Mortality of *Physalaemus cuvieri* exposed to acute concentrations of atrazine, cypermethrin, and tebuconazole

Agrochemical	Concentration mg/L	Time/mortality (h)						LC_{50} (mg/L)
		24	48	72	96	Total	Percent mortality	
Atrazine	0	0	0	0	0	0	0	
	3	0	0	0	0	0	0	
	12	0	0	1	5	6	20	19.69
	24	0	3	2	5	10	33.3	−95% LC: 16.49
	35	13	8	7	2	30	100	+95% LC: 23.52
	45	17	6	5	2	30	100	
	55	17	9	3	1	30	100	
Cypermethrin	0	0	0	0	0	0	0	
	0.14	0	3	4	5	12	40	0.24
	0.4	0	6	3	9	18	60	−95% LC: 0.12
	1.5	0	4	7	12	23	76.6	+95% LC: 0.46
	6	0	9	4	13	26	86.6	
Tebuconazole	0	0	0	0	0	0	0	
	0.4	0	0	0	0	0	0	
	0.7	0	0	0	0	0	0	0.98
	1	1	5	10	5	21	70	−95% LC: 0.90
	2	6	10	14	0	30	100	+95% LC: 1.07
	3	10	9	11	0	30	100	
	4	7	4	19	0	30	100	
	8	30	0	0	0	30	100	

UFFS, Erechim Campus (No of exposed = 30 for each concentration)

Table 3 Mortality of *Physalaemus cuvieri* tadpoles exposed to sublethal doses of atrazine, cypermethrin, and tebuconazole at different concentrations and exposure periods up to 7 days

Agrochemical	Concentration mg/L	Time/mortality (h)								Percent mortality
		24	48	72	96	120	144	168	Total	
Atrazine	0	0	0	0	0	0	0	0	0	0
	0.24	0	0	4	0	0	5	0	9	30
	0.48	0	2	1	0	1	1	2	7	23.3
	2.4	0	0	2	0	0	5	4	11	36.6
Cypermethrin	0	0	0	0	0	0	0	0	0	0
	0.014	0	1	2	1	1	5	4	14	46.6
	0.028	0	2	1	5	8	4	3	23	76.6
	0.14	0	3	4	5	2	3	5	22	73.3
Tebuconazole	0	0	0	0	0	0	0	0	0	0
	0.01	0	0	0	0	0	0	0	0	0
	0.02	0	0	1	0	0	0	1	2	6.6
	0.11	1	0	1	0	0	1	0	3	10

UFFS, Erechim Campus. (No of exposed = 30 for each concentration)

the first day, at the three tested concentrations. On the third day of testing, it was observed that, at the concentration 0.14 mg/L, some tadpoles were upside down before stirring the aquarium. On the fourth day, at the highest dose (0.14 mg/L), the tadpoles displayed standard 1, while at doses of 0.028 and 0.014 mg/L remained at standard 2. From the fifth to the seventh day, standard 1 was seen with 0.14 mg/L, 1 with 0.028 mg/L, and 2 at 0.014 mg/L. The tadpoles that remained alive until the end of the test responded with slow movements after a single stimulus.

In contrast to the other pesticides tested, when the sublethal doses of tebuconazole were evaluated, swimming activity remained the same as the control from the first to the seventh day. The control tadpoles did not change throughout the test period for the three pesticides.

Results for acute risk quotient (AHQ) was 0.003 for tadpoles for *P. cuvieri* exposed to atrazine, 0.80 for tadpoles exposed to cypermethrin and 0.02 for tadpoles exposed to tebuconazole. Only the AHQ of cypermethrin is higher than the level of concern (LOC = 0.5). For chronic risk, CHQ was 0.31 for swimming activity for *P. cuvieri* exposed to atrazine and 13.85 for cypermethrin.

For TER, results for acute risk was 262.5 for tadpoles exposed to atrazine, 1.23 for cypermethrin and 37.69 for tebuconazole. For swimming activity, TER was 3.2 for atrazine and 0.072 for cypermethrin.

Discussion

This study demonstrated differences in the toxicity of three pesticides evaluated in relation to acute toxicity, chronic toxicity and swimming activity in *P. cuvieri*. The LC_{50} 96 h of cypermethrin and tebuconazole showed higher toxicity than atrazine, according to the GHS categories (2011). Cypermethrin and tebuconazole showed high toxicity, whereas atrazine showed low toxicity.

