Differential uptake of endosulfan in embryo-larval development of the amphibian *Rhinella arenarum* under sublethal exposure.

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Highlights:

- Significant uptake of endosulfan in *Rhinella arenarum* embryos and larvae were reported.
- Stage-dependent uptake of endosulfan was evaluated and analyzed.
- Concentration, time and stage-dependent uptake of endosulfan were reported and discussed.
- The study showed the threat of endosulfan for *R. arenarum* populations.

Abbreviations: AS, AMPHITOX solution; BCF, bioconcentration factor; LC, lethal concentration; NOEC, no observable effect concentration.

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Abstract

Agroecosystems are usually polluted with a wide variety of contaminants, being pesticides very frequently detected. Endosulfan, an organochlorine pesticide, has been shown to cause both lethal and sublethal effects at environmentally relevant concentrations on aquatic organisms such as amphibians and especially on its early developmental stages. In this context, the aim of this study was to evaluate the uptake of environmentally relevant concentrations of endosulfan and its correlation with differential sensitivity to toxicity in the early development of the common South American toad, *Rhinella arenarum*. Embryos and larvae were exposed to acute, short-term chronic and chronic periods to sublethal concentrations of endosulfan. According to the developmental stage at which they were exposed, the uptake capacity was different. Bioconcentration Factors (BCFs) for embryos significantly decreased with exposure time and concentration ($p<0.05$), reaching a BCF of up to 1679 for embryos at 96 h exposure to the lowest concentration. BCFs for larvae significantly increased with exposure time ($p<0.05$), obtaining a maximum of 40 at 504 h. In our previous study we reported the higher resistance to endosulfan by embryos than larvae, in line with the main tendency of embryos to bioconcentrate endosulfan as observed in this study.

Keywords: Endosulfan; *Rhinella arenarum*; Amphibian embryo-larval development; Uptake; Bioconcentration Factor

1. Introduction

Over recent decades, soybean crops have had a much larger overall expansion than any other crop, threatening natural ecosystems. In the last fifty years, soybean world production has substantially increased from 27 to 269 millions of tons in 2012. A total of 93% of the soybean world production came from just six countries, among them Argentina provides the 21% of the total worldwide production. The area harvested for soybean in Argentina increased dramatically, with production rising from 8.5 million Ha in 1999-2000 to 19.5 million Ha in 2012-2013 (USDA, 2013). Soybean is attacked by a great diversity of defoliating caterpillars (Lepidoptera) during the growing season, whereas increased populations of bugs during the fruiting stage (Hemiptera) (Di Marzio et al., 2010). The main insecticides used to control pests in these crops are endosulfan, cypermethrin and chlorpyrifos. Also, mixtures of these pesticides are often used simultaneously (Aragón et al., 1998).

Endosulfan is an organochlorine compound, which mechanism of action is mainly due to over stimulation of the central nervous system, inhibiting calcium and magnesium ATPase (Paquette and Liem, 1999). Technical grade endosulfan is a mixture of alpha and beta isomers, being the ratio 7:3 the most frequent chemical form (ATDSR, 2000). After it release to the environment, endosulfan as other pesticides is subject to transport and transformation processes that, in addition to environmental parameters and physicochemical properties regulate their distribution and concentration in water, soil, sediments and biota (Van der Oost et al., 2003). The high molecular weight and the low water solubility of most organochlorine pesticides lead to their bioaccumulation in the biota, mainly in fatty tissues, and consequent biomagnification through the food web as well (Newman and Unger, 2003).

Endosulfan has been identified as a persistent organic pollutant (POP) due to its persistence, bioaccumulation, long-range transport, and adverse effects to human health and aquatic ecosystems (UN, 2011). For these reasons, it has been classified as highly toxic by the majority of environmental protection agencies (Sutherland et al., 2004) and, following the recommendation of the Scientific Committee, the United Nations Association in 2011 decided to promote the ban of the endosulfan worldwide use (UN, 2011). However, in spite of regulations and restrictions, it is still largely used, particularly in some developing countries as Argentina where it has been phased out just recently, since July 2013 (SENASA, 2013).

