Bull. Environ. Contam. Toxicol. (1996) 56:663-669


Bulletin of Environmental Contamination and Toxicology

# Stage-Dependent Uptake of Cadmium by *Bufo arenarum* Embryos

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Received: 8 November 1994/Accepted: 7 September 1995

Over the last several years, environmental contamination with cadmium has significantly increased because of its extensive use in anthropogenic activities. This heavy metal is a very toxic xenobiotic producing reproductive and developmental impairments in a wide spectrum of organisms (Chernoff 1973; Manson and O'Flaherty 1978; Rombough and Garside 1982; Pérez-Coll et al. 1985; Belmonte et al. 1989; Rivera et al. 1990). Within the life cycle of organisms, the embryo is the most sensitive period to adverse conditions (Olsson et al. 1990; EPA 1991). Moreover, stage-dependent susceptibilities to toxic agents in amphibian embryos treated with lead (Pérez-Coll and Herkovits 1990) cadmium (Herkovits and Pérez-Coll 1993) and aluminium (Beattie et al. 1992) were described. In the case of cadmium, this differential sensitivity could be related to changes in the metal accumulation through development (Herkovits et al. 1991) or in the induction of defense mechanisms against cadmium toxicity, such as metallothionein (Mt) synthesis, which seems to be developmentally regulated (Wilkinson and Nemer 1987; Olsson et al. 1990). In the case of the toad Bufo arenarum, susceptibility to cadmium seems to follow a biphasic pattern during embryonic development. From the two-cell stage to the neurula stage an increase in susceptibility occurs, whereas from the last stage onwards a gradual increase in the resistance against this heavy metal seems to be achieved (Herkovits and Pérez-Coll 1993).

From an ecotoxicological perspective, the threshold concentrations of xenobiotics for very short exposure periods are meaningful for establishing water quality criteria and water quality-based toxic-wastes control, while on the other hand amphibian embryos could be very useful for performing short-term toxicity tests. In this context, it could be of interest to correlate cadmium toxicity and uptake values in B. arenarum embryos exposed to short treatments, as in this case from 15 to 240 minutes. Considering that the high resistance against cadmium toxicity during blastula stage seems to be related to a very reduced uptake of this heavy metal in fish (Michibata 1981), as well as in amphibians (Herkovits et al. 1991), we report in the present study the uptake profile of cadmium at different post-hatching stages from the appearance of cardiac beat to the end of embryonic development.

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The relationship between the concentrations of cadmium in the external solutions and embryo is also reported.

## MATERIALS AND METHODS

Ovulation of B. arenarum females was induced by means of intraperitoneal injection of homologous hypophysis. Oocytes were fertilized "in vitro" with a sperm suspension made in 10% Holtfreter's solution (HS). The development of embryos was staged according to the table of Del Conte and Sirlin (1951). The uptake experiments were repeated three times with embryos obtained from different couples of parents and they were carried out at a controlled temperature of 20+/- 0.5°C. The following embryonic stages of different susceptibility to cadmium were selected to measure cadmium uptake: cardiac beat (S.19) gill circulation (S.20), open mouth (S.21) and complete operculum (S.25). Embryos were placed in glass petridishes in the proportion of 50 embryos/40 ml of solution (in duplicate) for embryos from S.19 to S.21, while at S.25 only 25 embryos/40 ml of solution (in quadruplicate) were placed due to the size of the embryos, Cadmium uptake was evaluated in the different batches of embryos for each developmental stage and exposure time tested. Embryos were exposed to cadmium for 15, 30, 60, 120, 180 and 240 min at different concentrations depending on the developmental stage as follows:

| Stage (S.) | mg $Cd^{++}$/L |
|---|---|
| Cardiac beat (S.19) | 0.06, 0.12, 0.25 and 0.50 |
| Gill circulation (S.20) | 0.06, 0.12, 0.25 and 0.50 |
| Open mouth (S.21) | 0.06, 0.12, 0.25 and 0.50 |
| Complete operculum (S.25) | 0.06, 0.12, 0.25, 0.50, 1.00, 2.00 and 3.00 |