Atrazine is being investigated as a potential endocrine disruptor in amphibians, and there is evidence that atrazine may adversely affect the reproductive capacity of anurans (Hayes et al. 2002; Tavera-Mendoza et al. 2002a, b) and, as a consequence, potentially contribute to population decline. In recent research on atrazine, using the concentrations of 1.5, 3.0, 6.0, 12.5, 19.0, and 25.0 mg/L for *Rhinella schneideri* and *Physalaemus nattereri* at 96 h of exposure, the LC_{50} was 22.18 and 33.71 mg/L, respectively (Pérez Iglesias 2015). The result for atrazine tested on *P. cuvieri* in the present study (19.69 mg/L) is similar to the values found for other amphibians (e.g., Brodeur et al. 2009; Ezemonye and Tongo 2009).

Amphibians show differences in sensitivity to cypermethrin (Biga and Blaustein 2013), which should also occur for other pesticides. In comparison with other species, *P. cuvieri* is more tolerant to cypermethrin (0.24 mg/L, this study). In *Physalaemus biligonigerus*, the LC_{50} was 0.12 mg/L (Izaguirre et al. 2000) and 0.11 mg/L in *Rhinela arenarum* (Izaguirre et al. 2006a). During acute exposure at concentrations of 10 µg/L of cypermethrin (0.01 mg/L), *Rana arvalis* presented 100% mortality, again demonstrating the high sensitivity of amphibians to this insecticide (Greulich and Pflugmacher 2003).

The fungicide tebuconazole has the ability to accumulate in amphibian tissues (Smalling et al. 2015). In fish, several papers detail the lethal concentration of tebuconazole, considered the closest group for comparison. For *Cyprinus carpio*, the LC_{50} was 2.37 mg/L (Toni et al. 2011), for *Rhamdia quelen* it was 5.3 mg/L (Kreutz et al. 2008) and for *Danio rerio* it was 19.6 mg/L (Sancho et al. 2010) of tebuconazole. These results show that tebuconazole is more toxic to *P. cuvieri* (0.98 mg/L) than to fish species tested in other studies.

In the chronic analysis, cypermethrin can be considered to be the most toxic, as it presented the highest mortality and caused a significant change in swimming activity. It has already been shown that this insecticide can causes high-death rates (Izaguirre et al. 2006a, b) and massive apoptosis in the central nervous system of anuran larvae (Izaguirre et al. 2000, 2001, 2006b).

Atrazine affected swimming activity, but not mortality, and tebuconazole did not affect any of these parameters. Thus, with chronic exposure, tebuconazole was the least toxic. These two agrochemicals are frequently detected in water (Gerónimo et al. 2014; Albuquerque et al. 2016), but at low concentrations. For atrazine, the maximum level reported in Brazil was 0.075 mg/L (Moreira et al. 2012) and for tebuconazole this was 0.026 mg/L (e.g., Toni et al. 2011). However, these concentrations do not refer to amphibian habitats and shows the need for further studies on the sub-lethal effects of concentrations found at development sites.

In the natural environment, the reduced mobility of tadpoles means less escape capacity, greater susceptibility to predation and reduced survival (Azevedo-Ramos et al. 1992). In addition, the physical structure is reduced due to a lack of nutrition, which can lead to death. Thus, changes in swimming due to the sublethal effects of agrochemicals can cause interruptions in the interactions between amphibian species and in the functioning of communities, because they affect trophic chains due to the absence of predators and prey (Howe et al. 1998; Peltzer et al. 2013). Thus, atrazine and cypermethrin are potential causes of reduced swimming activity in *P. cuvieri*, as has already been reported for *R. schneiderie*, *P. nattereri* (Pérez Iglesias 2015) and *P. gracilis* (Macagnan et al. 2017).

The calculated risk evaluation analysis indicated risks for acute and chronic toxicity (HQ) for tadpoles exposed to cypermethrin, and low risk for exposed to atrazine and tebuconazole in its natural habitat. But, analyzing the toxicity exposure ratio (TER), the situation is more complicated. Cypermethrin and Tebuconazole have risks for acute toxicity, and cypermethrin and atrazine for chronic toxicity, considered swimming activity. That is, cypermethrin appeared in all risk analyzes. The sublethal concentrations of cypermethrin tested in this study (0.014–0.14 mg/L) and the maximum acceptable toxicant concentration (0.021 mg/L) are close to those found in the environment (0.194 mg/L reported in Argentina; Marino and Ronco 2005), which leads to a real concern regarding the survival of *P. cuvieri* populations.

