An Acad Bras Cienc (2023) 95(4): e20191385 DOI 10.1590/0001-3765202320191385
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

CELLULAR AND MOLECULAR BIOLOGY

Chlorpyrifos toxicity and detoxifying enzymes activities in three native-aquatic species of macroinvertebrates from an agricultural area

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Abstract: Non-target species from agricultural areas might be exposed to sublethal pesticide concentrations favoring survival and reproduction of the resistance individuals. The objective of this study was to evaluate chlorpyrifos toxicity and detoxification enzymatic activities on three species (*Hyalella curvispina*, *Heleobia parchappii* and *Girardia tigrina*) from a drain channel with history of insecticide contamination (EF) and the Neuquén river (NR) in Argentina. Chlorpyrifos toxicity on amphipods (*H. curvispina*) and planarians (*G. tigrina*) from NR was about six- and two-fold higher than that of their counterparts from EF. Mean carboxylesterases (CarE) activities determined in the three species from NR were significantly different from EF, whereas mean glutathione-S-transferase (GST) activities were no significantly different. Finally, planarians from EF showed significantly higher mean 7-ethoxycoumarine O-deethylase (ECOD) activity than those from NR. Amphipods from both sites displayed similar ECOD activities. The present results suggest that chlorpyrifos resistance in amphipods from EF is not conferred by increased detoxification.

Key words: Chlorpyrifos, *Hyalella curvispina, Heleobia parchappii, Girardia tigrina,* resistance mechanisms.

INTRODUCTION

Agriculture is an important economic activity in the North Patagonian Region of Argentina, with significant implications to the environment quality.

Pesticides reach surface waters through various routes, but in particular through atmospheric drift after its application, by surface runoff and by seepage of contaminated groundwater (Gärdenäs et al. 2006, Loewy et al. 2011a, Phillips & Bode 2004). Studies from the area have demonstrated the presence of pyrethroids, organophosphates and carbamates in both groundwater and drain channels (Loewy et al. 1999, 2003, 2006, 2011a, b, Tosi et al. 2009). Between 2008 and 2010, the organophosphates azinphos-methyl and chlorpyrifos showed similar detection frequencies (more than 70%). During the last years, the detection in water of both pesticides was significantly reduced by restrictions of their use.

Aquatic invertebrates accomplish significant roles in the aquatic ecosystems such as decomposers, grazers, sediment feeders, parasites and predators. They also provide much of the food for vertebrates associated with these systems (Merrit & Cummins 1996).

Macroinvertebrates have been used to assess the effects of pesticides on aquatic ecosystems closed to agricultural areas (Liess and Carsten von der Ohe, 2005; Maltby & Hills

2008, Vranković et al. 2012, Wandscheer et al. 2017, Alavaisha et al. 2019).

These organisms from agricultural areas might be exposed to both lethal and sublethal pesticide concentrations according to the species susceptibility and life stage. During episodic peaks of pesticides, the acute lethal concentration median (LC_{50}) of the more susceptible species may be exceeded (Maltby & Hills 2008). Acute pesticide toxicity may eliminate an essential species affecting the functioning of the entire community by either promoting the dominance of undesired species or decreasing the community diversity (Zacharia 2011). Differences in pesticide metabolism by detoxifying enzymes is one of the factors that account for the intrinsic species sensitivity (Rubach et al. 2012). The detoxifying enzymes are classified as phase-I and -II reactions that occur consecutively to enable elimination of pesticides. The most relevant enzymes in aquatic insect larvae comprised oxidases (CYP450), esterases and glutathione-S-transferases (Katagi 2010).

Sublethal effects of pesticides may produce changes in behavior (Augusiak & Van den Brink 2016, Beketov & Liess 2008), reproduction (Bravo-Hernandez et al. 2014), development (Galvan et al. 2005) and population declines (Liess et al. 2013, Van Dijk et al. 2013). Sublethal effects elicited by pesticides on individual populations might equally impact at community level (Bridges 1997, Guedes et al. 2016).

Both lethal and sublethal insecticide concentrations may cause the development of insecticide resistance. The first (lethal) is the cause of resistance by the elimination of susceptible individuals and promoting the evolution of major resistant gene. The second (sublethal) favored the survival and reproduction of the resistant individuals and promote the accumulation of low-level resistance genes and mechanisms (Gressel et al. 2011, Guedes et al. 2017). Further, the stress caused by sublethal pesticide exposures might enhance both the mutation rates and the activity of the detoxification system, which might lead to pesticide resistance (Terriere & Yu 1974, Gressel 2011). Insecticide-resistance mechanisms usually involve enhanced activity of metabolic enzymes, which sequester or detoxify the insecticide, and insensitivity of the insecticide target site due to non-silent point mutations (Panini et al. 2016).