Aquatic ecosystems integrate the agricultural areas by providing water and drainage channels. As a consequence, these agroecosystems are continuously exposed to different types of toxic residues, being pesticides relevant contaminants. The impact of endosulfan on Argentinean agroecosystems is significant, taking into account that only in 2010 it was applied 5.5 million liters of endosulfan (CASAFE, 2012), being its residues found very frequently in the environment in the range of 0.01-26 $\mu g\ L^{-1}$ (Baudino et al., 2003; Dalvie et al., 2003; Wan et al., 2005b; Leong et al., 2007; Carriger and Rand, 2008; Woudneh et al., 2009; Leadprathom et al., 2009).

Amphibians, especially their embryo-larval development, are considered bioindicator species, helping to detect changes in environmental quality, both for their morpho-physiological characteristics and their requirements of aquatic and terrestrial habitats, making them more susceptible to physicochemical agents than other vertebrates. In light of changes in the environment (pollution, temperature variations, periods of drought, overgrazing, etc) can be seen direct/indirect changes in survival and reproduction patterns. Bionda et al. (2013) reported that amphibians in altered Argentinean agroecosystems had lower egg and larval survival compared to organisms from non-cultivated area, and population projections less favorable and tendency to extinction in crop dominated areas. Several laboratory studies reported adverse effects of endosulfan on amphibian embryo-larval development, *i.e.* lethality in various species at environmentally relevant concentrations (Jones et al., 2009; De Jong Westman et al., 2010), delay to complete metamorphosis (Brunelli et al., 2009), malformations of gills (Bernabó et al., 2008), failures in the endocrine system (Goulet and Hontela, 2003), and neurotoxic effects (Agostini et al., 2009; Denoël et al., 2013; Svartz et al., 2014). However, most toxicity studies evaluate adverse effects under acute exposure condition only in certain period of the life cycle. It is of concern evaluating an eventual differential sensitivity to pesticides among different developmental stages of a species in order to protect especially wild-living species. Although endosulfan toxicity was assessed through various studies, there is scarce information on the uptake of this compound in biota, and less in amphibians.

Moreover, an alarming amphibian population decline has been reported worldwide since 1969 (Blaustein and Wake, 1990; Alford and Richards, 1999; Houlahan et al.,

2000; Kiesecker et al., 2001). According to the IUCN, 36.9 % of amphibians species are under certain threaten degree, and agriculture and aquaculture activities are considered the main risk factors (IUCN, 2014). Although *R. arenarum*, the common South American toad, is included in the IUCN's Least Concern category, and presently considered as not threatened, so its use does not generate conflicts in their conservation.

Toxicity bioassays represent useful tools to evaluate the risk of exposure of ecosystems to different physicochemical agents. AMPHITOX is a set of toxicity tests that employs embryos and larvae of *R. arenarum* providing information on mortality, malformations, delayed development, reduced growth and behavioral abnormalities (Herkovits et al., 2002, Herkovits and Perez-Coll, 2003, Hutler Wolkowicz et al., 2013; Svartz et al., 2012). Moreover, AMPHITOX allows the evaluation of toxic uptake (Aronzon et al., 2014) as the assessment of stage-dependent susceptibility (Brodeur et al., 2009; Svartz et al., 2014), and by expanding the evaluation time allows obtaining acute, short-term chronic and chronic toxicity results. In this context, the aim of this study was to evaluate, in a comparative way, endosulfan uptake in the embryonic and larval development of *R. arenarum*. Also, to assessa correlation with the differential sensitivity to toxicity that we have previously reported (Svartz et al., 2014) and the uptake of the pesticide under acute, short-term chronic and chronic exposure periods by means of the AMPHITOX test.

