INTRODUCTION

Cadmium is a naturally ubiquitous heavy metal that is very toxic to aquatic and terrestrial wildlife. A xenobiotic with no known biological function, it causes a number of reproductive and developmental impairments [1]. It is found in elevated concentrations around the world because of human activities, such as mining, industrial dumping, and the application of municipal sludge and phosphate fertilizers to agricultural fields [1]. While the U.S. Environmental Protection Agency (U.S. EPA) has set an aquatic chronic cadmium criterion of 0.15 μg/L (hardness 50 mg/L; [2]), concentrations may surpass 400 μg/L in highly polluted areas [3] (http://pacific.fws.gov/ec/Restoration.htm). This value approaches or exceeds the concentration lethal to 50% of organisms in 96 h (96-h LC50) of many aquatic organisms. Temporary rain pools at contaminated sites may jeopardize colonizing species, such as breeding amphibians, if cadmium and other metals leach from the surrounding terrestrial environment. Soil and tailings around smelters and mines have been found to contain cadmium exceeding 300 mg/L as CaCO3, and alkalinity 258 mg/L as CaCO3) and allowed organisms to acclimate to the water hardness and temperature used during testing.

Burden increases with exposure time. Some evidence exists that the uptake [11] and toxicity [12] of cadmium is stage dependent. Increases in water temperature [8] and hardness [13] make cadmium less toxic and bioavailable, respectively. Speciation also has a large effect on cadmium bioaccumulation, retention, and depuration [14].

While some information exists on the toxicity of cadmium to amphibians, very little work has been done with native North American species. Because of differences in sensitivity and concern about metal contamination, it is important to conduct toxicity tests on additional native species to ensure that concentrations of cadmium in soil and surface waters are not deleterious to amphibians during any part of their life cycle. Chronic studies are also needed because they more accurately reflect environmental exposure and may reveal toxicity at lower concentrations than expected from acute tests. The objective of our study was to determine the effects of chronic cadmium exposure on the growth, survival, and metamorphosis of larval American toads (Bufo americanus). Abundant in the eastern United States and Canada, American toads frequently occupy disturbed habitats and oviposit in small, ephemeral pools containing runoff. These characteristics may make them very susceptible to cadmium exposure. We are unaware of any published studies that have experimentally assessed the effects of cadmium on American toads.

MATERIALS AND METHODS

Study organisms

Four B. americanus egg strings were collected from a stream at Grindstone Nature Area in Columbia (MO, USA) on April 23, 2001. Rearing and testing took place at the U.S. Geological Survey Columbia Environmental Research Center (Columbia, MO, USA). Clutches were mixed and eggs were placed in aquaria with well water (pH 7.8, hardness 286 mg/L as CaCO3 and alkalinity 258 mg/L as CaCO3) and allowed to hatch. Larvae were fed ground fish flakes ad libitum until they reached Gosner stage 25 [15], at which time the exposure began. During the 9-d holding period, organisms were gradually acclimated to the water hardness and temperature used during testing.
Toxicity test

Testing occurred in 36 19.6-L acid-rinsed glass jars (26 cm diameter) located in a water bath maintained at 23 ± 1°C. Jars were placed in two 3 × 6 blocks, and treatment and replicate positions were assigned randomly. Nominal cadmium concentrations and number of jars were as follows: <1 (n = 10), 5 (n = 10), 50 (n = 6), and 500 (n = 10) μg/L. Each jar contained 17 L of well water diluted with deionized water for a nominal hardness of 50 mg/L as CaCO₃. Stock solutions were made by adding cadmium (certified American Chemical Society CdCl₂·2.5H₂O crystals; Fisher Scientific, Fair Lawn, NJ, USA) to diluted well water. Different volumes of the same stock solution were used to dose jars at each concentration. Testing began on May 1, 2001, for 33 of the 36 jars. On May 11, three solution were used to dose jars at each concentration. Testing to diluted well water. Different volumes of the same stock

However, the text contains an error in formatting, particularly in the tables. The tables should be reformatted for clarity and correct presentation.

