

## Effects of Multiple Routes of Cadmium Exposure on the Hibernation Success of the American Toad (*Bufo americanus*)

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**Abstract.** The effects of multiple routes of cadmium exposure on juvenile American toads (*Bufo americanus*) were evaluated using environmentally relevant concentrations. During or after exposure, toads were individually hibernated for 172 days at approximately 4°C. The following experiments were conducted: (1) dermal exposure (hibernation in soil contaminated with up to 120 µg Cd/g (dry weight)); (2) injection exposure (single injection with cadmium to achieve a maximum whole-body nominal concentration of 3 µg Cd/g (wet weight) 12 days before hibernation in uncontaminated soil); and, (3) oral exposure (feeding with mealworms containing ≤16 µg Cd/g (dry weight) for 50 days before hibernation in uncontaminated soil). We hypothesized that sublethal levels of cadmium would become lethal during hibernation because of combined chemical and cold stress. No prehibernation mortality occurred in the injection and oral exposure studies. There was a significant treatment effect on whole-body cadmium concentration in toads orally or dermally exposed and on percent of cadmium retention in toads orally exposed. There was also a trend of increased time-to-burrowing and more toads partially buried with greater cadmium concentration in the dermal study, which indicated avoidance. In all 3 experiments, no significant differences were found among cadmium treatments in hibernation survival, percent of mass loss, or locomotor performance. However, toads fed mealworms averaging 4.7 µg Cd/g (dry weight) had only 56% survival compared with 100% survival for controls. Although our results suggest that environmentally relevant levels of cadmium do not pose a great risk to American toads, factors such as soil type or prey species may increase cadmium bioavailability, and other amphibian species may be more sensitive to cadmium than *B. americanus*.

activity (Carey and Bryant 1995). Most amphibian ecotoxicological research has focused on aquatic assessment and effects on the egg or larval stages. Although these studies have shown that expected environmental concentrations of some chemicals can be harmful, they do not provide a comprehensive evaluation of potential contaminant impacts on all amphibian life stages. Most amphibians breed and metamorphose in aquatic habitats, but many species are terrestrial the majority of their lives. The terrestrial environment can be a major source of pollutants (*e.g.*, agricultural fields, mines, industrial sites) and contaminant uptake may occur by oral, pulmonary, and dermal exposure from ingesting contaminated prey, receiving aerial deposition, and occupying polluted soil (Storm *et al.* 1994; Lambert 1997; Johnson *et al.* 1999). Toxicant avoidance may be difficult or impossible because of limited mobility, habitat barriers, inability to detect harmful contaminants, or failure to perceive contaminants as a threat. The loss of individuals old enough to occupy the terrestrial environment is possibly disproportionately detrimental to population dynamics for some amphibian species relative to the alteration of other vital rates (Biek *et al.* 2002). Indeed, some amphibian declines have been attributed to the loss of adults (Morell 1999; Carey *et al.* 2001). It is therefore important to understand the effects of contaminants on terrestrial life stages.

Hibernation can be a stressful period for amphibians because individuals rely on a limited supply of energy stores for their metabolic demands while occupying a severe environment. Beside the risk of starvation, amphibians may face predation, temperatures near or below freezing, oxygen depletion, and inadequate soil moisture. Little is known about overwintering survival, and study findings are mixed and weakened by small sample sizes. One hundred percent survival was reported in 6 radio-tagged toads (*Bufo bufo*; van Gelder *et al.* 1986), and the freezing of montane lakes can cause complete mortality in mountain yellow-legged frogs (*Rana muscosa*; Bradford 1983). Nine of 28 (32%) American toads (*B. americanus*) monitored in Minnesota for at least 1 winter during a 4-year period died during hibernation (Ewert 1969, unpublished PhD dissertation, University of Minnesota, Twin Cities, MN). Pickerel frog (*Rana palustris*) mortality increased dramatically during the last half of 1 winter, suggesting submission to winter hardship (Resetarits 1986). Juvenile amphibians appear to suffer greater

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Many amphibian populations and species have decreased or disappeared around the globe in the last few decades (Houlahan *et al.* 2000). Among the suggested causes of amphibian decline is the chemical contamination of habitat caused by human

mortality than do adults (Tester and Breckenridge 1964; Kelleher and Tester 1969; Resetarits 1986), possibly because of fewer energy reserves, decreased burrowing ability, and larger surface area-to-volume ratio.

Additional stress during hibernation resulting from a contaminant body burden may increase winter mortality or lower body condition at emergence. Physiological damage can result if toxicants reach target organs in sufficient concentration (Rozman and Klaassen 1996), thereby potentially compromising the ability of individuals to cope with typical winter conditions. Although amphibians possess detoxification enzymes and excretion mechanisms, depuration may be less effective during hibernation because metabolism is decreased (Pinder *et al.* 1992). Amphibians may enter hibernation already possessing a contaminant body burden or may absorb toxicants from polluted substrate during dormancy. Route and timing of exposure are important considerations for risk assessment because of potential differential effects on toxicodynamics and the rates of uptake and elimination (Harrison and Klaverkamp 1989).

Cadmium is a highly toxic, nonessential heavy metal that is naturally ubiquitous at low concentrations (Eisler 1985). Increased levels in water and soil result from airborne deposition, mining waste, industrial dumping, and the application of municipal sludge and phosphate fertilizers to agricultural fields. Dermal, oral, and respiratory uptake of cadmium by amphibians is possible, with accumulation, distribution, and toxicity varying depending on route of exposure (Hall and Swineford 1979; Vasil'eva *et al.* 1987). Although cadmium delivered orally (Linder *et al.* 1998) or through injection (Suzuki *et al.* 1986; Vasil'eva *et al.* 1987) has been found to accumulate in the liver and kidneys, aquatic submergence results in increased skin concentrations (Vasil'eva *et al.* 1987; Vogiatzis and Loumbourdis 1997). The amount of cadmium in tissue can increase with increasing cadmium in the surrounding medium (Vasil'eva *et al.* 1987; Nebeker *et al.* 1995). Exposure to cadmium-contaminated soils may result in decreased survival and altered growth, behavior (Lefcort *et al.* 1998), and competitive interactions (Lefcort *et al.* 1999). Cadmium adversely affects reproduction by altering oogenesis and the morphology of oocytes, as well as by accumulating in the ovaries and oocytes (Lienesch *et al.* 2000). Free-ranging amphibians have been found to possess up to 26  $\mu\text{g Cd/g}$  cadmium (whole body dry weight; Hall and Mulhern 1984). Because cadmium is eliminated slowly, prolonged sublethal exposure may eventually result in high tissue concentrations and subsequent organ dysfunction (Hammons *et al.* 1978). However, amphibians are capable of producing metal-binding compounds (*e.g.*, metallothionein) that decrease cadmium toxicity (Suzuki *et al.* 1986).