2. Materials and methods

2.1. Source of R. arenarum embryos

Three mating pairs of adult toads of *R. arenarum* of approximately 200–250 g were obtained in Moreno (Buenos Aires province, Argentina). Toad care, breeding, embryo acquisition and analysis were conducted according to the methods described in the AMPHITOX protocols (Herkovits et al., 2002; Herkovits and Pérez-Coll, 2003). Ovulation of females was induced by means of an intraperitoneal injection of a suspension of one homogenized toad pituitary gland in 1 mL AMPHITOX solution (AS) per female preserved according to Pisanó (1956), plus 5000 IU human chorionic gonadotropin (hCG). The AS composition is (in mg L^{-1}): Na^+ 14.75; Cl^- 22.71; K^+ 0.26; Ca^{2+} 0.36; HCO^-_3 1.45. Oocytes were fertilized *in vitro* using a testicular macerate homogenate suspended in AS, resulting in a spermatozoid suspension of 10%. The

sperm viability was confirmed by observing the spermatozoid morphology and movements under an optical microscope. The eggs were inspected for quality and fertility and were considered acceptable if the fertility rate was greater than 75% and embryo survival at the neurula stage was greater than 70%. The jelly coat was dissolved by immersing egg ribbons in a solution of 2% thioglycolic acid at pH 7.2 containing 1.35 mL saturated sodium hydroxide (NaOH) solution in 100 mL AS. This step was followed by a thorough wash of the embryos. Embryos were kept in AS and maintained at 20 ± 2 ºC. The AS was replaced entirely every three days and monitored weekly to ensure that the pH was at acceptable levels (7±0.5). Embryos were staged according to Del Conte and Sirlin (1951).

2.2. Test solutions

Test solutions were performed using technical-grade endosulfan (PS81; Supelco) with a purity of 99%. A stock solution containing 1000 mg endosulfan L^{-1} was prepared by dissolving endosulfan in analytical-grade acetone. The exposure concentrations were prepared by diluting the stock solution. Acetone concentration in test solutions was always lower than 1.1% to ensure no toxic effects from exposure to solvent (ASTM, 1993). Both AS and acetone treatments were simultaneously performed as controls. The concentration of endosulfan in stock solution was analyzed by high-performance liquid chromatography–electrospray ionization–mass spectrometry (negative mode), the identity of the compound was confirmed by scan detection, and the ion m/z=405 and m/z=407 were used for quantification (Chusaksri et al., 2006). The solution was analyzed daily and was found to be stable over the exposure time. The error between nominal and measured concentration of the stock solution did not exceed 5%.

2.3 Experimental design

R. arenarum embryos were exposed to endosulfan solutions from early blastula stage (S.4, embryos) and complete operculum stage (S.25, early larvae) onwards. For each experimental condition, sixty embryos or larvae were placed in triplicate 20 cm-diameter glass Petri dishes containing 200 mL of test solution which were entirely replaced every 48 h. The temperature between 20 ± 2 ºC and a 12:12 h light: darkness photoperiod were maintained throughout the experiments which lasted 504 h. Test

solutions ranged between 0.001-0.005 mg L^{-1} endosulfan and 0.0005-0.075 mg L^{-1} endosulfan for embryos and larvae, respectively. This concentration range was selected considering that the maximum exposure concentration was below the LC10-504 h value obtained in our previous study (Svartz et al., 2014). Tadpoles were fed with three granules of balanced fish food TetraColor (Tetra®) in each Petri dish. The uptake and wet weight were measured at three exposure times: 96, 240 and 504 h. At each time, 50 organisms were removed from each replicate, thoroughly washed with 200 mL of AS, blotted dry on absorbent paper and then weighed and stored at -20°C for analytical assessment.

2.4. Analytical method

Whole organism? tissue samples were used to determine endosulfan uptake in terms of BCFs expressed on wet weight basis. The tissues were thawed, dried, and 20–100 mg tissue was placed into 2 mL disposable polypropylene tubes. Then, 2 mL of acetone and Na_2SO_4 were added to the test tubes and the tissues were homogenized for 3 min. The extracted contents were sonicated twice for 10 min and then centrifuged for 5 min. The organic phase was separated and the procedure was repeated. The sample extracts were evaporated dry at 45 ºC and then solubilized in 2 mL of methanol, sonicated three times for 5 min and then filtered for subsequent analysis. Endosulfan isomers (α and β) were identified and quantified by Gas Chromatography (Hewlett Packard 5890-II) with ECD and a mass spectrometer (Hewlett Packard 5971). The analytical separation was performed with a HP-5 of 30 m x 0.32 mm column. Hydrogen was used as carrier at 0.8 ml/min, split injection at 1:50 and 220 ºC, oven ramp from 160 ºC to 250 ºC, at 10 ºC/min. Analyte identity was confirmed by mass spectrometry using a single quadrupole operating in SIM mode by monitoring ions m/z =304, 179, 137, previously characterized by scanning (between 70 and 550 umas). Recovery was 97.8%.