Previous studies showed a negative correlation between macroinvertebrates richness and chlorpyrifos and azinphos-methyl concentrations in drain channels (Macchi et al. 2018). Thus, the first objective of this study was to compare chlorpyrifos toxicity on three abundant species (Hyalella curvispina, Heleobia parchappii and Girardia tigrina) from a drain channel with history of insecticide contamination (EF) and the Neuquén river (NR) as the clean area. The second objective was to evaluate the activities of carboxylesterase (CarE), cytochrome P450 monooxygenases (CYP450) and glutathione S-transferases (GST) in these species from both sites. In this context, the first hypothesis was that macroinvertebrates from EF are more resistant to pesticides, especially to chlorpyrifos, than their counterparts form NR due to their long-term exposure. The second hypothesis was that resistance in the species from EF is associated to alterations in the activity of some of the detoxifying enzymes.

MATERIALS AND METHODS

Chemicals

The organophosphate chlorpyrifos (99.08% pure) was purchased from AccuStandard Inc., New Haven, CT, USA. Fast Garnet GBC salt, α-naphthyl acetate (α-NA), α-naphthol (α-N), 1,5-bis (4-allyldimethylammoniumphenyl) pentan-3-one dibromide (BW284C5), Triton X-100,

7-ethoxycumarine (7-EC), 7-hydroxycoumarine (7-OHC), reduced glutathione (GSH), 1-chloro-2,4-dinitrobenzene (CDNB), and bovine serum albumin were purchased from Sigma Chemical Co., Saint Louis, MO, USA.

Species and sampling sites

Adults of amphipods (*H. curvispina*), planarians (*G. tigrina*) and snails (*H. parchappii*) were sampled on February 2017, at the end of the production season, from EF and NR, the first discharging downstream of the selected site in the river. These sites are located in the Upper Valley of the Neuquén and Negro river, in northern Patagonia of Argentina (Fig. 1). These sites are included in an area of 110 ha which has been regularly monitored for insecticide residues for more than twenty years. This low-rainfall region is irrigated by a network channels and drains to support agriculture, one of the main activities in the area.

Toxicity assays

The acute toxicity of chlorpyrifos (99.8% purity) was evaluated in amphipods (6 mm), planarians (18 mm) and snails (4 mm) collected from both sites of study (EF and NR). The Organisms were acclimatized to the laboratory conditions, for 7 days at 21 °C before the beginning of the experiments. The acclimation was achieved by slowly adding dechlorinate filtered-tap water until the total volume of river water was changed. The bioassays were performed in groups of 10 organisms exposed for 48 h to different concentrations of chlorpyrifos (Table I) applied as 0.1 mL of acetone solution to 199