We selected the American toad as a model species for our studies of the effects of cadmium on the overwintering success of terrestrial hibernators. This species is common in a variety of habitats throughout eastern North America. American toads use agricultural fields and other disturbed areas for foraging, migration (Kolozsvary and Swihart 1999), breeding, and periods of dormancy and thus could be exposed to contaminants through several different routes and across all life stages. Terrestrial toxicant exposure can occur quickly after metamorphosis, for once toadlets reach 0.5 to 1.0 g in weight, they disperse into the uplands (Pough and Kamel 1984) and may travel a few hundred meters by late summer (Oldham 1985).

Juveniles and adults are fossorial and are often found in areas with loose soil and high insect abundance. During cold months, individuals hibernate below the frost line to prevent freezing and move vertically to adjust to thermal gradients (Breckenridge and Tester 1961; van Gelder *et al.* 1986).

The goal of this study was to evaluate the effects of different routes of cadmium exposure on the hibernation success of juvenile American toads. Three experiments were conducted in which toads were either hibernated in cadmium-contaminated soil, injected with cadmium before hibernation, or fed cadmium-contaminated prey before hibernation. Surviving toads were weighed to determine mass loss, tested for endurance with 2 types of locomotor performance trials, and analyzed for whole-body cadmium concentration. Cadmium exposure levels for all studies were within the range of values reported in the environment.

## Materials and Methods

### Test Organisms

Portions of 6 freshly deposited American toad clutches were collected in April and May 2000 at Grindstone Nature Area in Columbia, Missouri. The eggs were transferred to a laboratory at the United States Geological Survey Columbia Environmental Research Center (CERC) and allowed to hatch. Free-swimming larvae were transferred to outdoor polyethylene cattle tanks containing 2000 L uncontaminated pond water and 1 kg leaf litter, and they remained there until metamorphosis. Metamorphs were kept in 2-m<sup>2</sup> terrestrial enclosures from July to October, at which time they were transported back to the CERC and placed in 27-L aquaria with moist sphagnum moss. Feeding (cabbage loopers, *Trichoplusia ni*; crickets, *Acheta domestica*; mealworms, *Tenebrio molitor*) was discontinued 7 to 9 days before hibernation to allow gut clearance. Mass, tibia, and snout-vent length (SVL) were recorded the day hibernation began. Mass was determined again when toads were brought out of hibernation, and mass and SVL were measured the same day locomotor tests were performed.

### Hibernation Conditions

Hibernation took place in a dark room maintained at approximately 4°C and 35% to 65% relative humidity. Individual toads were kept in 1-L plastic containers with a fiberglass screen lid to allow air circulation and observation and to minimize fungal growth. Fortunately, the only fungi observed were on dead toads and the surrounding soil postmortem. Each container received (from top to bottom) a thin layer of sphagnum moss, 700 g (wet weight) soil, and 150 g (1.5 cm depth) rinsed gravel for filtration. Soil was collected in August 2000 from the University of Missouri Basket Wildlife Research Area (Boone County, Missouri). Once air dried and pulverized, it was sieved through 2-mm stainless steel screens and stored in polyethylene buckets. To prevent mortality from desiccation, soil moisture in the hibernation containers was maintained at 15% (125% water-holding capacity) by periodic misting with a spray bottle. Toads from all experiments were kept in hibernation for 172 days.

### Locomotor Performance Tests

Toads were tested for sprint performance by soaking their posterior end in an ink bath (water and food dye) and placing them on a table

covered with white laboratory countertop absorbent paper. Hopping was induced by gently tapping on the urostyle with a blunt probe. The first 4 hops were measured and average and total hop length were computed. Immediately afterward, endurance tests were conducted in a circular enclosed track that measured 9 cm wide and 260 cm in circumference. Individual toads were placed at a common starting point and induced to hop in the same manner as the sprint test. Testing ceased once the toad reached exhaustion (*i.e.*, no movement for 15 seconds) or 3 minutes had passed, and distance and duration were recorded. All tests were conducted indoors at 23 to 27°C, which falls within the range of active temperatures for this species (Fitch 1956). Toads were kept in the testing room for at least 10 days before the locomotor trials so they would acclimate to the temperature.

### Chemical Analysis

Intact toads that were killed for analysis were rinsed with deionized water and carefully inspected to ensure that soil particles had been removed before they were wet ground with an Ultra-Turrax tissue homogenizer. The resulting homogenate was acid digested and analyzed for cadmium using a Varian SpectrAA 20 Plus Atomic Absorption Flame Spectrometer and a Varian GTA-96 Graphite Furnace (Varian, Mulgrave, Victoria, Australia). Stock solutions were also analyzed with these instruments. Samples were run in duplicate on each instrument, and detection limits were 0.01 and 0.0001  $\mu\text{g/g}$ , respectively. Quality control and drift checks (standards) were run every 10 samples and the average spike recovery was 97%. Dry tissue weights were determined by drying a portion of each ground sample at 105°C until a constant weight was achieved. Soil was analyzed using only the Varian Flame Spectrometer, but preparation and analysis conditions were otherwise the same as that for tissue. All cadmium samples were submitted for analysis to the Mississippi State Chemical Laboratory (Mississippi State, MS).

### Contaminated Soil Experiment

A stock solution was made by adding cadmium (certified ACS  $\text{CdCl}_2 \cdot 2.5 \text{H}_2\text{O}$  crystals; Fisher Scientific, Fairlawn, NJ) to deionized water. Appropriate volumes of the stock were added to 5 different 13.5-L polyethylene buckets of soil (1/concentration) on September 7, 2000, to result in measured concentrations of <0.5 (control), 16, 30, 59, and 120  $\mu\text{g Cd/g}$  (dry weight). Treatment levels were based on cadmium found in soil around smelters ( $\leq 1020 \mu\text{g/g}$ ; Brown *et al.* 1994) and in agricultural fields ( $\leq 19 \mu\text{g/g}$ ; Hansen and Hinesly 1979). Additional deionized water was added to attain 15% soil moisture. A stainless steel auger (7-cm diameter, 29-cm bit length) attached to a hand-held drill was used to blend the soils immediately after dosing and once weekly for 5 weeks. The dosed soil was then allowed to equilibrate undisturbed at 4°C from September 7 to November 28, 2000, so that aqueous cadmium would partition into the soil and thereby more accurately reflect natural bioavailability. To verify cadmium concentration, single composite samples were analyzed from the <0.5-, 16-, and 59- $\mu\text{g/g}$  treatment buckets, whereas duplicate composite samples were analyzed from the 30- and 120- $\mu\text{g/g}$  treatments. A composite sample of undosed soil was analyzed for chemical and physical characteristics at the University of Missouri Soil Characterization Laboratory (Columbia, MO; Table 1).