2.5. Data analysis

The deviation from the ratio isomers of α-endosulfan:β-endosulfan in exposure solutions (7:3) was calculated, indicating either differential metabolism of isomers or differential uptake by the organisms. In this study, BCF (wet weight basis) =

concentration of the chemical in the organism (μg g^{-1}) / exposure concentration (mg L^{-1}) were calculated. Two-way ANOVA, repeated measures analysis was used to assess significant differences of endosulfan uptake among embryos and larvae exposed to different conditions (exposure time and concentration). Multiple comparisons were performed using Bonferroni's test. All data were expressed as mean ± SD and the statistical analyses were carried out using GraphPad Prism version 5.0. Significance level was set at 0.05.

3. Results

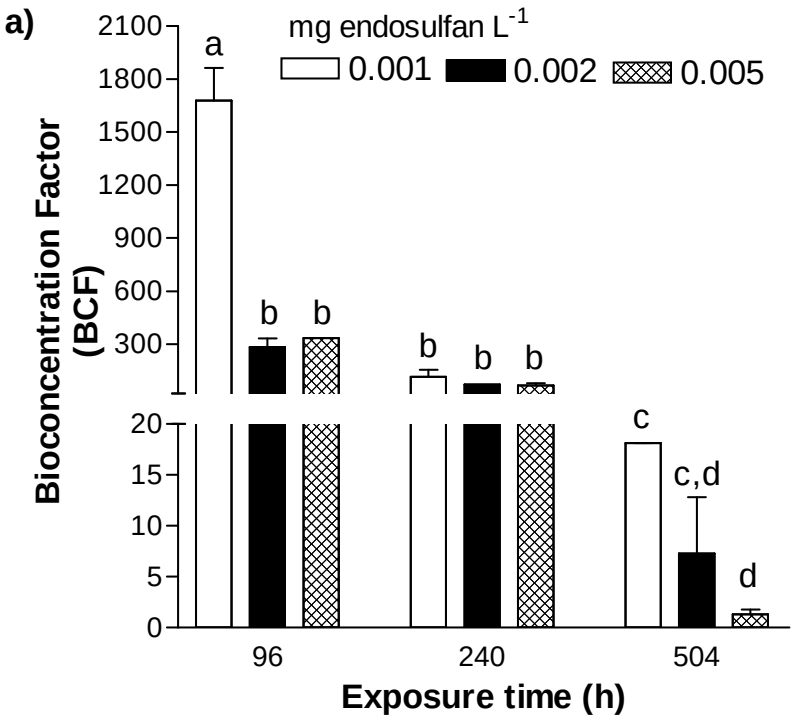
Because the two controls, AS and acetone solvent, did not differ statistically, both treatments were combined and reported as the 'control' in the rest of the manuscript.

In table 1 the levels of α- and β-endosulfan (μg/g), and total endosulfan (μg/g, wet weight) incorporated by embryos and larvae after different exposure times are indicated. Control embryos and larvae had endosulfan concentrations below detection limit (data not presented in the table), whereas all treatments showed detectable levels of both or one of the endosulfan isomers. In almost all treatments there were deviations in the isomer ratio α-endosulfan:β-endosulfan (7:3), indicating either differential metabolism of isomers or differential uptake in *R. arenarum*. For larvae, the ratio was deviated to β at 240 h and even at 504 h the α isomer levels were below the limit of detection.