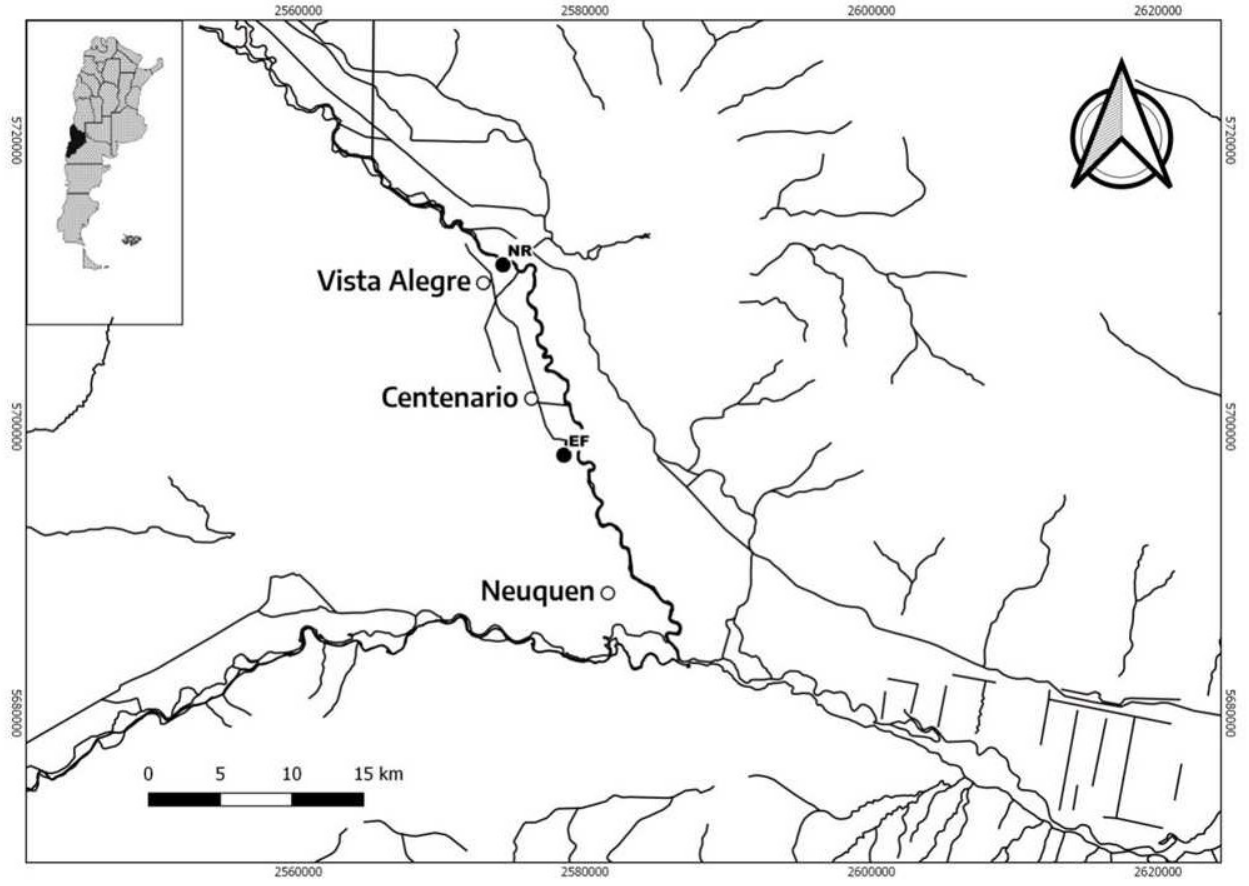

Figure 1. Sampling sites. Black circles indicate the sampling sites and the white circles show the locations.

mL of dechlorinated filtered-tap water. Control groups were exposed to 0.05 % of acetone. Each test was replicated 3 times on different days. Tests were run at 21°C and a photoperiod of 16:8 (L:D) h.

Enzyme activity

Carboxylesterase activity (CarE)

Organisms from each species and sampling site were homogenized in 0.1 M phosphate buffer (pH 6.5) plus 0.5% Triton X-100 with an electrical homogenizer PRO 200. Each homogenate consisted of 3 amphipods, 3 snails and 5 planarians in 500, 300 and 300 µL, respectively. Five independent homogenates were done for each species and collection site. Homogenates were centrifuged at 10,000 × g for 10 min at 4 °C and the supernatants were used as enzyme source. The activity of CarE was determined using α-NA as substrate (Dary et al. 1990). The assay was conducted in a final volume of 250 µL, with final concentrations of 2 mM α-NA and 0.002 mM of BW284C5 (an AChE inhibitor). After 15 min of incubation, 100 µL of freshly prepared 2.5 mM Fast Garnet GBC salt was added. Absorbencies were recorded 10 min later at 550 nm in a microplate reader (Molecular Devices, Sunnyvale, CA, USA). Absorbance values were transformed into µmol of α-N from an α-N standard curve (2-20 nmol) and activity was expressed as µmoles α-N min^{-1} mg of $protein^{-1}$.

Glutathione S-transferases (GST)

Groups of 3 amphipods, 3 snails and 4 planarians were homogenized in 500, 200 and 300 µL of buffer potassium phosphate (143 mM) and EDTA 6.3 mM (pH 7.5), respectively. Each homogenate was centrifuged at 10,000 x g during 30 min at 4 °C. The supernatant was used as enzyme source. The activity of GST was assayed according to Habig et al. (1974) using CDNB dissolved in acetonitrile as substrate. The reaction mixture in a final volume of 1000 µL consisted of 920 µL of 0.1 M phosphate buffer (pH 6.5), 20 µL of the enzyme source, 10 µL of CDNB (50 mmol L^{-1}) and 50 µL of GSH (2.5 mmol L^{-1}). Absorbance was recorded continuously at 340 nm for 1 min in a UV/visible spectrophotometer at 25 °C (Shimadzu, Kyoto, Japan). Rate measurements were corrected for the nonenzymatic reaction and transformed into µmol of CDNB conjugated min^{-1} mg of $protein^{-1}$ using the extinction coefficient 9.6 mmol L^{-1} cm^{-1}.

Ethoxycoumarine O-deethylase activity (ECOD)

The method to measure ECOD activity was adapted by De Sousa et al. (1995) for ex vivo, it was quite easy to perform and was characterized by a low chemical consumption and fast fluorescence reading. In addition, an advantage

Table I. Chlorpyrifos concentrations (mg/L) used in bioassays.

