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Uptake and Elimination of Cadmium in *Rana dalmatina* (Anura, Amphibia) Tadpoles

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Cadmium is widely distributed in the earth's crust, generally at relatively low concentrations. In most areas where it is concentrated, the concentrations are due to anthropogenic activities. In aquatic systems, organisms most commonly take it up directly from water through the gills and/or skin, but cadmium may also be ingested with contaminated food (Yost 1984). There is no evidence for the biomagnification of cadmium within marine and freshwater food webs; however, uptake of the free metal (Cd^{2+}) is often followed by accumulation in freshwater organisms. Cadmium is highly toxic to a wide range of animals. It has no known essential biological function and its presence in tissues reflects the exposure of the organisms to Cd in the environment (WHO 1992).

The liver and kidneys are the main organs for Cd accumulation in amphibians and the synthesis of metallothionein, a low molecular weight protein, seems to be of a major importance in their defence against the toxic cadmium ion (Vogiatzis and Loumbourdis 1997). The toxicity and toxic effects of cadmium generally result from binding of the metal with reactive and/or complexing groups leading to inhibition of enzymatic processes, possibly disturbing general growth, development and reproduction (WHO 1992).

The most common abnormalities in amphibians caused by exposure to sublethal concentrations are retardation of growth and limb regeneration (Nebeker et al. 1994; Nebeker et al. 1995), epidermic deformities (Ferrari and Salibián 1999) and malformations affecting the morphology and organisation of head, skeleton, eyes and tail formation (Calevro et al. 1998; Herkovits et al. 1997; Plowman et al. 1994).

In our study we a) evaluated the uptake of cadmium in *Rana dalmatina* tadpoles by exposing them to 0.5 mgCd^{2+}/L and 1 mgCd^{2+}/L for 24, 48, 72, and 96 hr; b) observed the behavioural responses of tadpoles during the period of exposure, and c) monitored the cadmium concentrations in tadpoles during detoxification after the 96-hr exposures in 0.5 mgCd^{2+}/L and 1 mgCd^{2+}/L. Uptake and elimination of cadmium was measured using the radioisotopic tracer ^{109}Cd.

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MATERIALS AND METHODS

A clutch of *Rana dalmatina* (Anura, Amphibia) was collected from stream Glinščica in the close vicinity of the Department of biology (Ljubljana, Slovenia) and brought to the laboratory. After hatching the larvae were kept at room temperature in a glass aquarium (60 x 29 x 26 cm) with stream water, aerated by the introduction of an air stone fed from an air pump. The larvae were fed every day with commercially available fish food (TetraPhyll). A total number of 104 two-month old tadpoles were used for the experiments. All experiments were conducted with animals acclimatized, 48 hr before the experiments began, to 10 % Holtfreter solution (HS) at room temperature without food. The composition of the stock HS was (in g/L): NaCl 3.50; KCl 0.05; $CaCl_2$ 0.10; $NaHCO_3$ 0.02. Before use, this solution was diluted (1:9) with double-distilled water to obtain the assayed concentration.

Prior to our cadmium uptake and elimination studies, we prepared two experimental solutions with different cadmium concentrations; 1 mgCd^{2+}/L (A) and 0.5 mgCd^{2+}/L (B). The basic composition of both waters was 10 % HS which was spiked with 5.45 mM $CdCl_2$ and cadmium radiotracer (^{109}Cd; 266 Bq/ml in 0.5 M HCl) and then adjusted to pH 6,5 by addition of 1% NH_3. To determine the specific activity of the tracer in experimental solutions A and B, 1 ml of each was transferred to clean polypropylene scintillation vials for measurement. A germanium well type detector, connected to a Canberra Series 90 multichannel analyzer, was used in the gamma ray activity measurements. Ten polypropylene beakers were filled with 200 ml of experimental water, five with experimental water A and five with experimental water B.