Table 1. Levels of α- and β-endosulfan expressed as μg/g, and total endosulfan (Σ α- and β- endosulfan) expressed as μg/g (wet weight) incorporated on *Rhinella arenarum* embryos and larvae after exposure to sublethal concentrations of technical endosulfan (α-:β-isomers 7:3 ratio) during different times. Data are expressed as mean ± SD. NA: not available. <LOD: below detection limit.

Stage	Exposure time (h)	Exposure concentrations (mg endosulfan L^{-1})	α-endosulfan (μg g^{-1})	β-endosulfan (μg g^{-1})	Σ endosulfan (μg g^{-1})	α–endosulfan: β-endosulfan	BCF
Blastula	96	0.001	1.246 ± 0.254	0.433 ± 0.081	1.679 ± 0.319	2.163 ± 0.395	1679.19 ± 319.15
		0.002	0.423 ± 0.108	0.143 ± 0.028	0.566 ± 0.136	2.935 ± 0.178	283.19 ± 67.93
		0.005	1.246 ± NA	0.420 ± NA	1.665 ± NA	2.964 ± NA	3 33.16 ± NA
	240	0.001	0.076 ± 0.057	0.038 ± 0.019	0.114 ± 0.072	1.879 ± 0,784	114.15 ± 72.33
		0.002	0.088 ± NA	0.055 ± NA	0.143 ± NA	1.608 ± NA	7 1.43 ± NA
		0.005	0.215 ± 0.087	0.106 ± 0.040	0.321 ± 0.126	2.012 ± 0.273	64.23 ± 25.10
	504	0.001	2.922 ± 4.105	0.012 ± 0.008	0.018 ± 0.019	1.619 ± NA	18.14 ± 0.02
		0.002	0.020 ± NA	0.005 ± 0.002	0.015 ± 0.016	4.307 ± NA	7.29 ± 7.79
		0.005	<LOD	0.007 ± 0.004	0.007 ± 0.004	<LOD	1.30 ± 0.81
Complete operculum	96	0.01	0.060 ± 0.038	0.023 ± 0.015	0.083 ± 0.052	2.059 ± 0.943	8.27 ± 5.19
		0.05	0.400 ± 0.102	0.189 ± 0.049	0.589 ± 0.143	2.144 ± 0.332	11.76 ± 2.85
		0.075	0.435 ± NA	0.186 ± NA	0.621 ± NA	2.344 ± ND	8 .28 ± 0.10
	240	0.0005	0.007 ± NA	0.008 ± 0.005	0.011 ± 0.000	0.611 ± ND	21.83 ± 0.34
		0.001	0.009 ± NA	0.012 ± 0.004	0.015 ± 0.002	1.2 ± ND	15.27 ± 2.47
		0.002	<LOD	0.012 ± 0.009	0.012 ± 0.009	<LOD	6.24 ± 4.39
	504	0.0005	<LOD	0.018 ± NA	0.018 ± NA	<LOD	35.21 ± NA
		0.001	<LOD	0.041 ± 0.023	0.041 ± 0.023	<LOD	41.22 ± 22.99
		0.002	<LOD	0.045 ± 0.023	0.045 ± 0.023	<LOD	22.56 ± 11.58

The BCFs calculated for embryos and larvae are shown in Figure 1. According to the developmental stage at which they were exposed, the uptake capacity was different. BCFs for embryos significantly decreased with exposure time and concentration ($p<0.05$), reaching a BCF of up to 1679 for embryos exposed at 96 h to the lowest concentration, and a minimum of 1.30 at 504 h in those exposed to the highest concentration. In contrast, BCFs for larvae significantly increased with exposure time ($p<0.05$), reaching a maximum near 40 at 504 h and a minimum of 6.24 at 240 h. These results highlight that the uptake in embryos exposed at 240 h (reaching larval stage, S.25) was significantly higher ($p<0.05$) than in larvae exposed from S.25.