Cadmium solutions were prepared from a standard solution for atomic absorption spectrophotometry (Sigma) and diluted with HS. The controls were groups of 50 embryos (in duplicate) from S.19 to S.21 or 25 embryos (in quadruplicate) from S.25 incubated in HS without additions and maintained simultaneously with treated embryos. At the end of treatment, embryos were twice washed with HS and digested with concentrated nitrosulfuric acid (1:1 v/v) until complete mineralization. Cadmium contents from 50 embryos at each measurement were analyzed with a Shimatzu AA640 atomic absorption spectrophotometer, at the wave length of 228,8 nm, detection limit 0.001 mg Cd++/L. Calibration was performed using a standard solution (Sigma) for atomic absorption spectrophotometry. The accuracy of the determination was checked by analyzing ten replicates of a solution containing 0.02 mg Cd++/L giving a mean value of 98.04% of the nominal concentration, with a coefficient of variation of 13.90%. Results were statistically analyzed with the non-parametric Kruskal Wallis test with multiple contrasts using the STATIGRAPHICS program, vs 4.0.

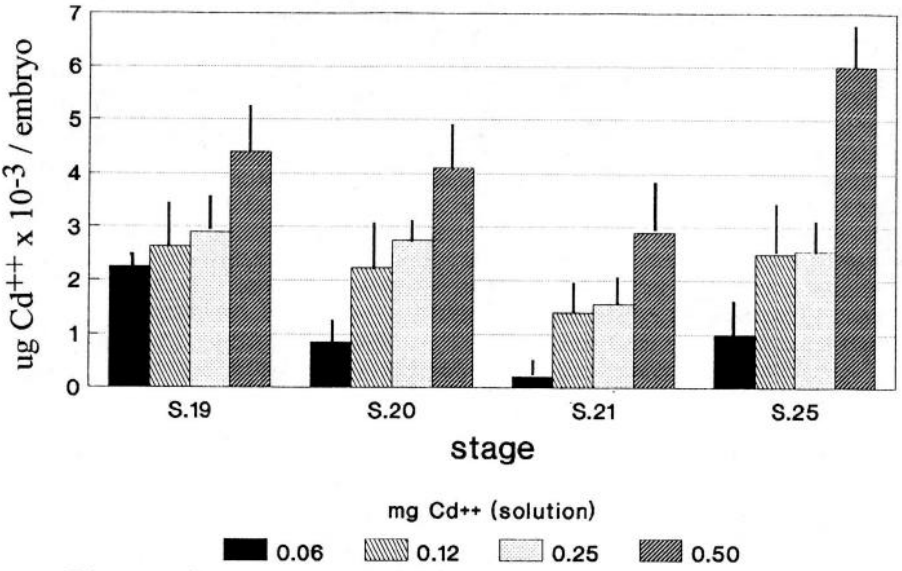


Figure 1.Cd uptake in different stages of Bufo arenarum at 60 min of exposure.

## RESULTS AND DISCUSSION

Within this short-term cadmium exposure and uptake study on B.arenarum embryos, the concentrations evaluated ranged between values which are below and above the 24-hr LC100, even considering the changes due to the stage-dependent susceptibility for these embryos (Herkovits and Pérez-Coll 1993). To compare uptake results of these developmental changes, four of the cadmium concentrations evaluated ranged from 0.06 mg $Cd^{++}$/L up to 0.5 mg $Cd^{++}$/L and were common for all stages evaluated in this study. Although several of these concentrations were higher than the 24-hr LC100 for most of the stages evaluated, only embryos at cardiac beat stage, exposed to 0.25 mg $Cd^{++}$/L expressed a lethal effect which occurred within the three hours of treatment. This result confirmed the higher susceptibility to cadmium of embryos at this stage (S.19) in relation to later development, while on the other hand, this also points out that stage-dependent susceptibility can be evaluated based on concentration as well as on time-exposure protocols (Herkovits and Pérez-Coll 1990)