Species	Site	Chlorpyrifos concentrations			
H. *curvispina*	NR	0.0025	0.125	0.025	1
(amphipod)	EF	0.75	1.5	3	
G. tigrina	NR	2	3	4	
(planarian)	EF	2	4	8	
H. parchappi	NR	0.65	1	2	3
(snail)	EF	0.5	1.5	5	

is the use of the NADPH provided by the insect that was alive just before the test. Comparing with the in vitro technique, which requires microsomes preparation, the ECOD activity is subject to interference from endogenous substances presented in homogenate. (Gottardi et al. 2016).

Fresh adults of amphipods and planarians from each sampling site were individually used for cytochrome P450 monooxygenase activity. Snails were not analyzed given the difficulty to completely remove the organism from its shell. The enzyme activity was assessed by a fluorometric protocol using 7-EC as substrate and expressed as 7-ethoxycoumarine O-deethylase activity (ECOD) using black flat bottom 96 multi-well microplate (Bouvier et al. 2002).

Each adult was cut into three fragments to maximize the enzyme source recovery and placed altogether in a well containing 50 µL of phosphate buffer (pH 7.2; 50 mM). The reaction was initiated by the addition of 50 µL of developing solution containing 7-EC at a final concentration in the well of 0.2 mM in phosphate buffer (pH 7.2; 50 mM). After 4 h incubation at 30 ºC, the reaction was stopped with 100 µL of 1:1 glycine (pH 10.4)/ethanol solution with a glycine well concentration of 0.017 mM. Subsequently, plates were centrifuged at 1,500 x g during 1.5 min to descend the biological tissues. Fluorescence was determined at 380 nm excitation and 460 nm emission in a spectrofluorometer (Wallac 1420 Multilabel, Turku, Finland). A standard curve was measured in every plate with 7-OHC (0.0125-1 nmol) and ECOD activity was expressed as pg of 7-OHC min^{-1} $adult^{-1}$.

Protein content

Protein concentration was determined using bovine serum albumin as the standard curve (5-40 µg) (Lowry et al. 1951).

Statistical analysis

Mortality data were subjected to probit analysis using Dr. Sakuma's PriProbit NM software (http://www.ars.usda.gov/Services/docs.htm?docid=11281). Values of median lethal concentration fifty (LC_{50}) between sites were significantly different if their 95% confidence intervals (95% CL) did not overlap. Data of CarE, GST and ECOD activities were tested for normality and homogeneity of variance using Kolmogorov–Smirnov test and the Levene test, respectively. Comparisons between populations were analyzed by one-way ANOVA with Bonferroni's multiple comparison tests using the GraphPad 5.0 software (Graphpad Software, San Diego, CA, USA).

Table II. Toxicity of chlorpyrifos in adults of amphipods, snails and planarians.

Species	Site	LC_{10} (CL 95%) (mg L^{-1})	LC_{50} (CL 95%) (mg L^{-1})	Slope	p
H. *curvispina*	NR	0.014 (0.0010-0.040)	0.25 (0.13-0.53)	1.02	0.35
(amphipod)	EF	0.79 (0.53-0.99)	1.58 (1.33-1.89)	4.28	0.54
H. parchappii	NR	0.68 (0.49-0.83)	1.38 (1.20-1.58)	4.18	0.10
(snail)	EF	0.49 (0.21-0.75)	1.44 (1.01-1.96)	2.74	0.98
G. tigrina	NR	1.79 (1.37-2.06)	2.64 (2.36-2.89)	7.63	0.58
(planarian)	EF	2.99 (2.09-3.65)	5.55 (4.72-6.69)	4.78	0.33