"Bioavailable" refers to the fraction of the total chemical in a medium available for uptake by an organism (Rand and Petrocelli 1985). To estimate bioavailability, we extracted cadmium from the <0.5-, 30-, and 120- $\mu\text{g/g}$  treatments on December 20, 2000 and June 27, 2001, using a technique similar to that of Weimin *et al.* (1992). Two grams dry, ground soil were added to glass beakers, wetted with

**Table 1.** Soil chemical and physical characteristics

|                                      |      |
|--------------------------------------|------|
| Clay (%)                             | 8.6  |
| Silt (%)                             | 42.7 |
| Sand (%)                             | 48.7 |
| Cation exchange capacity (mEq/100 g) | 8.6  |
| Organic carbon (%)                   | 0.9  |
| pH                                   | 7.0  |
| Salinity (mmho/cm)                   | 0.4  |
| Total N (%)                          | 0.1  |
| Ca (mEq/100 g)                       | 23.5 |
| K (mEq/100 g)                        | 0.2  |
| Mg (mEq/100 g)                       | 1.2  |
| Na (mEq/100 g)                       | TR   |

TR = trace amount.

methanol, and mixed with 100 ml 0.1 M  $\text{Na}_2 \text{EDTA}$  on a stir plate for 2 hours. After a brief settling period, 50 ml liquid was poured into centrifuge tubes and spun for 5 minutes at 1500 rpm. Twenty milliliters of the supernatant was drawn with a syringe and passed through a 0.45- $\mu\text{m}$  filter disc. Single samples were analyzed for cadmium, except for the 30- $\mu\text{g/g}$  treatment, which was sampled in duplicate.

Ten toads weighing 2 to 15 g each were randomly assigned to each treatment. Toads were put in individual hibernation containers within 2.5 hours of being placed in the cold room on November 29, 2000. Toads were observed for burrowing activity after 20, 60, 100, 180, and 260 minutes of being in the cold room to determine if cadmium in the soil affected burrowing behavior. We anticipated delayed burrowing with increasing cadmium concentration because bivalves have shown this response in cadmium-contaminated sediment (McGreer 1979). (This was the only study for which initial burrowing activity was assessed, but it would have been an appropriate end point for the other 2 studies because a cadmium body burden may affect movement.) Throughout hibernation, weekly checks were made to record specific toads that were less than half buried. Toads were removed from the cold room on May 20, 2001 and allowed 2 days to emerge from the soil. Toads that had not appeared by this time were dug out. Body mass was determined on May 21, and toads were transferred to individual plastic containers with sphagnum moss. Ad libitum feeding with mealworms occurred until locomotor tests were performed on May 31. All toads were killed on June 6 and frozen at  $-15^\circ\text{C}$ . For each noncontrol treatment, 4 toads that survived hibernation were randomly selected for individual whole-body cadmium analysis. Three control toads were pooled into 1 sample.

### Injection Experiment

Forty-four toads weighing 2 to 10 g each were injected once in the dorsal lymph sac with cadmium ( $\text{CdCl}_2 \cdot 2.5 \text{H}_2\text{O}$  dissolved in physiological saline: [0.9% NaCl]) at the following nominal whole body concentrations: 0, 0.4, 1.1, 3.0  $\mu\text{g/g}$  (wet weight). Injection volume was 0.5% of body weight to avoid detrimental side effects. Ten (0, 1.1  $\mu\text{g/g}$ ) or 12 (0.4, 3.0  $\mu\text{g/g}$ ) toads were randomly assigned to each treatment. Treatments were chosen based on whole-body cadmium concentrations in amphibians collected in the field ( $\leq 4.0 \mu\text{g/g}$  wet weight; Dmowski and Karolewski 1979; Hall and Mulhern 1984; Beyer *et al.* 1985; Storm *et al.* 1994). Once injected on November 17, 2000, each toad was placed in a plastic container with moist sphagnum moss and hand-fed 3 mealworms every other day until 7 days before hibernation began on November 29. The body burden of cadmium at hibernation initiation was determined by killing 2 toads each from the 0.4- and 3.0- $\mu\text{g/g}$  treatments the day after all other toads were placed in the cold room. Data were not collected on burrowing activity during

hibernation because the toads were kept on 2 shelves, and physical movement (*i.e.*, disturbance) of the containers located on the bottom shelf would have been necessary for observation. Toads were removed from hibernation on May 20, 2001, and treated the same as the soil-experiment toads, except that locomotor tests were performed on May 30. All toads were killed on June 6 and frozen at  $-15^{\circ}\text{C}$ . Three toads that survived hibernation were randomly selected from the 0-, 0.4-, and 3.0- $\mu\text{g/g}$  treatments and pooled for whole-body cadmium analysis. The toads killed at the beginning of hibernation were pooled by treatment. Toads were ground individually, and approximately 2 g wet homogenate from each toad was sampled.