Table 1. Sample size (n), mean, and standard error (SE) of water quality characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkalinity (mg CaCO₃/L)</td>
<td>366</td>
<td>48.34 ± 0.26</td>
</tr>
<tr>
<td>Ammonia (mg N/L)</td>
<td>24</td>
<td>0.40 ± 0.03</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>382</td>
<td>7.22 ± 0.04</td>
</tr>
<tr>
<td>Hardness (mg CaCO₃/L)</td>
<td>351</td>
<td>51.20 ± 0.25</td>
</tr>
<tr>
<td>pH</td>
<td>369</td>
<td>7.91 ± 0.02</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>396</td>
<td>22.59 ± 0.03</td>
</tr>
</tbody>
</table>

Table 2. Mean ± standard error for survival, metamorphosis, mass, and days to tail resorption. Sample size in parentheses

<table>
<thead>
<tr>
<th>Cd (μg/L)</th>
<th>% Survival</th>
<th>% Metamorphosis</th>
<th>Metamorph wet wt (mg)</th>
<th>Days to tail resorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1⁴</td>
<td>98.2 ± 0.9A</td>
<td>91.2 ± 2.8A</td>
<td>103.6 ± 2.2A</td>
<td>48.3 ± 0.6A</td>
</tr>
<tr>
<td>(4)</td>
<td>(167)</td>
<td>(155)</td>
<td>(155)</td>
<td>(155)</td>
</tr>
<tr>
<td>5 ± 0.4</td>
<td>98.2 ± 0.9A</td>
<td>95.9 ± 1.3A</td>
<td>110.8 ± 1.1B</td>
<td>45.5 ± 0.4B</td>
</tr>
<tr>
<td>(4)</td>
<td>(163)</td>
<td>(161)</td>
<td>(161)</td>
<td>(161)</td>
</tr>
<tr>
<td>54 ± 8.3</td>
<td>100 ± 0.0A</td>
<td>100 ± 0.0B</td>
<td>117.2 ± 4.3B</td>
<td>44.1 ± 0.8B</td>
</tr>
<tr>
<td>(4)</td>
<td>(85)</td>
<td>(85)</td>
<td>(83)</td>
<td>(83)</td>
</tr>
<tr>
<td>540 ± 28.3</td>
<td>22.4 ± 7.9B</td>
<td>7.1 ± 2.6C</td>
<td>79.9 ± 6.3</td>
<td>44.9 ± 1.6</td>
</tr>
<tr>
<td>(4)</td>
<td>(38)</td>
<td>(12)</td>
<td>(10)</td>
<td>(10)</td>
</tr>
</tbody>
</table>

* Differing letters indicate significant concentration differences according to least significant difference multiple comparison tests.

⁴ Control.
and the two lowest concentrations, 54 μg/L, had 22% survival (Table 2). The first metamorphs from the 540-μg/L treatment were also significantly influenced by cadmium concentration (Table 3). Tadpoles in the 54-μg/L treatment resorbed their tails sooner and at a greater mass than those in the two lower concentrations (Table 2). The first metamorphs from the 54-μg/L concentration on day 26. Control tadpoles were among the last to metamorphose and also emerged the smallest (Table 2). The reason for high mortality at 540 μg/L during the first 25 d of the test. However, on day 26, 19 mortalities took place from 6 of the 10 jars. Almost daily mortality continued for the remainder of the study at this concentration.

Percentage metamorphosis was likewise significantly reduced in the highest cadmium concentration (Table 3), with significant pairwise differences between it and all others (Table 2). Significant differences were also found between 54 μg/L and the two lowest concentrations, 54 μg/L having 100% metamorphosis (Table 2). Most tadpoles that survived also metamorphosed by the end of the experiment, with the exception of organisms in the highest concentration, which had 7% metamorphosis relative to 22% survival (Table 2). Mass and time to tail resorption were also significantly influenced by cadmium concentration (Table 3). Tadpoles in the 54-μg/L treatment resorbed their tails sooner and at a greater mass than those in the two lower concentrations (Table 2). The first metamorphs from the 54-μg/L concentration on day 26. Control tadpoles were among the last to metamorphose and also emerged the smallest (Table 2). The few metamorphs collected from the 540-μg/L treatment weighed an average of just 80 mg, which is 24 mg less than the controls. However, time to tail resorption (45 d) was comparable to the other concentrations.