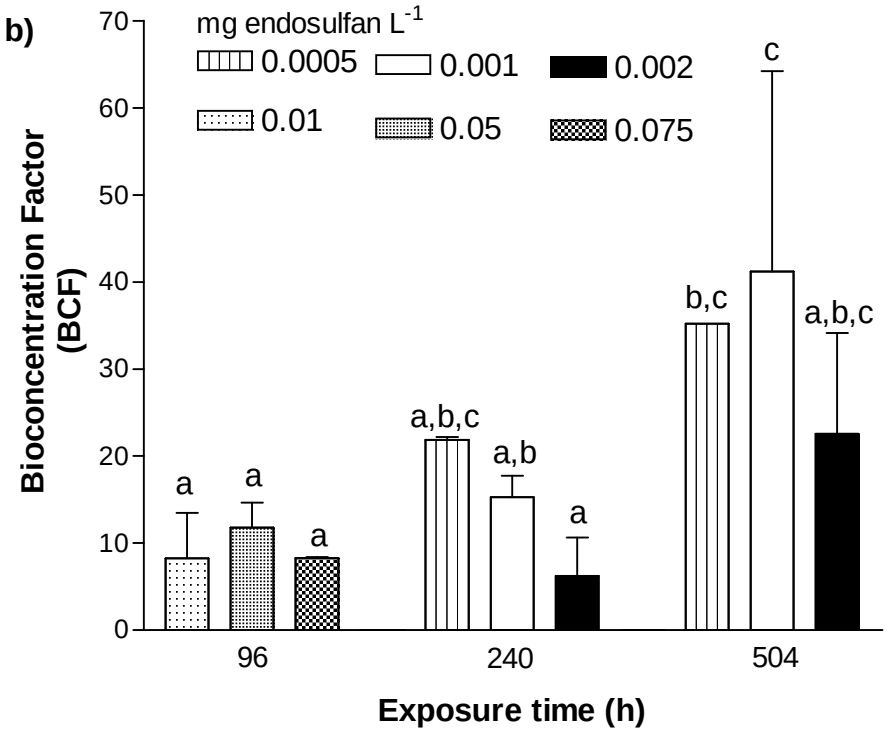


Figure 1 Bioconcentration Factors (BCFs) of endosulfan for *Rhinella arenarum* (a) embryos exposed from early blastula and b) larvae exposed from complete operculum stages. Different letters mean that values are statistically different at $p<0.05$.

Comparative results for embryos and larvae exposed to 0.001 and 0.002 mg endosulfan L^{-1} at 240 and 504 h are shown in Figure 2. Embryo BCFs were significantly higher ($p<0.05$) at the lower exposure concentration for both exposure times, being 1.60 and 2.35 times higher at 240 and 504 h, whereas for larvae, a similar tendency was also observed but values were not statistically different, being 2.45 and 1.82 times higher at 240 and 504 h respectively. Moreover, BCF values at 240 h for embryos were significantly higher than for larvae ($p<0.05$), up to 11.45 times, whereas this tendency was reversed at 504 h, being larvae BCF up to 3.09 times higher than embryos.