Within the first hour of exposure (Fig. 1), cadmium uptake increased significantly ($p<0.05$) in proportion to the external concentration (except for the case of 0.12 and 0.25 mg $Cd^{++}$/L that showed similar uptakes), but it is noteworthy that the amounts incorporated for each concentration were higher at S.19 and diminished gradually to S.21, resulting in a statistically significant ($p<0.01$) difference for 0.06

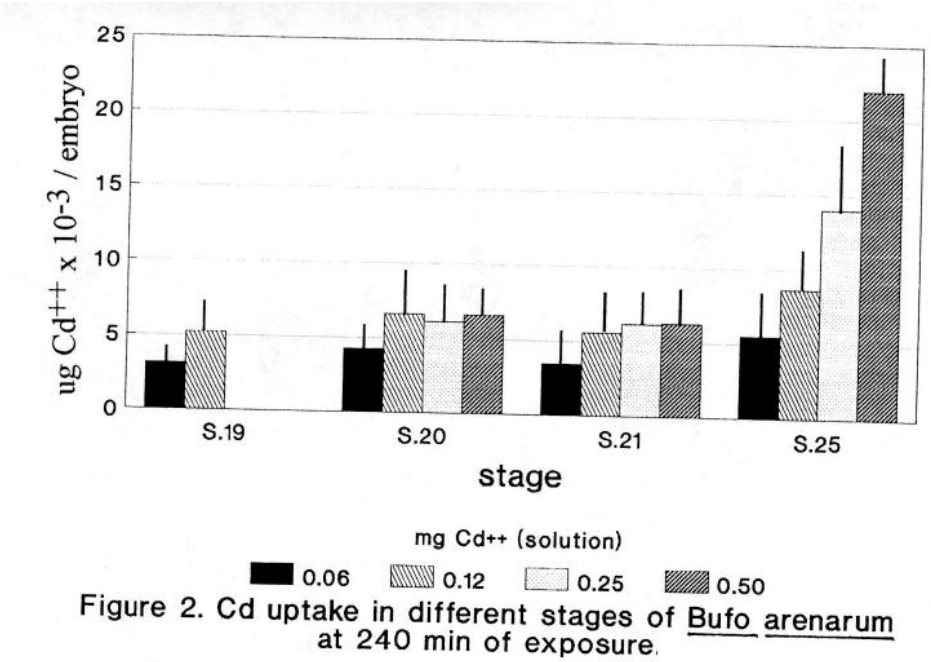


Figure 2. Cd uptake in different stages of Bufo arenarum at 240 min of exposure.

mg $Cd^{++}$/L between the uptake at S.19 and S.21. This tendency appeared to revert at S.25 and, moreover, for 0.50 mg $Cd^{++}$/L the uptake value was about two times the result obtained at S.21. Therefore, based on data of the first hour of cadmium exposure, the increasing resistance to cadmium toxicity from S.19 to S.21 could be related to a lower uptake of this heavy metal, while at S.25 the increasing resistance could have been related to other protective mechanisms of these embryos against cadmium toxicity. For example, changes in metallothioneins (Mt) could be involved, as these molecules seem to be developmentally regulated both in sea urchin (Wilkinson and Nemer 1987) and fish (Olsson et al. 1990). Moreover, other features such as cytosolic molecules, as well as subcellular compartmentation in membrane-limited vesicles (Coombs and George 1978) or even an increase in the extracellular compartment, which occurs as development advances, could also have been involved in the increase in uptake as well as in tolerance to cadmium at the last stages of embryonic development.

