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Levels of Serum Cholinesterase Activity in the Rococo Toad (*Bufo paracnemis*) in Agrosystems of Argentina

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Agrochemicals have contributed notably to the amphibian decline (Berrill et al. 1994; Carey and Bryant 1995; Alford et al. 2001). Although this vertebrate group is of common use in the laboratory toxicity tests (e.g., Frog Embryo Teratogenesis Assay - *Xenopus*, FETAX) and several anurans have been proposed as model species for studying endocrine disruption by pollutants (Kloas et al. 1999; Lutz and Kloas 1999), a few field studies have evidence a significant relationship between pollution and amphibian population decline (Power et al. 1989; Bridges 1997; Sparling et al. 2001).

A wide range of pesticides is commonly used in Argentina according to Bullacio and Panello (1999) and Arregui (2001). They listed a broad group of agrochemicals such as pyrethroid (e.g. deltamethrin, cypermethrin, alfamethrin), chlorinated (e.g. endosulfan, acetochlor), organophosphorus (e.g. monocrotophos, chlorpyrifos, fenitrothion) or carbamate (e.g. carbofuran, carbendazim) pesticides. Among these agrochemicals, organophosphorus (OPs) and carbamate (CB) compounds constitute an important group of pesticides with an intensive used in Argentina (CASAFE 1999). Despite this, there is no reliable database on the consumption and application rate of pesticides in Argentina. A study conducted at the provinces of Entre Ríos and Santa Fe (Argentina) during 2001-2002 revealed residues of organochlorine pesticides in amphibian tissues (Lajmanovich et al. 2002). In addition, malformations were reported in the anuran *Leptodactylus mystacinus* collected in the same region (Peltzer et al. 2001). These previous observations justify studies on toxic effects from pesticides in the amphibian population from surroundings of crop fields in Argentina (Izaguirre et al. 2000; Lajmanovich et al. 1998; 2003).

As part of a continuing study to assess the adverse impact from the anti-cholinesterase (anti-ChE) pesticides OPs and CBs on a natural population of anurans in the region, the aim of this work was to establish the normal levels of plasma ChE activity in the Rococo toad (*Bufo paracnemis*) and to compare plasma ChE in adult individuals from two agriculture areas and a pristine forest. Remarkably, this toad is uses as sentinel species of pesticide pollution. First, it is extensive distributed in the Neotropical region from northern Argentina, through

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Paraguay and southern Bolivia to the dry Caatinga areas of Bahia and Pernambuco in Brazil (Norman and Naylor 1994). Second, it is frequently found in agricultural lands and urban territories. Indeed, Guzman and Guardia (1978) have demonstrated that ChE activity response to known agents in a co-generic species. Third, the increasing concern in herpetofauna ecotoxicology (Sánchez-Hernández 2001) and the amphibian population decline justify, in part, our efforts in establishing whether pesticide use in Argentina has truly a real impact on amphibian population.

MATERIALS AND METHODS

The study area was situated in midwestern Entre Ríos Province (Argentina). This region is dominated by wetlands, fluvial forest, and temperate steppe vegetation (Bertonatti and Corcuera 2000). Because intensive agriculture was introduced to this area beginning in the 20th century, remaining forest is restricted primarily to riparian woodland surrounded by croplands and pastures.

Thirty six adult *Bufo paracnemis* were collected by hand from three sampling areas, one site was located in a pristine forest (PSM: a natural reserve "Parque General San Martín", 31° 40′ 29′′S; 60° 20′ 13′′O), and two sites (Villa Urquiza and La Picada) situated in suburban woodlands. These two areas differed in the degree of human disturbance, successional stages and crop. At Villa Urquiza (VU, 31° 40′ 29′′S; 60° 20′ 12′′O), the land is used for traditional crop (corn and soy bean) and livestock activities, predominately uplands with a native wood/shrub component. This site also contributed to wildlife habitat and recreation activities. "La Picada" (LP), 31° 43′ 37′′S; 60° 17′ 12′′O) was often used for intensive agricultural production with transgenic soy bean, corn, and sorghum. Moreover, in this site vegetables such as lettuce, tomato, and saltwort are also often cultivated.

The toads were captured in February 2003, with a mean air temperature of about 25 °C. Animals were quickly transported to the laboratory to avoid stress. Samples of blood (*ca.* 500 µL) were extracted in anesthetized animals (30 % ethyl alcohol) by cardiac puncture using a small needle previous locating the heart via palpation. The toads were then released in the same sites where they had been captured. The ChE activity was determined colorimetrically (Ellman et al. 1961) using the Cholinesterase® test provided by Wiener S.A. Lab. Group (Argentina). It is important to notice that Cazenave et al. (2000) and Parma de Croux et al. (2002) validated the kits for blood of another ectotherm vertebrate. The plasma was separated by centrifuging at 4500 r.p.m. for 10 min. Variations in optical density were measured in triplicate at 405 nm for 2 min at 25°C. AChE activity was expressed as catalytic concentration (µkat/L) (ICSH 1972). Data was statistically analyzed using the nonparametric Kruskal-Wallis *KS test*, pairwise comparisons between samples from the three sampling sites were tested by Dunn for post-hoc multiple comparisons test. The impact of sex and body length on serum ChE activity was tested statistically using the nonparametric Mann-Whitney *U* test and Pearson's correlation, respectively.