### Contaminated Prey Experiment

Twenty-seven toads weighing 10 to 28 g each were fed mealworm larvae raised on cricket meal dosed with cadmium at the following mean concentrations: 0 (control), 15, and 52  $\mu\text{g/g}$  (dry weight). Before dosing, the cricket meal was sieved through a 1-mm stainless steel screen. Nine toads were randomly assigned to each treatment. Treatments were based on levels of cadmium found in the soil and vegetation of contaminated sites ( $\leq 1020 \mu\text{g/g}$ ; Hammons *et al.* 1978; Hansen and Hinesly 1979; Brown *et al.* 1994). Radioactive Cd-109 (as  $\text{CdCl}_2$  in 0.5 M HCl, specific activity = 20.65  $\mu\text{Ci/g}$ ; Isotope Products, Burbank, CA) was added as a tracer to "cold" cadmium stock solutions ( $\text{CdCl}_2 \cdot 2.5 \text{H}_2\text{O}$  dissolved in deionized water) so that the labeled material made up 3.7% and 1.1% of the total cadmium content in the 15- and 52- $\mu\text{g/g}$  treatments, respectively. Cold cadmium was verified by analyzing a portion of the stocks before adding radioactive cadmium, and hot cadmium (Cd-109, a gamma-ray emitter) was determined by counting three 1- $\mu\text{l}$  samples from each stock. Every week for 4 weeks, a 25-ml stock was made for each concentration and poured over 75 g dry meal in a plastic cup. The meal was then dried on a hotplate for 24 hours and thoroughly mixed. Cadmium concentration and homogeneity were confirmed by counting 3 samples (80 to 200 mg dry weight) from each cup. An equal mass of mealworms (13 to 17 g) was then added to each cup and the mealworms were allowed to feed for a minimum of 3 weeks before being fed to the toads. The mealworm mass constituted a 1-week feeding ration for the toads; consequently, a different set of cups was used for each week of feeding. Cups from weeks 1 through 3 were restocked with uncontaminated mealworms and used to feed the toads during weeks 5 through 7. Cadmium uptake rate by the mealworms was determined for the week 1 and 2 cohorts by taking 3 samples (300 to 500 mg wet weight) from each cup weekly for 4 weeks. On March 27 and April 17, 2001, mealworm exoskeletons from the set of cups actively being used to feed the toads were collected, weighed, and counted for radioactivity. The cadmium content of all samples in this experiment was calculated by measuring radioactivity in a Beckman 8000 gamma detector (Beckman Instruments Inc., Fullerton, CA). Counting efficiency was determined by counting solutions of known activity.

Once the feeding trials began on March 17, 2001, toads were kept individually in 1.5-L plastic containers with a small tray of moist sand and fed 3 times weekly for 50 days. Toads were fed 2% of their body wet weight (0.021 g mealworm/g toad/feeding day). On the days toads were fed, worms were weighed out into 27 scintillation vials (1/toad) and counted for radioactivity. Occasionally, samples from the dosed treatments had little or no activity. These samples were returned to the cups and replaced by others so that toads of the same treatment received mealworms containing similar concentrations of cadmium. Hence, mealworm selection was not random. Noncontrol toads always ate every mealworm, so it was unnecessary to account for unconsumed cadmium. Fecal pellets were collected throughout the study and counted for radioactivity. This provided a conservative estimate of total excreted cadmium because the toads often defecated in the sand (making collection difficult), moisture came off of the pellets, urine was not monitored, and some pellets probably went undetected during and after hibernation. Feeding trials ended May 5, after which toads were fed uncontaminated meal-

worms until May 16. The mass and SVL of each toad were measured before and after the feeding trials to document growth.

Toads were placed in the cold room on May 25 and removed November 13. Throughout hibernation, weekly checks were made to record specific toads that were less than half-buried. Surviving toads were fed 1% of their body weight 3 times before being tested for sprint performance on November 23. Toads were killed on either November 26 or 28 and stored at  $-15^{\circ}\text{C}$  until they were individually analyzed for whole-body cadmium. Contrary to our other experiments, reported tissue means included all toads in the study, and entire carcasses were dried to determine dry weight. After obtaining body counts, partial egg clutches were removed from either 2 (15  $\mu\text{g/g}$ ) or 3 (52  $\mu\text{g/g}$ ) female toads and counted separately.

### Statistical Analysis

Reported cadmium for the feeding study was based on values derived from measuring the amount of radioactive Cd-109. Any cadmium already present in the meal, mealworms, or toads was considered a constant across treatments and was not included in the analyses or tables. Toads in this experiment likely had a background level of cadmium approximating 0.5  $\mu\text{g/g}$  (dry weight) *i.e.*, the concentration observed in control toads from the soil and injection studies. No radioactivity significantly above background was detected in the control organisms; the range of radioactivity in control toads (56 to 65 cpm) fell within the range of blanks counted throughout the study (51 to 73 cpm). Control toads were considered to contain 0.000  $\mu\text{g Cd/g}$  when making statistical comparisons with the other treatments.

Results of all 3 studies were analyzed with the software package SAS (1989; SAS Institute, Cary, NC). Analysis of variance (ANOVA) was used to determine whether cadmium treatment had significant effects on percent of mass loss during hibernation, time-to-burrowing (on ranks), locomotor performance, and whole-body cadmium concentration (on ranks for feeding study only). The few toads that refused to hop or that never buried were excluded from the locomotor performance and time-to-burrowing analyses, respectively. Additional variables analyzed only for the feeding study using ANOVA were percent change in mass and SVL and percent food assimilation for the 50-day period of experimental feeding, as well as percent cadmium retention (on ranks). Percent food assimilation is body mass gained divided by prey mass consumed, and percent cadmium retention is cadmium mass present in the whole body after hibernation divided by cadmium mass consumed. Whole-body cadmium concentration (soil study only) was log-transformed to establish normality and homogeneity of variance (Zar 1999). Type III SS were used to account for unequal sample sizes. Pairwise differences were determined with a least significant difference test. Hibernation survival and the number of toads observed less than half buried were analyzed with the  $\chi^2$  statistic. Simple linear regression was also used to analyze toad burrowing activity (soil study only). Toad bioaccumulation factor (BAF) and bioconcentration factor (BCF) values for cadmium were calculated by dividing toad tissue concentration by mealworm tissue and soil concentration, respectively. Mealworm BAFs were determined by dividing mealworm tissue concentration by cricket meal concentration.

## Results

### Contaminated Soil Experiment

The dosed soils were well homogenized because duplicate soil samples analyzed for cadmium differed  $<1 \mu\text{g/g}$ . The whole-body cadmium concentrations in toads increased with soil

concentration (Table 2). Toads hibernated in soil containing 59 and 120  $\mu\text{g Cd/g}$  had significantly greater body burdens than the control and the two lower soil concentration groups (Tables 3 and 4). Despite a nearly 2-fold difference in soil concentration, toads from the 2 highest treatments did not significantly differ in cadmium uptake and averaged just slightly more than 2  $\mu\text{g Cd/g}$  (dry weight). Bioconcentration factors were less than 1, although almost all of the total soil cadmium was "bioavailable" according to the ethylenediamino tetra-acetic acid (EDTA) extraction method used (Table 2). There was a strong trend of increased partial burial with increasing soil concentration ( $r^2 = 0.89$ ,  $df = 1,3$ ,  $P = 0.0154$ ), but the differences were not significant ( $\chi^2 = 4.50$ ,  $df = 4$ ,  $P = 0.3425$ ; Table 3). Survival ( $\chi^2 = 0.16$ ,  $df = 4$ ,  $P = 0.9969$ ), time-to-burrowing, percent mass loss, and locomotor performance did not differ significantly by treatment (Table 4), although mass loss and burrowing time showed a general trend of increasing with increasing cadmium concentration (Table 3).

