Effect of Cr(VI) on Early Life Stages of Three Species of Hylid Frogs (Amphibia, Anura) from South America

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ABSTRACT: Hylidae are one of the most diverse and widely distributed frog families in the world. The species selected in the present study is representative for the subtropical wetland area of South America. Considering the global decline of amphibian populations, the present contribution is aimed towards the sensitivity of three frog species to a common pollutant, chromium [Cr(VI)]. Toxicity tests were done exposing organisms at two different stages of development (from 24 h embryos to 120 h larvae and from 144 to 240 h tadpoles) using *Hyla pulchella, H. nana, and Scinax squalirostris*, and measuring mortality and morbidity as end points. Organisms used for testing were obtained from mating pairs collected at a local pond. Assays were done under laboratory standardized conditions with three replications and at least five organisms per replication, using eight dilutions of the toxicant and negative controls. Results of mortality were expressed as 96 h 50% lethal concentration (LC_{50}). Slope of dose−response plots for the three species were obtained by regression analysis. Comparisons were done by simple linear regression equations. Results from regression analysis show that sensitivity of the three species differs significantly ($p < 0.01$). Although the presence of malformations was observed, organisms at earlier stages present a lower sensitivity to chromium. Regarding lethal effects, *S. squalirostris* is more sensitive ($LC_{50} = 4.72$ mg/L) than the other species (*H. nana* $LC_{50} = 10.99$ mg/L and *H. pulchella* $LC_{50} = 19.67$ mg/L) in tests with organisms of the second stage. The present communication contributes to knowledge on the biological effects of Cr(VI) on poorly studied neotropical frog species.   © 2000 by John Wiley & Sons, Inc. Environ Toxicol 15: 509−512, 2000

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INTRODUCTION

Amphibians are key components of many ecosystems, and their disappearance may complicate efforts to manage ecosystems on a sustainable basis (Corn, 1994). The widespread decline of amphibian species has re-

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cently received considerable attention, although this phenomenon has been perceived by American researchers about 30 years ago (Gibbs et al., 1971; Hine et al., 1981). At present, it is accepted that amphibian decline is a global problem, and a variety of explanations have been offered. The proposed causes for decline are either anthropogenic or natural (Corn, 1994). Among the human-induced causes of habitat alteration, pollutant discharges are considered as one of the

major responsible agents of species decline. The study of the effect of different contaminants, such as heavy metals, to the development of amphibians will help us to identify the various causes of declining populations.

Most studies concerning the effect of heavy metals on frog development have been carried out considering frogs from the Holarctic Region. In the neotropics, researchers have centered their attention mostly on toad species of the genus *Bufo* (Bufonidae), because they are easy to maintain and grow in captivity; only a few studies were carried out on tree frogs of the family Hylidae (Natale, Basso, and Ronco, unpublished results). Hylidae is one of the most diverse and widely distributed frog families in the world, and they are extremely variable in size and external appearance, being largely arboreal. Some hylid species from southern South America seem to be very sensitive to habitat degradation and the study of the effect of pure compounds on their early life stages is relevant in order to monitor the health of the habitats where they dwell.

Hyla pulchella, *H. nana*, and *Scinax squalirostris* are neotropical hylid frogs that can be easily found in sympatry at several wetlands of Buenos Aires province in Argentina and northern localities. In this contribution an analysis was conducted to determine the sensitivity of these tree-frog species to a common pollutant chromium (VI) [Cr(VI)] by means of laboratory toxicity bioassays using early life stages of these organisms.

MATERIALS AND METHODS

Test Species

The species used in this study are *H. pulchella*, *H. nana*, and *S. squalirostris*. These species are members of the tree frogs of the family Hylidae. They are slender, medium-sized to small frogs with smooth and permeable skin. The three species lay their eggs in masses attached to the submerged stems of aquatic plants, and they develop into aquatic larvae (tadpoles). The breeding season occurs during the austral spring and summer periods, corresponding to the maximum yearly rainfalls (Cei, 1980).