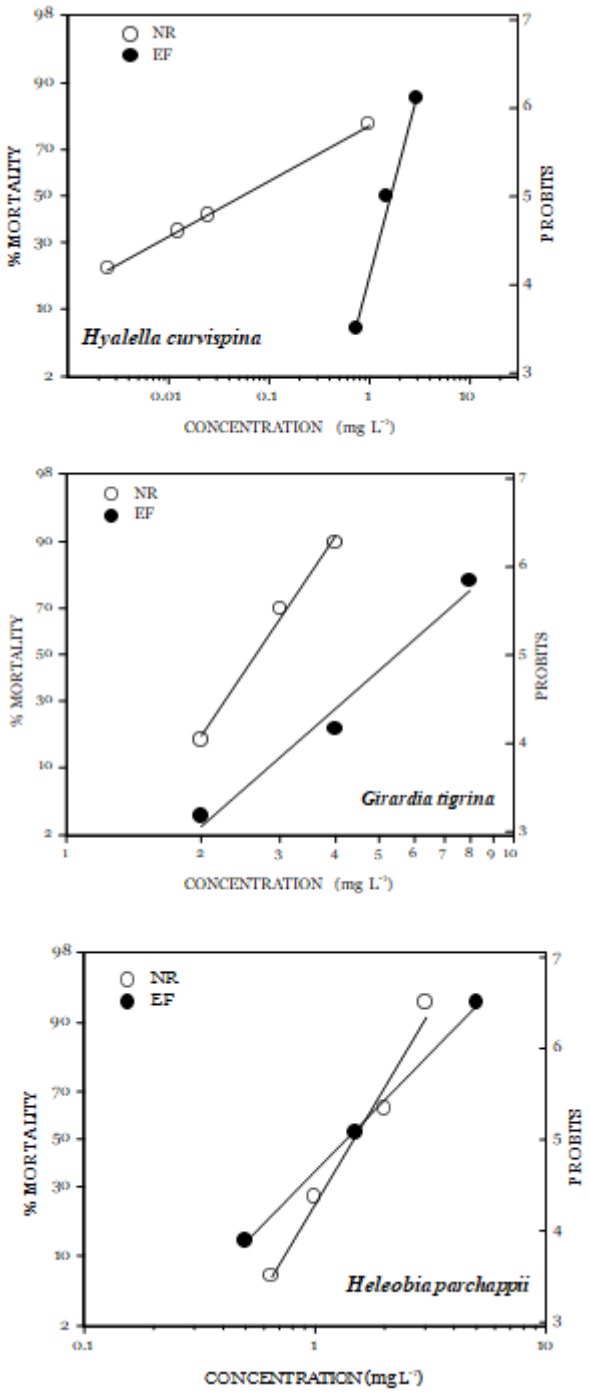

Figure 2. Concentration–response lines of *H. curvispina*, *G. tigrina* and *H. parchappii* from both NR and EF sites.

RESULTS AND DISCUSSION

Toxicity of chlorpyrifos in native species

The response to chlorpyrifos of adult *H. curvispina*, *H. parchappii* and *G. tigrina* from both EF and NR is shown in Table II. Concentration–response lines of all three species and from both sites fitted the probit model ($p > 0.05$) (Fig. 2). Chlorpyrifos toxicity was significantly lower in both amphipods and planarians from EF than their counterparts from NR, according to their LC_{50} values and not-overlapping confidence intervals. Conversely, no significant differences on chlorpyrifos toxicity were found on snails from both sites. The slopes of the regression lines of snails and planarians from NR were steeper than the ones observed for these species from EF, suggesting more genetic homogeneity (Brown & Pal 1971)

The intensive use of insecticides causes a selection pressure leading to the development of resistance (Zhu et al. 2016). Basically, resistance is an adaptive mechanism to withstand a severe level of environmental stress (Denholm & Rowland 1992). The development of insecticide resistance is a dynamic and complex process that directly depends on genetic, physiological, behavioral and ecological factors from the organisms. Indirectly, operational factors, such as the type of insecticide used, time and application rate, coverage and application method, also influence the resistance development (Denholm & Rowland 1992, Rust 1996). Even though selection for insecticide resistance is often associated with differential mortality among individuals, the phenomenon refers to differential survival and reproduction. Therefore, insecticide resistance can be achieved by it sublethal exposure favoring survival and reproduction of the resistant individuals (Guedes et al. 2017). According to these authors, sublethal insecticide exposures may delay selection of a major single gene while

favoring multifactorial or polygenic resistance and promote mutation rates of genes involved in DNA repair. Further, sublethal insecticide exposure may influence insecticide resistance by insecticide-induced hormesis and induction/cross-induction of detoxification enzymes. Some factors that increase the concentration and/or frequency of the lethal and sublethal pesticide exposures and therefore, the development of resistance to chlorpyrifos in amphipods and planarians are: (a) chlorpyrifos is adsorbed to sediments and particulate matter which increases the persistence in the aquatic environment by reducing its availability of dissipation (Gebremariam et al. 2012), (b) life cycles that overlaps with the insecticide applications against agricultural pests (Macchi et al. 2018), (c) no refuge for these species since they accomplish their entire life cycle in the aquatic environment (Garcia et al. 2010, Stocchino & Manconi 2013); and therefore, they are not able to escape the insecticide residues that arrive by air drift or natural runoff .

Resistance in non-target species is one of the many consequences from indiscriminate use of pesticides. For example, resistance in blackflies (Andrade & Castello Branco Junior 1990, Osei-Atwenwboana et al. 2001) and some mosquito populations (Hemingway et al. 1997) has apparently arisen from agricultural pesticide use. Previous studies showed that non-target *Simulium* larvae and *H. curvispina* collected from an irrigation channel nearby the study area were resistant to the organophosphate azinphosmethyl (Anguiano et al. 2008). Moreover, simuliids from this channel were highly resistant to the pyrethroid fenvalerate (400-fold), which was intensively used for more than twenty years (Montagna et al. 2012). The frequency of resistant genotypes increases in populations under insecticide pressure and the regression lines shift to the right with lower slope values. As the selection pressure continues, increased LC_{50} and LC_{95} values, associated with higher slopes, are indicative of the progression of resistance to a higher intensity and frequency of resistant genotypes. A subsequent decrease in the slope value would be indicative of higher intensities of resistance (Immaraju et al. 1989).