For cadmium uptake studies, 80 tadpoles of comparable size were placed, in groups of tens, into eight polypropylene beakers, each containing 200 ml of experimental water A or B (40 animals for each cadmium concentration). The tadpoles were not fed during the experiment, which was performed at room temperature (ca. 20 ± 1°C). During the period of exposure the behavioral responses of tadpoles were recorded. The uptake of ^{109}Cd by the tadpoles was measured at 24, 48, 72 and 96 hr exposure periods. At the end of each exposure period 10 animals were transferred firstly to a polypropylene beaker filled with 10% HS (to remove surface-bound cadmium tracer) for five minutes, secondly to polypropylene beaker containing the sedative ethyl 3-aminobenzoate methane sulphonic acid salt (MS-222) for one minute, and thirdly to polypropylene scintillation vials where they were weighed and the total body ^{109}Cd radioactivity was counted for 300 s in the gamma counter. The experimental water with animal excrementa was filtered through a 0.45 μm filter (Nalgene). 1 ml of filtered water and filter with excrementa were transferred to polypropylene scintillation vials and counted for ^{109}Cd for 300 s in the gamma counter.

For cadmium elimination studies 24 tadpoles of comparable size were placed, in groups of twelve, into two polypropylene beakers, each containing 200 ml of experimental water A or B (12 animals for each cadmium concentration). The

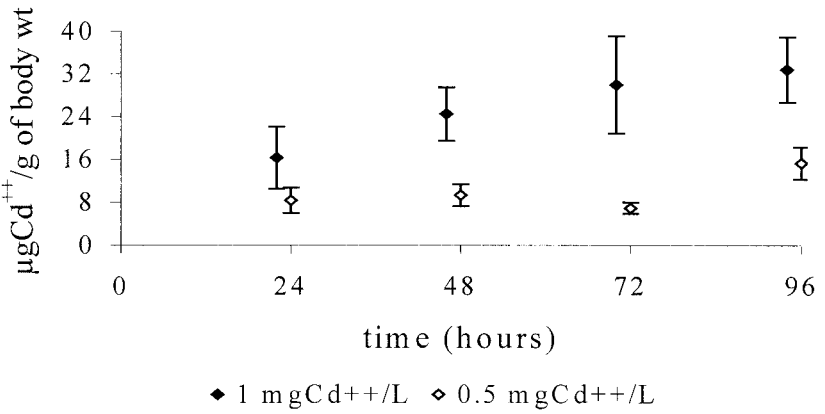

Figure 1. Cadmium concentration (mean ± SE) in *Rana dalmatina* tadpoles exposed to 1 mgCd^{2+}/L and 0.5 mgCd^{2+}/L for 24, 48, 72 and 96 hr.

tadpoles were exposed to ^{109}Cd labeled cadmium for 96 hr. The experiment was again performed at room temperature without feeding the animals.

Following exposure, tadpoles were twice washed with 10% HS and then transferred to polypropylene scintillation vials where they were weighed and the total body ^{109}Cd radioactivity was counted for 300 s in the gamma counter. After the measurements were taken, the animals were individually placed into polypropylene beakers containing 50 ml of 10% HS and were fed with commercially available fish food (TetraPhyll). On days 3, 6, 10, 14, 19, and 24, the total body ^{109}Cd was counted. On each occasion after counting and weighing, tadpoles were returned back to the polypropylene beakers with 50 ml of clean 10 % HS and fed.

Calculation of the quantitative uptake and elimination of cadmium was based on the known relationship between the initial concentration of added cadmium in the water and the radioactivity of ^{109}Cd radiotracer. The mass of cadmium in the tracer solution itself was negligible in comparison to the added Cd. Thus, the measured activity of ^{109}Cd in water, tadpoles, and excreta corresponded to a known mass of Cd. Corrections for radioactive decay was applied.