The cypermethrin is the one with the lower half-life among the tested pesticides. It is not only more toxic, but also in exerts its toxicity in a shorter period after application. This insecticide has several uses in agriculture, veterinary medicine and in public health (insects control) (Montanha and Pimpão 2012), because this, is applied all year round. It has been detected in aquatic environment at concentrations sufficient to cause toxicity to anuran larvae. Atrazine and tebuconazole can be less toxic, but can exert their effects all over the year after the application.

The tested agrochemicals have possibility of coming into contact with non-target species, in this case amphibians in the natural environment. *Physalaemus cuvieri* is a common and widely distributed pond-breeding frog that reproduces in altered environments and agroecosystems (Frost 2017; Silva et al. 2009). Because of this, it comes into constant contact with pesticides. In Brazil, the legislated maximum permitted level of atrazine in any water is 0.002 mg/L (Brasil 2005), and 0.18 mg/L for tebuconazole in water for human consumption (Brasil 2011); there is no set limit for cypermethrin. Only in the state of Rio Grande do Sul is there a limit for cypermethrin in water for human consumption, which is 0.3 mg/L (Brasil 2014). The chronic concentrations tested in this study are close to the maximum permitted levels for tebuconazole and cypermethrin.

Conclusions

In this study, in addition to providing acute toxicity data for three pesticides, it was possible to observe chronic effects in terms of swimming activity in *P. cuvieri*, related to exposure to atrazine and cypermethrin. Atrazine was weakly toxic and had an effect on the swimming activity of *P. cuvieri*. The use of this herbicide was banned in the European Union in 2004 (Bethsass and Colangelo 2006), but it is used in Brazil and other countries. The presence of atrazine may not affect the immediate survival of tadpoles, but may decrease fitness and reduce the ability to feed and escape from predators, causing populations to decrease over time. The risk evaluation analysis indicated risks for tadpoles exposed to three tested pesticides, specially cypermethrin. Cypermethrin was the most toxic pesticide tested, with high mortality at the environmental contaminant concentrations allowed by legislation in water for human consumption and found in rivers. This means that the environmental contaminant concentration of cypermethrin can has effects in a non-target vertebrate.

Of the three pesticides studied, we call special attention to the insecticide cypermethrin and the possibility of environmental contamination causing localized declines in amphibian populations.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was licensed by IBAMA (45786-1) and authorized by the Ethics Committee for Animal Use of the Federal University of Fronteira Sul.

References

Abraham M, Rodrigues MT, Baldo D (2010) *Physalaemus cuvieri*. The IUCN Red List of Threatened Species 2010: https://doi.org/10.2305/IUCN.UK.2010-2.RLTS.T57250A11609155.en. Accessed 22 Jun 2017

Albuquerque AF, Ribeiro JS, Kummrow F, Nogueira AJA, Montagner CC, Umbuzeiro GA (2016) Pesticides in Brazilian freshwaters: a critical review. Environ Sci Process Impacts 18:779–787

Allran JW, Karasov WH (2000) Effects of atrazine and nitrate on northern Leopard frog (*Rana pipiens*) larvae exposed in the laboratory from post hatch through metamorphosis. Environ Toxicol Chem 19:2850–2855

Andrei E (2017) Compêndio de defensivos agrícolas: guia prático de produtos fitossanitários para uso agrícola. Andrei, São Paulo

Azevedo-Ramos C, Sluys MV, Hero IM, Magnusson W (1992) Influence of tadpole movement on predation by odonate naiads. J Herpetol 26:335–338

Bernabò I, Guardia A, Macyrella R, Sesti S, Crescente A, Brunelli E (2016) Effects of long-term exposure to two fungicides, pyrimethanil and tebuconazole, on survival and life history traits of Italian tree frog (Hyla intermedia). Aquat Toxicol 172:56–66

Bethsass J, Colangelo A (2006) European union bans atrazine, while the United States negotiates continued use. Int J Occup Environ Health 12:260–267

Biga LM, Blaustein AR (2013) Variations in lethal and sublethal effects of cypermethrin among aquatic stages and species of anuran amphibians. Environ Toxicol Chem 32:2855–2860

Bishop CA, Mahony NA, Struger J, NG P, Pettit KE (1999) Anuran development, density and diversity in relation of agricultural activity in the Holland river watershed, Ontario, Canada (1990–1992). Environ Monit Assess 57:21–43

Boone MD, Bridges CM, Rothermel BB (2001) Growth and development larval green frogs (*Rana clamitans*) exposed to multiple doses of an insecticide. Oecologia 129:518–524