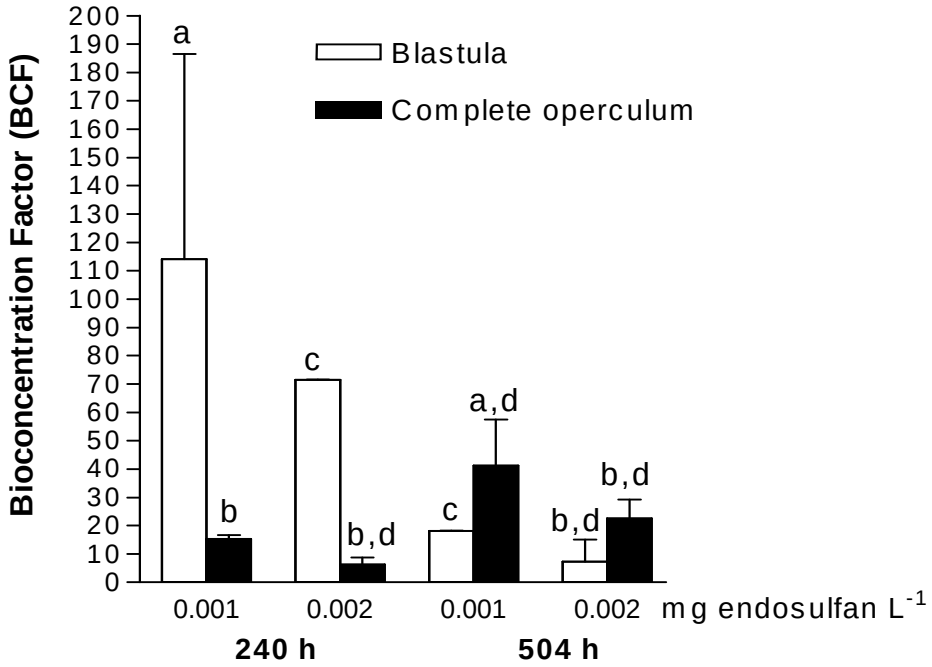


Figure 2 Comparative bioconcentration factors at 240 h and 504 h for embryos and larvae exposed to 0.001 and 0.002 mg endosulfan L^{-1}. Different letters means that values are statistically different at $p<0.05$.

4. Discussion

Despite endosulfan is already banned worldwide, its high environmental persistence, bioconcentration and biomagnification capacity through the trophic web, and its long range transport through the atmosphere, make the ecotoxicity assessment of this pesticide still remains of high relevance.

In a recent publication (Svartz et al., 2014) we reported the high toxicity of endosulfan to *R. arenarum* embryos and larvae with LC50 at the chronic exposure (504 h) of 0.03 (0.014-0.05) and 0.01 (0.006-0.015) mg L^{-1} respectively. Moreover, we had characterized both lethal and sublethal effects of endosulfan on the embryo-larval development of *R. arenarum,* whereas at present study we attempt to elucidate if any correlation between sensitivity and uptake of the pesticide occurs. For this purpose, we evaluated the uptake in *R. arenarum* embryos and larvae exposed to sublethal endosulfan concentrations, being the maximum exposure concentration under the LC10-504 h value obtained in the previous study. The results highlight the differential uptake of endosulfan in the amphibian *R. arenaum* according to the developmental stage at

which they were exposed. In this sense, embryonic uptake of the pesticide decreased with exposure time and concentration, whereas larval uptake increased with exposure time.

BCF is used to evaluate the ability of the aquatic organism to accumulate chemicals from the water environment. Thus, BCF is defined as the ratio of the concentration of the chemical in the organism, to its concentration in the surrounding water. If BCF>1, it indicates that the organism has a potential to accumulate the chemical but is generally not considered to be significant unless the BCF exceeded 100 (USEPA, 1991). All of the calculated BCFs in this study were over one, but only the values obtained for acute exposed embryos were over 100, reaching up to 1679 for the lowest exposure concentration. The fact that the higher BCF values were related to the lower exposure concentrations was also observed for *R. arenarum* embryos exposed to nickel, an essential trace element that also caused, at lower concentrations, enhancing embryonic survival (Pérez Coll et al., 2008). The maximum BCFs for endosulfan obtained in this study were higher than the maximum values reported for other amphibians as *Bufo regularis* adults (BCF=27) (Ezemonye and Tongo, 2010) and were in the range of those reported for saltwater fish species, in which BCFs were in the range between 328 and 2755 (Schimmel et al., 1977; USEPA, 1980; Toledo and Jonsson, 1992).

R. arenarum larval period was the most sensitive developmental stage to endosulfan, being at acute and short-term chronic exposures almost 26 times and 3 times more sensitive than embryos, respectively (Svartz et al., 2014). Moreover, embryos sensitivity significantly increased at short-term chronic period coinciding with neuromuscular development, the main toxicity target of this pesticide (LC50-96 h=17.62 (14.65-20.53) mg endosulfan L^{-1}; LC50-240 h=0.81 (0.26-1.25) mg endosulfan L^{-1}). It is interesting to note that this highest endosulfan resistance of embryos is in line with the great tendency to bioconcentrate the pesticide reported in this study, reaching BCF values up to 1679 at 96 h of exposure. In contrast, larvae exposed from the beginning of their development, showed a high sensitivity to the pesticide even with a low bioconcentration expressing lethal and sublethal effects (Svartz et al., 2014).