RESULTS AND DISCUSSION

There was statistically significant difference ($p<0.05$) between the cadmium content of the tadpoles exposed to the two different Cd^{2+} concentrations during the uptake experiments at 48, 72, and 96 hours (Fig.1). The mean cadmium concentration in tadpoles exposed to 1 mg Cd^{2+}/L was approximately twice as high after 24 and 96 hours, three-times after 48 hours, and four-times after 72 hours of exposure than in tadpoles exposed to 0.5 mg Cd^{2+}/L.

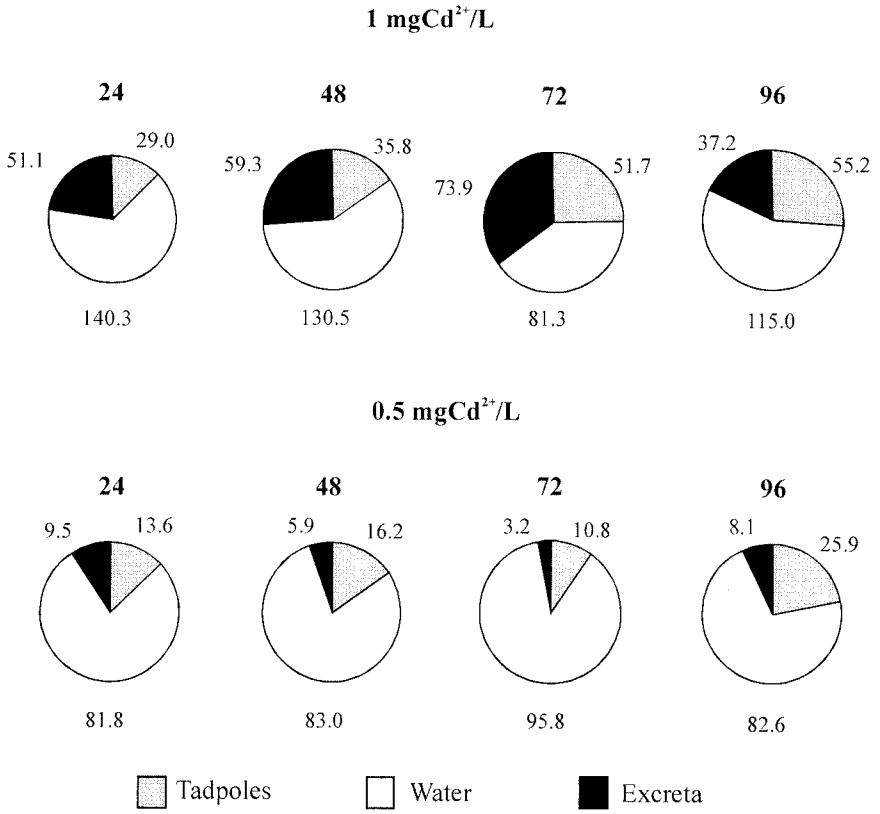

Figure 2. Distribution of cadmium (in μg) in experimental water, excrementa, and tadpoles after 24, 48, 72, and 96 hr of exposure to 1 $mgCd^{2+}/L$ and 0.5 $mgCd^{2+}/L$.

The uptake of cadmium occurred via water through the gills and skin because the tadpoles were not fed during the experiments. We also presume that the uptake of cadmium mainly occurred through the gills, while the uptake through the skin was a minor source. Namely, a layer of mucus, which decreases the diffusion rate of cadmium, covers the external surface of tadpoles. It is well known that Cd^{2+} forms strong covalent bonds with SH- groups of a wide range of biological molecules and for this reason, mucus form a strong complexing agent for cadmium (Pärt and Lock 1983).

Mean cadmium content in tadpoles exposed to 1 $mgCd^{2+}/L$ increased gradually with exposure time, while in tadpoles exposed to 0.5 $mgCd^{2+}/L$ a decrease in the mean cadmium concentration was observed after the 72 hours of exposure. This may be related to metal excretion from the tadpoles into the experimental water, since an increase in the water activity (Fig. 2) after 72-hr of exposure was observed.