RESULTS AND DISCUSSION

Mean (± SD) length and weight of *B. paracnemis* was 210 ± 50.5 mm and 750 ± 450 g, respectively. The proportion of collected females to males was not significantly different from 50:50 [Chi-squared (with Yates correction) = 1.63; $p > 0.05$]. Although, the number of toads collected in the reference area was relatively low, no significant differences were found in plasma ChE activity between sex (Mann-Whitney U test = 27; $p > 0.05$) of the toads collected in the non-contaminated area, and no significant correlation was found between ChE activity and length (Pearson's correlation coefficient = 0.24; $p > 0.05$). Serum ChE activity of the lizard *Gallotia galloti* does not show difference between male and female, and the enzyme activity does not correlate with the length of the reptile (Sánchez-Hernández et al. 1997). High levels of dietary fat not alter serum ChE activity (Van Lith et al. 1992). However, avian serum (e.g. *Anser caerulescens caerulescens, Coturnix japonica*) ChE generally shows a significant variation with the size of the animal and the sex (Bargiello et al. 1977; Hill 1989; Thompson and Walker 1994).

The mean values of the ChE activity in the plasma of *B. paracnemis* varied from 310.1 μkat/L for the toads collected at LP to 1885.4 μkat/L for individuals captured at PSM (Fig. 1). The mean ChE activity found in the animals captured in the agriculture area (LP) was significantly lower than those collected in the other two sampling sites varied significantly among sites (Kruskal-Wallis: $KS = 11.91$; $p < 0.01$) but remained relatively similar between the pristine forest (PMS) and the site with low agricultural activities (VU) (Dunn: *Mean Rank* = 3.8; $p > 0.05$). Moreover, this ChE depression was higher in the toads from the site with intensively cropping (LP) respect to other sites (*Mean Rank* = 10.6 and 6.5; $p < 0.05$, respectively).

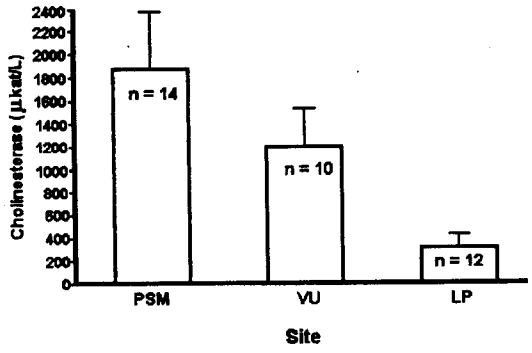

Figure 1. Comparative mean values of ChE activity in three populations of *Bufo paracnemis*. PSM: "Parque San Martín", VU: "Villa Urquiza", and LP: "La Picada". Error bars indicated + 1 SE.

In amphibian populations exposure to anti-ChE pesticides generally is evaluated by comparison of ChE activities from animals collected in non-polluted areas with those from agriculture areas (Sparling et al. 2001). A statistical criterium or diagnostic threshold is commonly used, which is based in the mean ChE activity of the control group (Sánchez-Hernández 2003). Toads having ChE inhibited are accepted to be due to anti-ChE pesticides exposure when ChE activity is depressed by more than 2 standard deviations (SD) below the mean activity in reference toad plasma. All toads from the intensively agricultural site (LP) showed the plasma ChE activity below the diagnostic threshold (reference group mean -2 SD = 929.7 μkat/L). By contrary, only three toads from Villa Urquiza (an area with a relatively low agriculture activity) had plasma ChE activity level less than the diagnostic threshold.

Avian AChE activity depressed by more than 50% is forensic evidence that birds have been exposed to OP pesticides (Hill and Fleming 1982). Sublethal exposures to pesticides could result in ChE depression and related effects (Sánchez-Hernández 2001). In our study, the pristine forest (PSM) and the site with low agricultural activities (VU) appeared to provide suitable habitats that resulted in a low anti-ChE pesticide exposure and consequently in a normal plasma ChE activity, whereas ChE activity in toads collected at the intensively cropped site (LP) decreased by more than 50 % respective to the group caught at the control site.

Although these results are preliminary, the low levels of plasma ChE in the toads from the sampling site LP may be due to a high pesticide impact from an intensively cropping in midwestern Entre Ríos Province of Argentina. However, further studies are necessary to validate this hypothesis and to confirm that plasma ChE depression in this vertebrate species is due to inhibition by anti-ChE pesticides. The close relationship between anurans and agriculture has many potential implications for management (Knutson et al. 1999). Perhaps anurans should be included in the testing of new agricultural chemicals prior to government approval.

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