### Injection Experiment

On the final feeding day before hibernation, 30% and 50% of toads injected with 1.1 and 3.0  $\mu\text{g Cd/g}$  refused food, compared with 10% and 0% in the control and 0.4 treatments, respectively. At the initiation of hibernation, toads possessed a wet-weight body burden of 0.4 and 2.3  $\mu\text{g Cd/g}$ , relative to the nominal injection values of 0.4 and 3.0  $\mu\text{g Cd/g}$ . Whole-body dry weight tissue concentrations of toads administered the highest nominal injection were 25 times greater than controls after emergence from hibernation (Table 3). There was no significant treatment effect on survival ( $\chi^2 = 0.54$ ,  $df = 3$ ,  $P = 0.9094$ ; Table 3), percent mass loss, or locomotor performance (Tables 3 and 4). Of note, toads injected with 0.4  $\mu\text{g Cd/g}$  had the highest mortality and mass loss and the poorest locomotor performance.

### Contaminated Prey Experiment

Mealworms used for the feeding trials had a mean dry weight body burden of 4.7 and 16  $\mu\text{g Cd/g}$  for the 15 (low)- and 52 (high)- $\mu\text{g Cd/g}$  cricket meal treatments, respectively (Table 5). Their uptake of cadmium appeared to level off after approximately 3 weeks (Fig. 1). Toads in the high-cadmium treatment accumulated the greatest body burdens, and the 3 treatments differed significantly from each other (Tables 3 and 4). Within each treatment, toads that died during hibernation had significantly greater tissue contamination than those that survived (low treatment:  $F = 27.36$ ,  $df = 1,7$ ,  $P = 0.0012$ ; high treatment:  $F = 7.22$ ,  $df = 1,7$ ,  $P = 0.0312$ ). However, some surviving toads had higher body burdens than ones that had died. Eggs from 2 low-treatment female toads were found to contain 0.270 and 0.371  $\mu\text{g Cd/g}$  (dry weight), and 3 high-treatment female toads had eggs with 0.179, 0.279, and 7.088  $\mu\text{g Cd/g}$ . Cadmium retention averaged <20% (Table 5), and toads in the low-cadmium treatment group retained a significantly greater proportion of ingested cadmium than those in the high-treatment group (Table 4). All BAFs were <1: the mealworm BAF was equivalent for the 2 cadmium exposure con-

centrations, whereas the toad BAF was higher in the low-treatment group (Table 5). The mealworm BAFs were perhaps slightly inflated because mealworm sampling was not random. The 2 collections of mealworm skins averaged 12 and 36  $\mu\text{g Cd/g}$  (dry weight) for the low and high treatments, respectively. Although the 2 collections produced similar numbers, these averages may also be inflated because the skins were not thoroughly checked for meal particles. Toad fecal pellets contained a large proportion of the total cadmium ingested, and mealworm skins were often observed in the pellets, but defecation frequency was similar among treatment groups (Table 5). Percent change in mass and SVL during the feeding trials, as well as percent food assimilation, were not significantly different by treatment (all  $p > 0.05$ ; Table 5).

There was no significant difference or trend in the total number of toads observed less than half buried on at least 1 occasion during hibernation (Table 3). Survival ( $\chi^2 = 1.30$ ,  $df = 2$ ,  $P = 0.5220$ ; Table 3), percent mass loss, and locomotor performance did not differ significantly by treatment (Tables 3 and 4), but toads in the low-cadmium treatment always fared worst. Although not statistically significant, toads fed cadmium had <70% survival relative to 100% survival for controls. A significant negative effect may have been observed with greater replication and higher power; our power ( $1 - \beta$ ) to detect a large effect size of 40% was <0.50 (Cohen 1988).

### Discussion

Three different routes of cadmium exposure were investigated for effects on hibernating juvenile American toads. Control survival ranged from 80% to 100%, which is quite high relative to what it can be in nature (Bradford 1983) and likely indicates that hibernation conditions were adequate for this species, particularly given our small sample size and long duration of exposure. Significant treatment effects were observed for whole-body cadmium concentration (soil and feeding studies) and percent cadmium retention (feeding study). Toads hibernated in contaminated soil possessed mean whole-body cadmium values of 0.500 to 2.198  $\mu\text{g/g}$ . Toads fed contaminated prey averaged 0.698 and 1.376  $\mu\text{g/g}$  higher than the controls for the low- and high-cadmium treatments, respectively. The 2 different pathways of exposure resulted in similar tissue concentrations, but the BAFs were higher for the feeding study than were the BCFs for the soil study. Previous research with amphibians has shown that cadmium will partition into different tissues depending on exposure route (Vasil'eva *et al.* 1987). Therefore, comparable whole-body levels may not always result in similar toxicity because some organs and physiological processes may be more impacted and more sensitive than others. Significant cadmium uptake in our studies did not translate into significantly decreased survival, growth, or locomotor performance, *i.e.*, factors that may affect fitness and population dynamics. However, the presence of increased cadmium in eggs (feeding study) may be an indication of impaired reproduction.

Toads hibernated in contaminated soil possessed higher cadmium levels as soil concentration increased. Toads in the 2 highest cadmium concentrations accumulated similar body burdens, which may indicate that equilibrium or saturation was

**Table 2.** Measured soil and tissue dry weight concentrations, bioconcentration factors, and bioavailable fraction of cadmium for hibernating juvenile *B. americanus* (soil study)

| Soil ( $\mu\text{g/g}$ ) | Toads <sup>a</sup> ( $\mu\text{g/g}$ ) | Bioconcentration factor <sup>b</sup> | Bioavailable fraction <sup>c</sup> ( $\mu\text{g/g}$ ) |           |
|--------------------------|--|--------------------------------------|--|-----------|
|                          |  |                                      | 12/20/2000   | 6/27/2001 |
| <0.5                     | 0.503                                  | —                                    | 0.5  | <0.5      |
| 16                       | 0.500 $\pm$ 0.132                      | 0.031                                | ND   | ND        |
| 30                       | 0.879 $\pm$ 0.104                      | 0.029                                | 27   | 26        |
| 59                       | 2.037 $\pm$ 0.484                      | 0.035                                | ND   | ND        |
| 120                      | 2.198 $\pm$ 0.190                      | 0.018                                | 105  | 100       |

ND = no data.

<sup>a</sup> Whole body; mean  $\pm$  1 standard error.

<sup>b</sup> Mean toad cadmium concentration/soil cadmium concentration. Not calculated for controls because exact soil concentration was unknown.