BRASIL (2005) Resolução 375 do Conselho Nacional do Meio Ambiente, CONAMA. Brasília. http://www.mma.gov.br/port/conama/res/res05/res35705.pdf. Accessed 01 Aug 2017

BRASIL (2011) Portaria No. 2914 do Ministério da Saúde. Brasília. http://portalarquivos.saude.gov.br/images/pdf/2015/maio/25/Portaria-MS-no-2.914-12-12-2011.pdf. Accessed 01 Aug 2017

BRASIL (2014) Portaria Nº 320 da Secretária de Estado da Saúde do Rio Grande do Sul. Porto Alegre. https://www.legisweb.com.br/legislacao/?id=269539. Accessed 01 Aug 2017

BRASIL (2017) Ministério da Agricultura e Pecuária (Ministry of Agriculture and Livestock). AGROFIT. http://www.agricultura.gov.br. Accessed 01 Aug 2017

Brodeur JC, Svartz G, Perez-Coll CS, Marino DJG, Herkovits J (2009) Comparative susceptibility to atrazine of three developmental stages of Rhinella arenarum and influence on metamorphosis: non-monotonous acceleration of the time to climax and delayed tail resorption. Aquat Toxicol 91:161–170

Candioti JV, Natale GS, Soloneskia S, Ronco AE, Larramendy ML (2010) Sublethal and lethal effects on *Rhinella arenarum* (Anura, Bufonidae) tadpoles exerted by the pirimicarb-containing technical formulation insecticide Aficida®. Chemosphere 78:249–255

Carneiro FF, Rigotto RM, Augusto LGS, Friedrich C, Búrigo AC (2015) Dossiê ABRASCO : um alerta sobre os impactos dos agrotóxicos na saúde. Expressão Popular, EPSJV, Rio de Janeiro, São Paulo, http://www.abrasco.org.br/dossieagrotoxicos/wp-content/uploads/2013/10/DossieAbrasco_2015_web.pdf Accessed 01 Aug 2017

Chung SW, Gu RR (2003) Estimating time-variable transformation rate of atrazine in a reservoir. Adv Environ Res 7:933–947

Damalas CA, Eleftherohorinos IG (2011) Pesticide exposure, safety issues, and risk assessment indicators. Int J Environ Res Public Health 8:1402–1419

EPA. United States Environmental Protection Agency. Available at http://www.epa.gov/pesticides. Accessed 01 Aug 2017

Ezemonye L, Tongo I (2009) Lethal and sublethal effects of atrazine to amphibian larvae. Jordan J Biol Sci 2:29–36

Ferracini VI, Pessoa MCYP, Silva AD, Spadotto CA (2001) Risk analysis of contamination of ground water and surface waters of Petrolina (PE) and Juazeiro (BA). R Ecotoxicol 11:1–16

Frost DR (2017) Amphibian Species of the World: Online Reference. Version 6.0 http://research.amnh.org/herpetology/amphibia/index.html. Accessed 01 Aug 2017

Gerónimo E, Aparicio VC, Bárbaro S, Portocarrero R, Sebastián J, Costa JL (2014) Presence of pesticides in surface water from four sub-basins in Argentina. Chemosphere 107:423–431

GHS (2011) Globally Harmonized System of Classification and Labelling of Chemicals. United Nations, New York and Geneva

Gosner K (1960) A simplified table for staging Anuran Embryos and larvae with notes on identification. Herpetologica 16:183–189

Greulich K, Pflugmacher S (2003) Differences in susceptibility of various life stages of amphibians to pesticide exposure. Aquat Toxicol 65:329–336

Grizolia CK (2005) Agrochemical mutations, cancer and reproduction. UNB, Brasília

Hayes TB, Collins A, Lee M, Mendonza M, Noriega N, Stuart AA, Vonk A (2002) Hermaphroditic, demasculinized frogs after exposure to the herbicide atrazine at low ecologically relevant doses. Proc Natl Acad Sci USA 99:5476–5480.

Hayes T, Haston K, Tsui M, Hoang A, Haeffele C, Vonk A (2003) Atrazine-induced hermaphroditism at 0.1 ppb in American leopard frogs (*Rana pipiens*): laboratory and field evidence. Environ Health Perspect 111:568–575

Herrero-Hernández E, Andrades MS, Marín-Benito JM, Sánchez-Martín MJ, Rodríguez-Cruz MS (2011) Field-scale dissipation of tebuconazole in a vineyard soil amended with spent mushroom substrate and its potential environmental impact. Ecotoxicol Environ Safe 6:1480–1488.