The enormous difference in the pesticide accumulation between embryos and larvae could be due to the high lipid content of the yolk of embryos. Thus, as previously stated,

the lipophilic nature of endosulfan may allow it to easily partition from aqueous media into the lipid components of living cells (Rao and Lal, 1987; Nagel and Loskill, 1991). Furthermore, this differential accumulation between embryos and larvae was also observed for *R. arenarum* exposed to diazinon (Aronzon et al., 2014), although much less pronounced in the case of diazinon in which embryos uptake could be up to 27 times higher than larvae. In contrast, it was seen that both *R. arenarum* (ex *Bufo arenarum)* and *Xenopus laevis* exposed to cadmium at different developmental stages, showed an increase in metal uptake at later developmental stages that were thought to be related to the gradual increase in the external surface of the larvae, the development of gills and the possible transport of the contaminant across the membrane from the surface to inner organs (Herkovits and Perez Coll, 1996; Herkovits et al., 1998).

The bioaccumulation of organic contaminants depends on the uptake and elimination rates of tissues/organs that are related to their abilities to metabolically transform the chemical (Nowak, 1997). Once absorbed, a rapid transport of endosulfan throughout the body takes place. The pesticide may be transported via the lymphatic system or blood stream and distributed to various body tissues, including those of storage depots and sites of metabolism or biotransformation (Landis and Yu, 2003). In this sense, one of the factors influencing the endosulfan decline observed in embryos could be the metabolic transformation of endosulfan to its metabolites and excretion, as it was observed for other insecticides in fishes (Kanazawa, 1975). The assessment in Atlantic salmon (*Salmo salar*) to dietary endosulfan exposure revealed a significant induction of the CYP1A enzyme family at 710 μg/kg, suggesting that CYP1A-mediated hydroxylation may be important for the metabolic transformation of endosulfan to the toxic metabolite, endosulfan sulfate (Glover et al., 2007). Moreover, the study on stereoselective metabolism of endosulfan by human liver microsomes and human CYP450 isoforms showed that the intrinsic clearances of endosulfan sulfate from β-endosulfan were 3.5-fold higher than those from α-endosulfan, suggesting that β-endosulfan would be cleared more rapidly than α-endosulfan (Lee et al., 2006). This fact is the opposite that occurs in this study, in which α-endosulfan:β-endosulfan ratios were below 7:3 both in embryos and larvae exposed to the pesticide, suggesting that α-endosulfan would be metabolized more rapidly than β-endosulfan or could be an isomer differential uptake in the organisms. Also, α-endosulfan has been reported as the isomer

most toxic in fishes and aquatic invertebrate organisms; sulfate endosulfan has a similar toxicity to the technical mixture (7:3), whereas the commercial formulations and β-endosulfan presents the least toxicity (Wan et al., 2005a). Endosulfan as a persistent organic pollutant could be retained on amphibian body tissue and when they are fed on by predators, the chemical concentrates from one trophic level to the next, leading to biomagnification causing severe ecological consequences (Newman and Unger, 2003). Although endosulfan is already banned, due to its persistence capability will still cause toxic effects at ecological levels.

Taking into account endosulfan levels were reported in the range from 0.1 to 26 μg L^{-1} in ground and surface water near agroecosystems (Baudino et al., 2003; Dalvie et al., 2003; Wan et al., 2005b; Leong et al., 2007; Carriger and Rand, 2008; Woudneh et al., 2009; Leadprathom et al., 2009), bioconcentration in embryos and larvae of *R. arenarum* could be occur. Moreover, this phenomenon could be amplified by biomagnification, being a great concern not only the risk of *R. arenarum* populations that normally develop in those ecosystems but also for trophically associated species. The results obtained in this study confirm the important potential uptake of *R. arenarum* embryo-larval development and are in line with the decisions to restrict and promote the ban of its worldwide use.

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