DISCUSSION

Percentage survival and metamorphosis was less than 25% at the highest cadmium concentration while exceeding 90% in all other treatments. At the three lowest concentrations (0, 5, and 54 μg/L), a tendency was observed for animals to weigh more and resorb their tails sooner as cadmium increased. This may have been a hormetic stress response that was antagonistic to toxicant exposure. Cadmium causes hormesis in other biological processes and species [18,19]. Studies with contaminants [20], predators [21], and shortened hydroperiods [22] have shown that tadpoles will metamorphose faster in a stressful environment. In field settings characterized by relatively low levels of chemical stressors, amphibians that metamorphose larger and more quickly may reach reproductive maturity sooner [23,24]. Additional studies should be undertaken to address the relationship between cadmium exposure and reproductive fitness. Adverse toxicological effects on endpoints other than growth and the energetic costs associated with exposure must be clearly characterized before the role of cadmium as a hormetic agent is understood.

Exposed metamorphs carry a cadmium body burden that may be physiologically stressful and cause reduced success in the terrestrial environment. The metabolism of toxicants could elevate maintenance costs and decrease the amount of energy put toward growth and survival [25]. Cadmium accumulates in the liver and kidneys of amphibians [26], with organ dysfunction, lesions, and numerous other ailments a potential consequence. Sublethal cadmium stress may reduce the ability of metamorphs to cope with natural terrestrial stressors (e.g., desiccation, temperature extremes, pathogens; [27,28]). Salamander larvae exposed for 24 d to mean cadmium concentrations of 48.9 and 193.1 μg/L (hardness 45 mg/L as CaCO3) were found to contain 1.62 and 4.70 μg/g Cd whole-body wet weight, respectively [10]. While it is unknown at what whole-body concentration cadmium becomes lethal to larval and postmetamorphic amphibians, it has been suggested that concentrations over 5 μg/g Cd wet weight in any vertebrate are life threatening [1]. Terrestrial American toads collected upwind of two zinc smelters had a mean whole-body cadmium burden of 1.5 μg/g dry weight, whereas not enough toads were observed to sample at a site closer to and downwind of the smelters [4]. Because cadmium accumulates with age [1], high tissue concentrations obtained in the larval stage may result in amphibians exceeding threshold toxicity levels later in life if additional exposure occurs. Amphibian predators may be at risk as well, though biomagnification is unlikely [29].

Many toxicity tests are conducted for 96 h or less, with chronic exposures being far less common. In our study, only one tadpole died during the first 4 d across all treatments. Yet by the end of 60 d, 78% of tadpoles exposed to 540 μg/L had died. While the 96-h LC50 is unknown for larval American toads, studies with other species and our data indicate it would be in the mg/L range. Hazard assessments based on short-term
exposures would therefore greatly underestimate the risk of cadmium to larval amphibians. Effects on organisms exposed as both eggs and larvae may also be quite different from what we report here.

Because ambient aquatic cadmium concentrations are generally well below 5 μg/L and the U.S. EPA chronic cadmium criterion is 0.15 μg/L at the hardness tested, American toads exposed only as larvae should be protected in most cases. However, larvae may be at risk of depressed survival, percentage metamorphosis, and body condition in surface waters that exceed 54 μg/L. At certain concentrations, hidden or delayed costs may be associated with hormesis (e.g., altered body fat) or a contaminant body burden (e.g., organ dysfunction). Bioavailable cadmium appeared to increase with cadmium concentration based on the dose responses observed. To better characterize the risk of uptake from aequous exposure, factors known to affect the bioavailability of cadmium (e.g., hardness, temperature, speciation) should be manipulated. Cadmium uptake may also occur by ingestion of food and sediment, and bioavailable cadmium can vary with route of exposure [14]. Increased body burdens from multiple exposure routes could have detrimental effects not predicted by the aquatic concentration of cadmium alone.

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