Howe GE, Gillis R, MowbraY RC (1998) Effect of chemical synergy and larval stage on the toxicity of atrazina and alachlor to amphibian larvae. Environ Toxicol Chem 17:519–525

IBAMA (2010) Brazilian Institute of Environment and Renewable Natural Resources. Pesticides and related products marketed in 2009 in Brazil: an environmental approach. www.ibama.gov.br/areas-tematicas-qa/relatorios-de-comercialzaçao-de-agrotoxicos/pagina2. Accessed 01 Aug 2017

IBAMA (2014) Brazilian Institute of Environment and Renewable Natural Resources Pesticides and related products marketed in 2014 in Brazil. http://www.ibama.gov.br/agrotoxicos/relatorios-de-comercializacao-de-agrotoxicos. Accessed 01 Aug 2017

Izaguirre MF, Lajmanovich RC, Peltzer PM, Soler AP, Casco VH (2000) Cypermethrin-induced apoptosis in thetelencephalon of *Physalaemus biligonigerus* tadpoles (Anura: Leptodactylidae). Bull Environ Contam Toxicol 65:501–507

Izaguirre MF, Lajmanovich RC, Peltzer PM, Peralta Soler A, Casco VH (2001) Induction of cell death by the synthetic pyrethroid insecticide cypermethrin in the developing brain of Physalaemus biligonigerus tadpoles from Argentina. Froglog 43:3–4

Izaguirre MF, Marín L, Vergara MN, Lajmanovich RC, Peltzer PM, Casco VH (2006a) Experimental models of anurans to study pyrethroid effects. Cienc, Docencia Tecnol 32:181–206

Izaguirre MF, Vergara MN, Casco VH (2006b) CAS role in the brain apoptosis of *Bufo arenarum* induced by Cypermethrin. Biocell 30:309–320

Jardim ANO, Caldas ED (2012) Brazilian monitoring programs for pesticide residues in food e results from 2001 to 2010. Food Control 25:607

Kreutz LC, Barcellos LJG, Silva TO, Anziliero D, Martins D, Lorenson M, Marteninghe A, Silva LB (2008) Acute toxicity test of agricultural pesticides on silver catfish (*Rhamdiaquelen*) fingerlings. Ciênc Rural 38:1050–1055

Macagnan N, Rutkoski CF, Kolcenti C, Vanzetto VV, Macagnan L, Sturza P, Hartmann PA, Hartmann MT (2017) Toxicity of cypermethrin and deltamethrin insecticides on embryos and larvae of *Physalaemus gracilis* (Anura:Leptodactylidae). Environ Sci Pollut Res https://doi.org/10.1007/s11356-017-9727-5

Marino D, Ronco EA (2005) Cypermethrin and chlorpyrifos concentration levels in surface water bodies of the Pampa Ondulada, Argentina. Bull Environ Contam Toxicol 75(4):820–826

Montanha FP, Pimpão CT (2012) Toxicological effects of pyrethroids (cypermethrin and deltamethrin) on fish—Review. Rev Cient Elet Med Vet 18:1–58

Moreira JC, Peres F, Simões AC, Pignati WA, Dores EC, Vieira SN, Strüssmann C, Mott T (2012) Contaminação de águas superficiais e de chuva por agrotóxicos em uma região do estado do Mato Grosso Ciência e Saúde Coletiva 17:1557–1568

Moutinho MF (2013) Comparative ecotoxicity of sugar cane herbicides for amphibian larvae. Dissertation, Universidade de São Paulo

Peltzer PM, Junges CM, Attademo AM, Bassó A, Grenón P, Lajmanovich RC (2013) Cholinesterase activities and behavioral changes in *Hypsiboas pulchellus* (Anura: Hylidae) tadpoles exposed to glufosinate ammonium herbicide. Ecotoxicology 22:1165–1173

Pérez Iglesias JM (2015) Exposure and effect biomarkers to assess the effects of atrazine at different levels on neotropical anthill tadpoles. Dissertation, Universidade Estadual Paulista Julio de Mesquita Filho

Pignati W, Pereira N, Cândido A (2014) Vigilância aos agrotóxicos: quantificação do uso e previsão de impactos na saúde-trabalho-ambiente para os municípios brasileiros. Ciência Saúde Coletiva 19:4669–4678

Saha S, Kaviraj A (2009) Effects cypermethrin on some biochemical parameters and its amelioration through dietary supplementation os ascorbic acid in freshwater catfish *Heteropneustes fossilis*. Chemosphere 74:1254–1259

Sancho E, Villarroel MJ, Fernández C, Andreu E, Ferrando MD (2010) Short-term exposure to sublethal tebuconazole induces physiological impairment in male zebrafish (*Danio rerio*). Ecotoxicol Environ Saf 73:370–376

Sancho E, Villarroel MJ, Ferrando MD (2016) Assessment of chronic effects of tebuconazole on survival, reproduction and growth of Daphnia magna after different exposure times. Ecotoxicol Environ Saf 124:10–17.