The two most common forms of resistance are target-site modifications that prevent the insecticide binding or interacting at its site of action and enzyme-based resistance (esterases, CYP450, GST) caused by enhanced or modified activities of detoxification enzymes that prevents the insecticide from reaching its molecular target (Hardy 2014). Organophosphate and carbamate insecticides inhibit acetylcholinesterase (AChE), a key enzyme in the cholinergic system that catalyze the hydrolysis of acetylcholine. Generally, AChE insensitivity confers high levels of organophosphate resistance (Bisset et al. 2006, Chen et al. 2007, Djogbenou et al. 2007).

Enzyme activity

The metabolism of most xenobiotics occurs in two phases. The first phase includes oxidations, reductions or hydrolyses or a combination of any of those, and the second phase consists mostly of conjugations. In the first phase, biologically active compounds may be detoxified, and biologically inactive compounds may be activated. The second phase seems to be an inactivating process in the majority of cases (David Josephy et al. 2005).

Esterase activity

Mean CarE activities ± standard error (± SE) of amphipods, snails and planarians from both sites are exhibited in Fig. 3. Contrary to expectations, mean CarE of amphipods from NR (0.018 ± 0.0014 µmoles min^{-1} mg of $protein^{-1}$) was significantly higher than the corresponding counterpart from EF (0.0081 ± 0.0012 µmoles

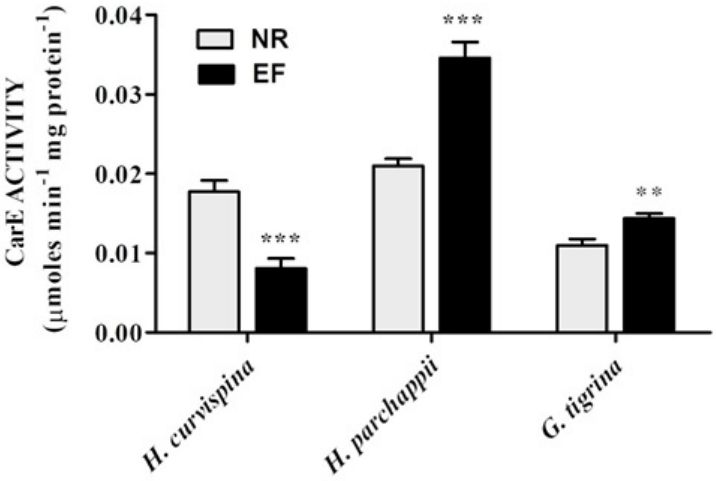

Figure 3. Carboxylesterase activities of amphipods (*H. curvispina*), snails (*H. parchappii*) and planarians (*G. tigrina*). The values were analyzed by one-way ANOVA followed by Bonferroni's multiple comparison test. Each column and bar represent the mean and SE of 5 independent replicates. Asterisks are indicative of significant mean values differences between sites (= P < 0.01; *** = P < 0.001).**

min^{-1} mg of $protein^{-1}$) (F = 26.71; df = 8; p= 0.00085). In contrast, mean enzyme activity ± SE of snails from NR (0.021 ± 0.00089 µmoles min^{-1} mg of $protein^{-1}$) was significantly lower than snails from EF (0.035 ± 0.0020 µmoles min^{-1} mg of $protein^{-1}$) (F = 39.83; df = 8; p= 0.00023). Likewise, CarE activity of planarians from NR (0.011 ± 0.00077 µmoles min^{-1} mg of $protein^{-1}$) was lower and significantly different than the one determined on planarians from EF (0.014 ± 0.00061 µmoles min^{-1} mg of $protein^{-1}$) (F = 11.03; df = 8; p= 0.010).