<sup>c</sup> Bioavailable fraction measured by EDTA soil extraction.

**Table 3.** Least squares means for survival, mass loss, locomotor performance, time-to-burrowing, whole-body tissue concentration, and number of individuals less than half buried for hibernating juvenile *B. americanus* (soil, injection, and feeding studies)<sup>a</sup>

| Concentration <sup>b</sup><br>( $\mu\text{g Cd/g}$ ) | Survival<br>(%) | Mass loss<br>(%) | Sprint average hop<br>length (cm) | Endurance hop<br>distance (cm) | Time-to-burrowing<br>(min) | Toad tissue<br>concentration <sup>cd</sup><br>( $\mu\text{g Cd/g}$ dry<br>weight) | No. less<br>than half<br>buried |
|--|-----------------|------------------|-----------------------------------|--------------------------------|----------------------------|---|---------------------------------|
| Soil study   |                 |                  |                                   |                                |                            |   |                                 |
| Control  | 80              | 9.6 $\pm$ 2.1    | 9.6 $\pm$ 1.2                     | 1486 $\pm$ 138                 | 20 $\pm$ 21                | 0.503 <sup>ab</sup>   | 2                               |
| 16   | 70              | 12.4 $\pm$ 2.3   | 12.5 $\pm$ 1.2                    | 1268 $\pm$ 138                 | 51 $\pm$ 22                | 0.500 <sup>ab</sup>   | 5                               |
| 30   | 70              | 12.7 $\pm$ 2.3   | 9.9 $\pm$ 1.3                     | 1407 $\pm$ 138                 | 76 $\pm$ 21                | 0.879 <sup>ab</sup>   | 5                               |
| 59   | 70              | 13.4 $\pm$ 2.3   | 11.0 $\pm$ 1.2                    | 1617 $\pm$ 139                 | 68 $\pm$ 21                | 2.037 <sup>bc</sup>   | 8                               |
| 120  | 80              | 13.1 $\pm$ 2.1   | 9.1 $\pm$ 1.1                     | 1454 $\pm$ 129                 | 73 $\pm$ 22                | 2.198 <sup>bc</sup>   | 8                               |
| Injection study                                      |                 |                  |                                   |                                |                            |   |                                 |
| Control  | 90              | 8.5 $\pm$ 3.1    | 9.2 $\pm$ 1.1                     | 1371 $\pm$ 147                 | ND                         | 0.440   | ND                              |
| 0.4  | 70              | 16.4 $\pm$ 3.5   | 9.2 $\pm$ 1.2                     | 1136 $\pm$ 167                 | ND                         | 1.149   | ND                              |
| 1.1  | 100             | 11.5 $\pm$ 3.0   | 9.2 $\pm$ 1.0                     | 1339 $\pm$ 140                 | ND                         | ND  | ND                              |
| 3.0  | 90              | 11.5 $\pm$ 3.1   | 9.7 $\pm$ 1.1                     | 1372 $\pm$ 147                 | ND                         | 10.929  | ND                              |
| Feeding study  |                 |                  |                                   |                                |                            |   |                                 |
| Control  | 100             | 9.3 $\pm$ 2.2    | 15.7 $\pm$ 1.3                    | ND                             | ND                         | 0.000 <sup>ab</sup>   | 6                               |
| 15   | 56              | 13.2 $\pm$ 3.0   | 15.4 $\pm$ 1.7                    | ND                             | ND                         | 0.698 <sup>bc</sup>   | 7                               |
| 52   | 67              | 9.0 $\pm$ 2.8    | 17.5 $\pm$ 1.6                    | ND                             | ND                         | 1.376 <sup>cd</sup>   | 7                               |

ND = no data; SE = standard error.

<sup>a</sup> Some means followed by  $\pm$  1 SE.

<sup>b</sup> Cadmium concentrations in measured dry weight soil (soil study), nominal wet weight tissue (injection study), and measured dry weight cricket meal (feeding study).

<sup>c</sup> Differing letters within the column and within a study indicate significant concentration differences according to least significant difference multiple comparison tests.

<sup>d</sup> Values reported for feeding study represent only the cadmium detected using calculations based on measured radiation from Cd-109. Control reported as 0.000 because no cadmium above background was detected.

reached. Although bioconcentration factors were  $<0.04$ , amphibians can absorb cadmium from the soil through the skin, and this exposure route may be as important as oral uptake. The increase in mass loss with increased soil cadmium concentration may be caused by water balance impairment or increased metabolism to combat toxicity. The dermal absorption of cadmium may result in a decrease in the normal uptake of water occurring in amphibians exposed to cold (Sinsch 1991). The trend for toads to delay time-to-burrowing and to increase partial burial with higher soil cadmium suggests that cadmium was detected and perceived as something to avoid. It is unlikely that cadmium absorption caused these behaviors by impairing movement because the locomotor results were insignificant and the toads eventually did bury themselves after initial introduc-

tion to the containers. Midge larvae also increasingly avoid contaminated sediment with increasing cadmium concentration, but their estimated threshold level for avoidance (213 to 422  $\mu\text{g/g}$ ) exceeds that of our highest treatment (Wentzel *et al.* 1977). In the field, avoidance could translate into more time and energy expended to locate hibernation sites in uncontaminated areas. Studies should be conducted to determine whether this is a real occurrence and the associated costs. Cadmium is just one of many soil contaminants that amphibians may encounter. The dermal absorption by salamanders of 2,4,6-trinitrotoluene and Aroclor 1260 from soil has also been shown (Johnson *et al.* 1999). Nitrogen fertilizers cause avoidance behavior or even lethality when amphibians are exposed by way of soil or moist towels (Oldham 1997; Marco *et al.* 2001).