Source of Organisms

Adult frogs were collected from a clean permanent pond located near La Plata, Province of Buenos Aires (35°01′S, 57°51′W). Mating pairs from all the studied species were captured during amplexus and placed in 3 L glass jars containing water and vegetation from the site. Once the pair was conditioned to the jar, it was deposited within the natural habitat until egg laying was observed. Eggs obtained by this procedure were transferred to the laboratory for bioassay studies.

Maintenance of Organisms in the Laboratory

Eggs were kept in the laboratory at 25°C ± 0.5, with a light:dark period 16:8, in dechlorinated tap water with a pH of 7.5 saturated with oxygen. After acclimatization during 24 h, embryos were randomly sorted, a portion used in toxicity tests, and another portion left for development to stage 25 (Gosner, 1960), also used for testing. Feeding of tadpoles was not necessary.

Experimental Design and Acute Toxicity Test Conditions

Toxicity tests were done exposing organisms at two different stages of development: from 24 h embryos to 120 h larvae and from 144 to 240 h tadpoles (stages 0−25 and 25 on, respectively) for the three species during 96 h. Assays were performed in 3 L jars filled with 1 L dechlorinated tap water with five organisms per jar. Conditions were the same as those used for maintenance. Media were completely replaced every day during exposure tests. Toxicity tests were done using eight concentrations of Cr(VI) based on a previous experiment (1.75, 5.25, 8.75, 12.25, 15.75, 19.25, 22.75, 26.25 mg/L) with three replications and negative controls. Concentrations after exposure were confirmed by chemical analysis of Cr(VI) using atomic absorption spectrophotometry. Solutions of Cr(VI) were prepared from $K_2Cr_2O_7$ (Merck, analytical grade). Assessment of mortality as the endpoint was done by visual observation of immobility after gently prodding the organisms with a glass rod. Readings of mortality were done every 24 h during exposure.

Statistical Analysis of Results

Log−probit transformation of mortality data was done before regression analysis (Finney, 1971). Regression and correlation analyses were done for each assay according to the stage of development of organisms used in tests and the species. LC_{50} estimation for the three species and confidence interval were obtained by inverse prediction (Sokal and Rohlf, 1981; Zar, 1998) and also by means of the PROBIT program to corroborate results.

A comparison of the linear regression equations for the three species was done to assess significant differences (Zar, 1998).

RESULTS AND DISCUSSION

Negative control tests with embryos and tadpoles did not show mortality. Exposed embryos of *H. pulchella*, *H. nana*, and *S. squalirostris* showed up to 20, 33, and

100% mortality, respectively, within the tested concentration range. The 96 h LC_{50} for *S. squalirostris* was 9.0 [7.5−10.1] mg Cr(VI)/L. Surviving embryos from the three species after exposure evidenced different types of malformations involving their heads, notochords, fins, and skin.

Results of toxicity tests to Cr(VI) with tadpoles, expressed as LC_{50}, confidence interval, regression, and correlation parameters for the three species are included in Table I and Fig. 1. Results of the comparison between slopes corresponding to the set of data from bioassays with tadpoles of the three species can be seen in Table II.

LC_{50} data from tadpoles included in Table I agree with the LC_{50} values estimated by means of the PROBIT program. These values and confidence limits are for *S. Squalirostris*, *H. nana*, and *H. pulchella*: 4.18, 10.99, and 19.67 mg Cr(VI)/L, respectively. These results show differences in sensitivity to Cr(VI) with no overlap between confidence limits. According to the results, *S. squalirostris* is the most sensitive species, followed by *H. nana* and *H. pulchella*.

Results from regression analysis (Table I), showing significant differences in slope from zero, indicate differences of response for each concentration of toxicant per species. The fit of the data sets to a linear model can be assessed by r and r^2 values from Table I.

Comparisons from linear regression equations (Table II) indicate no differences between slopes and significant differences between elevations. The Tuckey test of elevations also indicates differences between the responses for the three species. This last analysis agrees with the one done with the results of LC_{50} (Fig 1).