Carboxylesterases are members of the α, β-serine hydrolase multigene family and are widely expressed in multiple tissues. CarE are multifunctional enzymes that catalyze the hydrolysis of substrates containing ester, amide, and thioester bonds, including carbamate, pyrethroid and others insecticides. These enzymes are irreversibly inhibited by organophosphates during attempted catalytic turnover of these substrates, or reversibly inhibited by carbamates due to slow decarbamylation rates (Ross et al. 2010). Previous studies on azinphosmethyl resistant populations *H. curvispina* and *Simulium* larvae from the area have shown that the organisms from pesticide-contaminated channels exhibited significantly higher CarE activities than those from uncontaminated sites (Anguiano et al. 2008, Montagna et al. 2012). Insects resistance to organophosphate has been associated with changes in CarE activity due to overexpression of carboxylesterase genes attributed to transcriptional up-regulation (Cao et al. 2008), gene amplification (Grigoraki et al. 2017) or both (Pan et al. 2009). The overexpressed CarE proteins increase the sequestration of organophosphates, preventing inhibition of acetylcholinesterase target site (Jokanovic, 2001). The second mechanism of organophosphate resistance associated with CarE refers to amino acid substitutions that converts the enzyme to an organophosphorus hydrolase (Cui et al. 2011). However, the changes in the enzymatic properties of esterases that confers high activities towards organophosphates lead to low activities to common substrates, such as naphthyl acetate (Cui et al. 2015). Further studies on CarE of amphipods from EF site are required to determine if chlorpyrifos resistance is associated to those qualitative changes (CarE gene mutation).

GST activity

Mean GST ± SE activities of amphipods, snails and planarians from both sites is shown in Fig. 4. The average of GST in the amphipods from NR (0.21 ± 0.033 µmol min^{-1} mg $protein^{-1}$) was not

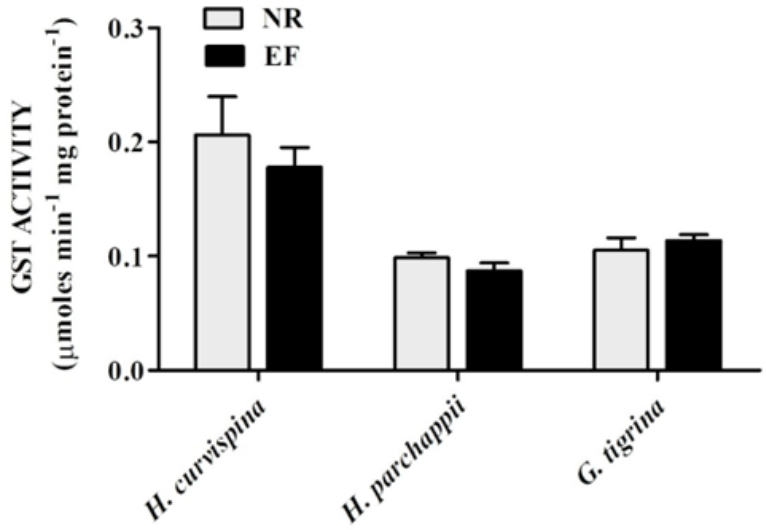

Figure 4. GST activities of amphipods (*H. curvispina*), snails (*H. parchappii*) and planarians (*G. tigrina*). The values were analyzed by one-way ANOVA followed by Bonferroni's multiple comparison test. Each column and bar represent the mean and SE of 5 independent replicates.

significantly different from those collected in EF (0.18 ± 0.0017 µmol min^{-1} mg $protein^{-1}$). Likewise, the average GST activity in snails from NR (0.098 ± 0.0043 µmol min^{-1} mg $protein^{-1}$) was similar, and not significantly different, to the one determined in this species from EF (0.087 ± 0.0071 µmol min^{-1} mg $protein^{-1}$). Finally, the mean GST activity in planarians from NR (0.10 ± 0.011 µmol min^{-1} mg $protein^{-1}$) did not show significant differences to the activity of its counterpart from EF (0.11 ± 0.0054 µmol min^{-1} mg $protein^{-1}$).

GST are multifunctional enzymes that catalyzes the conjugation of reduced glutathione (GSH) to a variety of compounds containing an electrophilic center and in protecting cells from damage and peroxidative products of DNA or lipids (van der Oost et al. 2003). GSH is also a cofactor of glutathione peroxidase and an important nonenzymatic scavenger by donating an electron to other unstable molecules, such as reactive oxygen species (ROS) (Elia et al. 2001). Antioxidant defense is crucial for the organisms to respond to pollutants contamination, including pesticides (Bhagat et al. 2016). The GST response of annelids, mollusks, crustaceans and aquatic insects to pesticides exposure is very diverse and chemical dependent. In many cases, pesticides have a low capacity to induce GST (Domingues et al. 2010). GST enzymes usually provide limited levels of resistance to DDT, organophosphates and pyrethroids. Resistance based on GST seems to be associated with enzyme overproduction as a result of gene duplication or increased transcription rates (Labbé et al. 2011). In simuliids highly resistant to DDT and pyrethroids from irrigation channels in the region, GST activity fluctuated slightly over the years without a direct association to resistance (Montagna et al. 2003, 2012).