**Table 4.** Summary table of ANOVA analyses for soil, injection, and feeding studies

| Variable                  | Source        | <i>df</i> | MS         | <i>F</i> | <i>P</i> |
|---------------------------|---------------|-----------|------------|----------|----------|
| Soil study                |               |           |            |          |          |
| Toad tissue uptake        | Concentration | 4         | 1.880      | 7.86     | 0.0024   |
|                           | Error         | 12        | 0.239      |          |          |
| Time-to-burrowing         | Concentration | 4         | 295.950    | 2.23     | 0.0817   |
|                           | Error         | 43        | 132.784    |          |          |
| Percent mass loss         | Concentration | 4         | 0.002      | 0.50     | 0.7360   |
|                           | Error         | 32        | 0.004      |          |          |
| Sprint average hop length | Concentration | 4         | 12.921     | 1.33     | 0.2817   |
|                           | Error         | 30        | 9.717      |          |          |
| Endurance hop distance    | Concentration | 4         | 112465.420 | 0.85     | 0.5072   |
|                           | Error         | 31        | 133004.222 |          |          |
| Injection study           |               |           |            |          |          |
| Percent mass loss         | Concentration | 3         | 0.008      | 0.95     | 0.4281   |
|                           | Error         | 31        | 0.009      |          |          |
| Sprint average hop length | Concentration | 3         | 0.632      | 0.06     | 0.9795   |
|                           | Error         | 31        | 10.220     |          |          |
| Endurance hop distance    | Concentration | 3         | 96155.293  | 0.49     | 0.6891   |
|                           | Error         | 31        | 194646.035 |          |          |
| Feeding study             |               |           |            |          |          |
| Toad tissue uptake        | Concentration | 2         | 603.000    | 38.90    | <0.0001  |
|                           | Error         | 24        | 15.500     |          |          |
| Toad cadmium retention    | Concentration | 2         | 630.778    | 47.92    | <0.0001  |
|                           | Error         | 24        | 13.164     |          |          |
| Percent mass loss         | Concentration | 2         | 0.003      | 0.65     | 0.5353   |
|                           | Error         | 17        | 0.005      |          |          |
| Sprint average hop length | Concentration | 2         | 7.703      | 0.53     | 0.5984   |
|                           | Error         | 16        | 14.526     |          |          |

ANOVA = analysis of variance.

**Table 5.** Mean  $\pm$  1 SE for cadmium dynamics, bioaccumulation factors, and biological effects (feeding study)

| Cadmium treatment level                                  | Control         | Low                | High               |
|--|-----------------|--------------------|--------------------|
| Meal <sup>a</sup> ( $\mu\text{g Cd/g dry weight}$ )      | BG              | 15.034 $\pm$ 0.792 | 51.822 $\pm$ 3.161 |
| Mealworms <sup>b</sup> ( $\mu\text{g Cd/g dry weight}$ ) | BG              | 4.664 $\pm$ 0.158  | 16.073 $\pm$ 0.598 |
| Toads <sup>c</sup> ( $\mu\text{g Cd/g dry weight}$ )     | BG              | 0.698 $\pm$ 0.091  | 1.376 $\pm$ 0.291  |
| Toad feces ( $\mu\text{g Cd}$ )                          | BG              | 5.115 $\pm$ 0.625  | 17.003 $\pm$ 1.701 |
| Ingestion by toads ( $\mu\text{g Cd}$ )                  | BG              | 16.840 $\pm$ 1.114 | 53.456 $\pm$ 3.726 |
| Retention by toads <sup>d</sup> (% Cd)                   | —               | 15.6               | 9.3                |
| Mealworm bioaccumulation factor <sup>e</sup>             | —               | 0.31               | 0.31               |
| Toad bioaccumulation factor <sup>f</sup>                 | —               | 0.15               | 0.09               |
| Average total number of toad fecal pellets <sup>g</sup>  | 2.0             | 3.0                | 3.1                |
| Toad mass change <sup>h</sup> (%)                        | 31.3 $\pm$ 6.2  | 34.8 $\pm$ 5.7     | 30.3 $\pm$ 4.1     |
| Toad SVL change <sup>h</sup> (%)                         | 13.5 $\pm$ 2.0  | 10.5 $\pm$ 1.6     | 11.8 $\pm$ 1.9     |
| Food assimilation by toads <sup>h,i</sup> (%)            | 54.6 $\pm$ 10.3 | 61.9 $\pm$ 9.6     | 54.0 $\pm$ 6.9     |

BG = background: no counts above background detected; SE = standard error.

<sup>a</sup> Mean of cricket meal when mealworms first added.

<sup>b</sup> Mean of mealworms at time of use for feeding.

<sup>c</sup> Mean includes toads that died during hibernation and those that were killed after hibernation.

<sup>d</sup> Mean of the  $\mu\text{g Cd}$  in toad/ $\mu\text{g Cd}$  ingested (mean percent cadmium accumulation).

<sup>e</sup> Mean mealworm cadmium concentration/mean cricket meal cadmium concentration (dry weights).

<sup>f</sup> Mean toad cadmium concentration/mean mealworm cadmium concentration (dry weights).

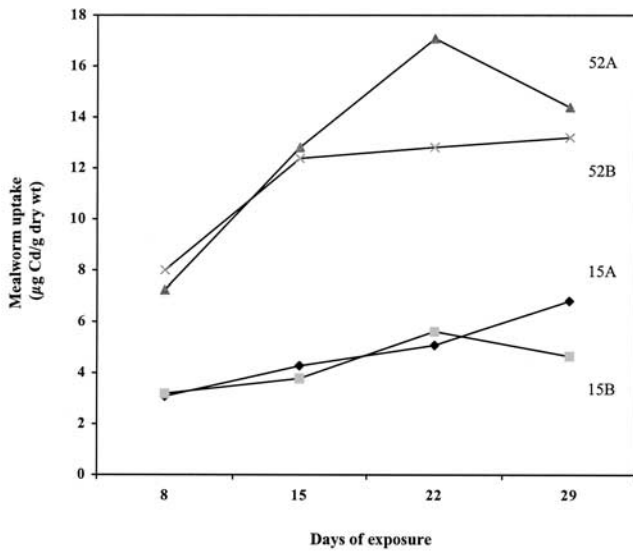
<sup>g</sup> Collected March 24 through May 25, 2001.

<sup>h</sup> From March 16 through May 25, 2001.

<sup>i</sup> Mean of the mass change/mass of food ingested.

Malathion inhibited brain cholinesterase activity when introduced on a paper substrate, but this effect was not observed in salamanders occupying sprayed outdoor enclosures (Baker 1985).

Toads injected with cadmium exhibited no treatment differences in any of the measured variables. This result is somewhat surprising for the toads in the highest treatments given the whole-body contaminant levels endured and the cessation of



**Fig. 1.** Cadmium uptake by mealworms over time. Mealworms exposed to low (15 µg/g) or high (52 µg/g) cadmium in their diet were sampled from the week 1 (A) and 2 (B) cohorts. Each point represents the mean of three 300- to 500-mg (wet weight) samples

eating before hibernation. Adult *B. regularis* injected once with cadmium were found to have a 96-hour  $LD_{50}$  of 6.2 µg/g (wet weight) (Hilmy *et al.* 1986), which is approximately twice that administered in our highest treatment. American toads may be tolerant of cadmium because there was not a single death in the 12-day period before hibernation. However, the highest mean posthibernation body burden of 11 µg/g (dry weight) is within the range found in terrestrial amphibians occupying polluted sites (*e.g.*, 1.3 to 14.4 µg/g; Dmowski and Karolewski 1979). Free-ranging American toads in an area upwind of 2 zinc smelters contained  $\leq 2.1$  µg Cd/g but were rarely encountered in the more polluted areas downwind (Beyer *et al.* 1985). It is unclear why toads injected with 0.4 µg Cd/g fared the worst during and after hibernation. Future manipulative laboratory research should include multiple sublethal injections of lower doses over time; such an exposure regime may be more realistic and may impose less stress on the organisms.