It can be concluded that the tested hylid frog embryos are less sensitive to Cr(VI) than tadpoles. Also, *S. squalirostris* tadpoles are at least four times more sensitive than *H. pulchella*. If the sensitivity to other toxicants is similar, tadpoles of *S. squalirostris* could be

TABLE I. Results of toxicity tests with Cr(VI) (mg/L) to tadpoles of three species of hylid frogs. b = slope (regression coefficient), a = elevation, r = correlation coefficient, r^2 = determination coefficient, n = number of pairs of x and y values. (*) Significantly different from zero slope

| Species | LC_{50} Analysis | | Regression and Correlation Analysis | | | | |
	LC_{50}	Confidence Interval (95%)	b	A	r	r^2	n
S. squalirostris	4.05	2.89−5.63	3.57*	1.19	0.99	0.99	3
H. nana	10.48	0.52−14.44	5.07*	−2.46	0.96	0.93	3
H. pulchella	16.26	12.18−31.59	4.49*	−2.83	0.93	0.86	5

S. squalirostris (y=1.19+3.57*x)

H. nana (y=−2.46+5.07*x)

H. pulchella (y=−2.83+4.49*x)
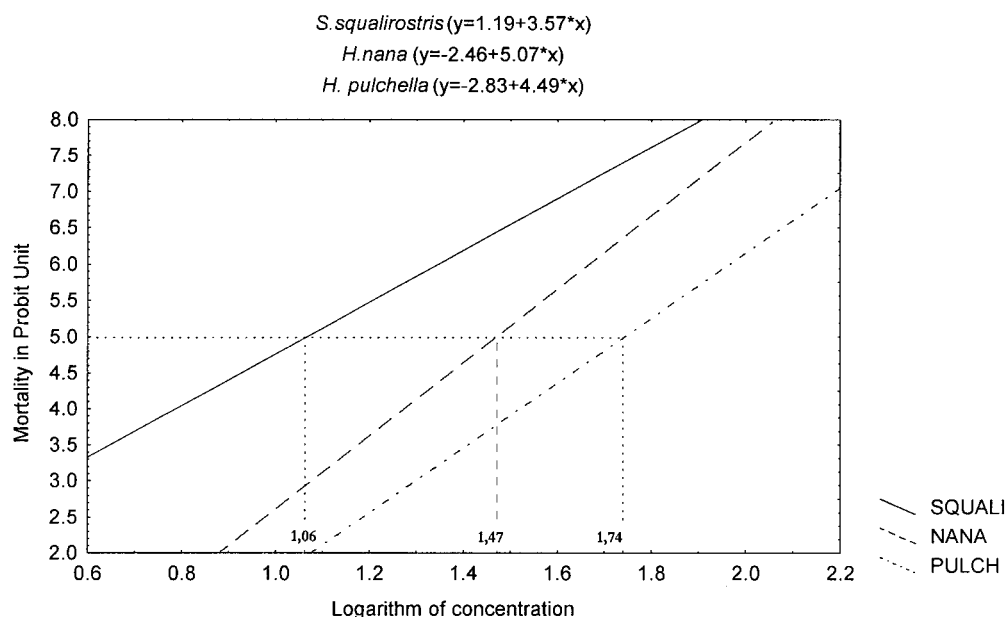
Fig. 1. Comparison of regression equations of Cr(VI) lethality for *H. pulchella*, *H. nana*, and *S. squalirostris*.

TABLE II. Comparison of the linear regression equations for three species of hylid frogs expressed as Cr(VI)

Test		Null Hypothesis	Statistic Test	Critical Value	Accept/Reject
For differences among slopes		$b1 = b2 = b3$	$F = 0.70$	$F = 5.78$	A
For differences among elevations		$a1 = a2 = a3$	$F = 51.10$	$F = 4.73$	R
Multiple	A	1 vs. 2	$q = 6.41$	$q = 3.63$	R
comparisons	B	1 vs. 3	$q = 10.23$	$q = 3.63$	R
among elevations	C	2 vs. 3	$q = 12.41$	$q = 3.63$	R

considered adequate reference organisms in regional environmental monitoring programs. Sensitivity of these species to Cr(VI) is higher compared to that found with other genera of anurans such as *Bufo*, *Rana*, and *Xenopus* (Schuytema et al., 1996) and some fish species (Indorato et al., 1984) and lower than microcrustaceans (Bulus, Rossini, and Ronco, 1996).

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