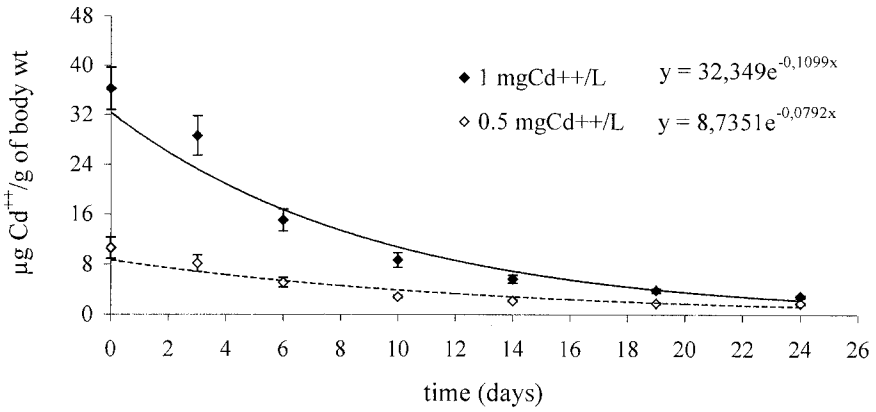

Figure 3. Cadmium concentration (mean ± SE) in *Rana dalmatina* tadpoles at different days of depuration after 96 hr of exposure in 1 $mgCd^{2+}/L$ and 0.5 $mgCd^{2+}/L$.

Within the life cycle of an organism, embryos are often times the most sensitive developmental stage subject to chemical exposure. During the experiments no tadpole mortality was observed. Reductions in activity and atypical swimming position were observed among tadpoles exposed to 1 mg Cd^{2+}/L and 0.5 mg Cd^{2+}/L, while gulping of air from the surface and intensive excretion were noted only among tadpoles exposed to 1 mg Cd^{2+}/L. These changes were became more apparent during the course of the experiment. Muiño et al. (1990) reported that mortality of *Bufo arenarum* tadpoles was less than 10% at 0.5 and 1 $mgCd^{2+}/L$. A concentration of 0.2 $mgCd^{2+}/L$ arrested the development of the *Xenopus laevis* embryos at stage 40 (blood circulation in gills) in 50 % of cases (Herkovits et al. 1998), while a concentration of 1 $mgCd^{2+}/L$ was effective in 100 % of cases within 24 hours (Herkovits et al. 1997). An increased resistance to cadmium toxicity in amphibians was observed after pre-treatment with low Cd/Zn concentrations (Herkovits and Pérez-Coll 1995), or after administration of the chelating agent EDTA (Hilmy et al. 1986).

Variability in the uptake of Cd^{2+} within tadpoles exposed to the same cadmium concentration at the same exposure time may be related to the fact that each tadpole is a unique and complex organism with a different susceptibility to cadmium. On the other hand, it may also be related to the different swimming activity of the tadpoles during the exposure tests, because some of them were much more active than those lying at the bottom of the beakers most of the time.

When the tadpoles were placed into clean water in the detoxification study (Fig. 3), 50 % of the accumulated metal was discharged during the first 6 days in the group exposed to 1 $mgCd^{2+}/L$, whereas in the group exposed to 0.5 $mgCd^{2+}/L$ elimination was slower, taking about 10 days to be reduced by half. At the end of

24 days only 8 % of the metal remained in the tadpoles exposed to 1 mgCd^{2+}/L, while in the tadpoles exposed to 0.5 mgCd^{2+}/L the concentration of cadmium dropped just to 17 % of the original value at the end of 24 days of depuration. Cadmium concentrations in tadpoles by the end of the detoxification period were 7-20 (1 mgCd^{2+}/L) and 3-12 (0.5 mgCd^{2+}/L) times lower compared to the concentrations of the heavy metal at the end of the exposure period.

The phenomenon that more rapid elimination of a heavy metal occurs at higher concentrations (or at the beginning of the elimination process) is well known, but in the case of tadpoles few experimental data are available and more information would be needed before speculating on possible mechanisms.

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