Toads fed contaminated mealworms retained <20% of the ingested cadmium. Much of the cadmium was excreted in the feces as has been documented in fish (Harrison and Klaverkamp 1989). Cadmium retention was greatest in the low cadmium group, but toad whole-body cadmium concentration increased with mealworm contamination level. Within the low- and high-cadmium treatments, toads that died during hibernation had significantly greater cadmium body burdens than those that survived. Although toads in both cadmium treatments did not exceed 3.2 µg/g (dry weight; based only on radioactivity), hibernation survival was notably decreased relative to that of the controls. This was not expected given the results of the soil and injection exposures and suggests that cadmium may be most toxic to American toads when obtained orally. The distribution of cadmium into different organs depending on exposure route could be an explanation. Toads did not avoid the contaminated prey because they readily ate all provided mealworms. This suggests that toads in the field might consume

prey containing at least 16 µg Cd/g. Because our study was of short duration and because available prey can possess >100 µg/g cadmium (Beyer *et al.* 1985), free-ranging toads may obtain much higher body burdens than we observed and be more at risk than our data indicate. It is unknown whether our results for this experiment were affected by the unnatural timing of feeding and hibernation.

Other studies using juveniles indicate that amphibians are tolerant of prey highly contaminated with cadmium. *Xenopus laevis* fed earthworms containing 609 µg Cd/g (dry weight) for 28 days did not suffer any mortality but did accumulate cadmium in the liver and eggs (Linder *et al.* 1998). *Xenopus* fed earthworms containing 72 µg/g had a mean of 0.5 µg/g (dry weight) in their eggs (Linder *et al.* 1998), which is in agreement with our egg data. Salamanders fed pellets containing 982 to 5701 µg Cd/g (wet weight) for 22 days all survived and had no differences in growth relative to controls (Nebeker *et al.* 1995). Bioaccumulation factors varied from 0.02 to 0.1 (Nebeker *et al.* 1995), which is comparable to but somewhat lower than what we found.

Choice of prey used for oral uptake studies may be important because of differences in digestibility by the amphibian and bodily distribution and uptake of the contaminant by the prey. In our study, low BAFs for the mealworms resulted in lower toad exposure levels than anticipated. We determined that mealworm exoskeletons contained cadmium concentrations in excess of twice that of the whole body, which suggests that mealworms absorb or excrete cadmium through the dermis. Because they periodically shed their exoskeletons, their whole-body burden is likely to be highly variable, as we found. For this reason and because toads cannot digest the exoskeletons, a nonmolting species may be a better choice. Invertebrates have been found to contain as much as 140 µg Cd/g (dry weight), with the highest concentrations reported in earthworms (Beyer *et al.* 1985). Linder *et al.* (1998) found that the earthworm *Eisenia foetida* accumulates 2 to 5 times the concentration of cadmium in the soil, although gut content makes up approximately half of the total body metal load. Because many amphibian species eat earthworms and they accumulate considerable cadmium, they would be good for a worst-case assessment of uptake by way of prey intake.

We tested exposure routes separately, but in reality organisms may be exposed to contaminants through multiple media and habitats throughout their lives. Therefore, single-route exposures may greatly underestimate the ability or tendency of organisms to accumulate harmful chemicals. Johnson *et al.* (1999) found that salamanders both orally and dermally exposed to 2,4,6-trinitrotoluene (TNT) and Aroclor 1260 (PCB mixture) had higher body burdens of TNT and 2 primary metabolites than the sum of what was found in individuals exposed to either pathway alone. Of the common measured end points for our dermal and oral studies, only whole-body cadmium concentration was significantly affected by treatment. Hence, we cannot conclude whether 1 exposure route is generally more harmful than the other at the cadmium levels tested. Nevertheless, there is the potential for cumulative toxicity because amphibians may obtain a body burden while feeding and aestivating in the active months and then add to that body burden during hibernation. As a result of chronic low exposure, terrestrial amphibians as well as their predators may

ultimately be negatively affected. Increased cadmium levels in eggs may also pose a risk to offspring.

Our experiment tested multiple stressors by incorporating both a chemical and a physical factor. In the case of the feeding study, there was some indication that cadmium toxicity increased during hibernation. No toads died in any treatments before overwintering began, but survival was only 56% or 67% in toads fed contaminated mealworms compared with 100% survival for controls. Although not statistically significant, any decrease in the number of postmetamorphic amphibians may be biologically significant. The feeding study demonstrated the importance of observing organisms after exposure in realistic or stressful (*i.e.*, challenging) environmental conditions (see also Johnson and Prine 1976). Studies that terminate on the completion of exposure may fail to detect latent or sublethal effects that become harmful given certain situations. The duration or environmental conditions chosen for the postexposure period should depend on the life cycle and ecology of the organism and the nature of the contaminant. Hibernation end points may be particularly important for lipophilic contaminant studies because of the use of fat bodies for energy and the associated opportunity for stored contaminants to spread throughout the body as fat is metabolized.

Because juveniles were exposed to high but environmentally relevant levels of cadmium, it appears that American toads are fairly tolerant of cadmium contamination. However, additional research is needed with other soil types, prey, exposure regimes, and environmental conditions because of possible increases in toxicity. Soil characteristics such as moisture, pH, particle composition, percent organic matter, and cation exchange capacity may affect bioavailability (Alloway 1995). Different prey species vary in their uptake and distribution of contaminants and digestibility. Long-term studies should be conducted in which toads are subjected to multiple routes of exposure simultaneously or sequentially across more than one life stage because this is likely a more realistic scenario and cumulative contamination may exceed threshold levels of tolerance with time. Finally, environmental conditions such as temperature and season affect amphibian physiology and hence may alter susceptibility to contamination. Because of species differences in hibernation ecology and physiology (Pinder *et al.* 1992) and in the uptake and distribution of contaminants (Harrison and Klaverkamp 1989), research with other amphibians should also be conducted to better understand whether current levels of terrestrial contamination pose a risk to amphibians in general.

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