At four hours of exposure (Fig. 2) the pattern of uptake did not show any significant differences ($p<0.05$) between S.19 (in conditions which allow embryo survival) and S.21, and it is noteworthy that uptake at these stages appeared to be not related to the external concentration, while for stage 25 there was a conspicuous increase in cadmium uptake. This result points out the existence of a sharp stage-dependent phenomenon with saturation of the cadmium transport system at S.19-S.21 and not saturation at S.25, in which the embryos exposed to

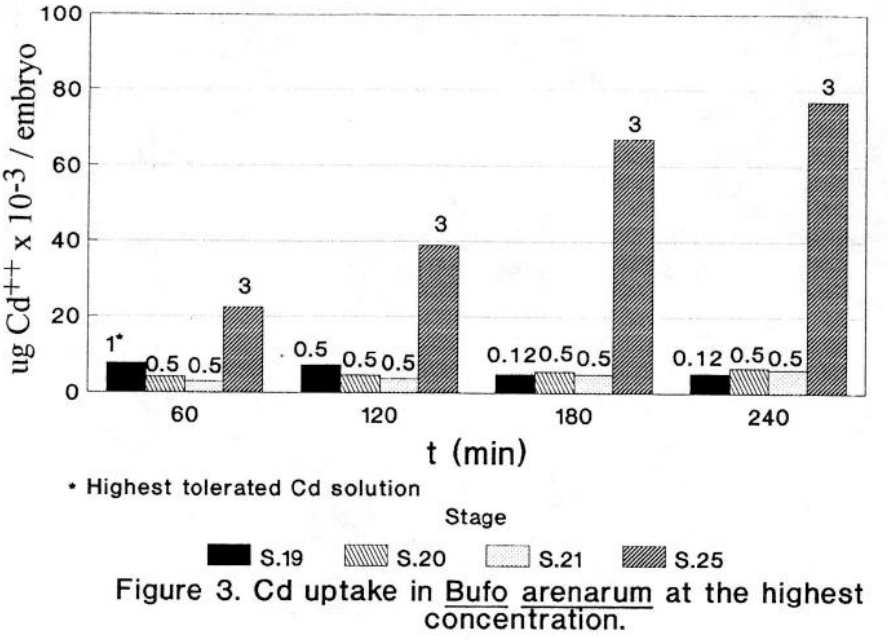


Figure 3. Cd uptake in Bufo arenarum at the highest concentration.

0.5 mg $Cd^{++}$/L during 240 min attained an uptake value three times higher than in previous stages.

In cadmium-exposed Mytilus edulis there was no energy, temperature, aerobic dependency or competition with other cations such as zinc, concluding that cadmium uptake was by diffusion (Carpene and George 1981). However, in other experimental systems using simultaneous incubations of human erythrocytes with Zn or Cu, a decrease in the cadmium uptake rate and transport constant was observed, suggesting that the cadmium uptake occurred by a mediated transport via cations chemically similar to it (Nguyen and Chien 1988). The relationship between the concentration of cadmium in the external solutions and in the embryos is shown in Fig. 3. It is noteworthy that the embryos exposed over four hours in only few days of development (from S.19 to S.25) increased their resistance to Cd at least about 25 times (from 0.12 to 3 mg $Cd^{++}$/L), while the uptake value increased only about 15 times (from 5.2 to 76.8 x 10-3 ug $Cd^{++}$/embryo). The fact that a direct relationship between Mt-bound cadmium and tissue-cadmium concentration is generally accepted (Nöel-Lambot et al. 1980) also supports a possible stage-dependent increase in Mt at the end of B.arenarum embryonic development.

Acknowledgments. We wish to acknowledge Dr. Susana Zeni and Lic. Laura Ruiz for colaborating in this study and thank Ing. Federico Ushase of Cátedra de

Fisiología, Facultad de Veterinaria, Universidad de Bs. As. for his assistance in the atomic absorption spectrophotometry measurements. We also thank anonymous reviewers for their comments. The work was supported by a grant from CONICET.

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