ECOD activity

Non-significant differences in mean ECOD activity was found between amphipods from NR (9.44 ± 2.00 pg 7-OHC min^{-1} $adult^{-1}$) and EF (7.02 ± 1.21 pg 7-OHC min^{-1} $adult^{-1}$) (Fig. 5). On the other hand, the ECOD activity of planarians from NR (28.13 ± 2.06 pg 7-OHC min^{-1} $adult^{-1}$) was significantly lower ($p = 0.000078$) than the one determined in planarians from EF (44.53 ± 2.73 pg 7-OHC min^{-1} $adult^{-1}$).

In aquatic invertebrates, the first step in xenobiotic detoxification is mainly governed by CYP450 system (Gottardi et al. 2016). The CYP450 enzymes are a diverse class of enzymes involved in the metabolism of both endogenous and exogenous compounds. CYP450 genes are under

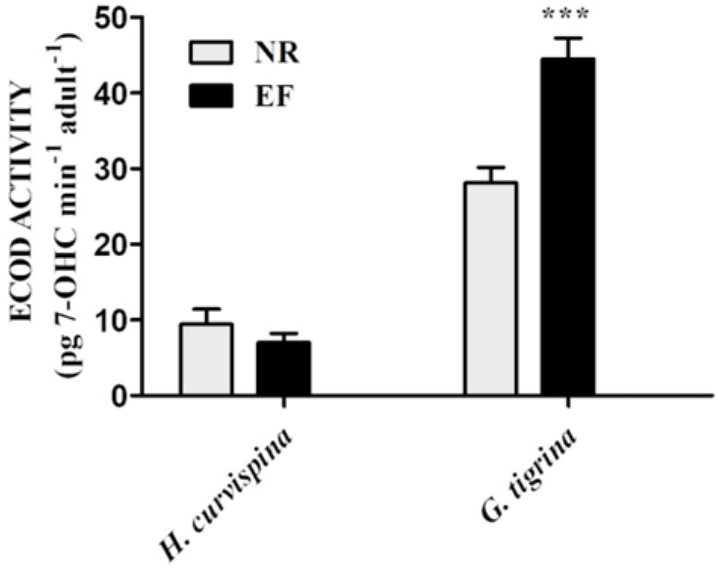

Figure 5. CYP450 activities of amphipods (*H. curvispina*) and planarians (*G. tigrina*) from EF and NR. The values were analyzed by one-way ANOVA followed by Bonferroni's multiple comparison test. Each column and bar represent the mean and SE of individual samples (total individuals analyzed varied between 10-30 according to the available organisms). Asterisks are indicative of significant mean values differences between sites (*** = P < 0.001).

complex regulation where induction play a central role in the adaptation to plant chemicals and regulatory mutations as responsible of insecticide resistance (Feyereisen 1999). CYP450 enzymes have been implicated in resistance of many insect pests by either upregulation, amplification or gain-of-function mutation, which enable them to rapidly metabolize insecticides compared with their susceptible counterparts (Chan et al. 2014). In *Simulium* from the area, piperonyl butoxide (a CYP450 inhibitor) produced high levels of synergism to DDT and the pyrethroid fenvalerate, indicating the role of CYP450 in the detoxification of both pesticides (Montagna et al. 2003).

LIMITATIONS

The number of macroinvertebrate species used for toxicological and biochemical analysis could be underrepresented. Future research should incorporate less abundant species which are probably most susceptible than the ones utilized in the present study.

CONCLUSIONS

Amphipods and planarians inhabiting a drain channel from an agricultural area have developed resistance to chlorpyrifos as a result of its long-term exposure. The present results suggest that chlorpyrifos resistance in amphipods from EF is not conferred by increased detoxification. Therefore, resistance in this population could be attributed by amino acid substitutions in either CarE or acetylcholinesterase (AChE) proteins. On the other hand, chlorpyrifos resistance in planarians may be attributed to the increased of both CYP450 and CarE activities. Although snails from EF were as susceptible as their counterparts from NR, they showed a significant increase in mean CarE activity.

Acknowledgments

Betsabé Lares, Laura B. Parra-Morales and Josefina Del Brio are a fellowship holder of the CONICET for they postdoctoral and doctoral studies. The financial support for this research was provided by a grant from the Universidad Nacional del Comahue Proyect 04/I200 and 04/I237.This project was funded by the National University of Comahue (Grant 04/I196).

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How to cite

LARES B, DEL BRIO J, PARRA-MORALES L, FERNÁNDEZ H & MONTAGNA C. 2023. Chlorpyrifos toxicity and detoxifying enzymes activities in three native-aquatic species of macroinvertebrates from an agricultural area. An Acad Bras Cienc 95: e20191385. DOI 10.1590/0001-3765202320191385.

Manuscript received on November 11, 2019;
accepted for publication on March 30, 2020

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