Sass JB, Colangelo A (2006) European Union bans Atrazine, while the United States negotiates continued use. Int J Occup Environ Health 12:260–267

Schottler SP, Eisenreich SJ (1997) Mass balance model to quantify atrazina sources, transformation rates, and trends in the Great Lakes. Environ Sci Technol 31:2616–2625

Silva JF, Ferreira LR, Ferreira FA (2007) Herbicides: absorption, translocation, metabolism, formulation and mixtures. In: Silva

AA, Silva JF (eds) Topics in weed management. Universidade Federal de Viçosa, Viçosa, Brazil, p 367

Silva HSVP, Loiola C, Pereira SRF, Santos RL, Andrade GV, Nunes SG (2013) Acute toxicity and genotoxicity of Folisuper 600BR commercial pesticide to tadpoles of Physalaemus cuvieri (Anura: Leiuperidae). Pestic: R Ecotoxicol 23:1–10

Silvano DL, Segalla MV (2005) Conservation of amphibians in Brazil. Mega Divers 1:79–86

Silva FR, Santos RS, Nunes M, Rossa-Feres DC (2009) Anuran captured in pitfall traps in three agrossystem in Northwestern São Paulo State, Brazil. Biota Neotrop 9:253–255

Singh PB, Singh V (2008) Cypermethrin induced histological changes in gonadotrophic cells, liver, gonads, plasma levels of estradiol-17β and 11-ketotestosterone, and sperm motility in Heteropneustes fossilis (Bloch). Chemosphere 72:422–431

Smalling KL, Reeves R, Muths E, Vandever M, Battaglin WA, Hladik ML, Pierce CL (2015) Pesticides concentrations is frog tissue and wetland habitats in a landscape dominated by agriculture. Sci Total Environ 502:80–90

Sparling DW, Fellers GM (2009) Toxicity of two insecticides to California, USA, anurans and its relevance to declining amphibian populations. Environ Toxicol Chem 8:1696–1703

Sparling DW, Linder G, Bishop CA, Krest SK (2010) Ecotoxicology of Amphibians and Reptiles. CRC Press, SETAC Books, New York

Svartz G, Meijide F, Coll CP (2016) Effects of a fungicide formulation on embryo-larval development, metamorphosis, and gonadogenesis of the South American toad *Rhinella arenarum*. Environ Toxicol Pharmacol 45:1–7. https://doi.org/10.1016/j.etap.2016.05.008

Svartz GV, Herkovits J, Pérez-Coll CS (2012) Sublethal effects of atrazine on embryo-larval development of Rhinella arenarum (Anura: Bufonidae). Ecotoxicology 21:1251

Svartz GV, Pérez-Coll, CS (2013) Comparative toxicity of cypermethrin and a commercial formulation on *Rhinella arenarum* larval development (Anura: Bufonidae). Int. J. Environ Health 6. https://doi.org/10.1504/IJENVH.2013.056973

Tavera-Mendoza L, Ruby S, Brousseau P, Fournier M, Cyr D, Marcogliese D (2002a) Response of the amphibian tadpole (*Xenopus laevis*) to atrazine during sexual differentiation of the testis. Environ Toxicol Chem 21:527–531

Tavera-Mendoza L, Ruby S, Brousseau P, Fournier M, Cyr D, Marcogliese D (2002b) Response of the amphibian tadpole (*Xenopus laevis*) to atrazine during sexual differentiation of the ovary. Environ Toxicol Chem 21:1264–1267

Toni C, Ferreira D, Kreutz LC, Loro VL, Barcellos LJG (2011) Assessment of oxidative stress and metabolic changes in common carp (*Cyprinus carpio*) acutely exposed to diferente concentrations of the fungicide tebuconazole. Chemosphere 83:579–584

Vieira EFS, Cestari AR, Chagas RA, Cortes GKR (2014) Collection and characterization of suitable matrix for long-release systems—release studies of Atrazine and Diuron herbicides. Quim Nova 37:398–403