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SOIL INGESTION MAY BE AN IMPORTANT ROUTE FOR THE UPTAKE OF CONTAMINANTS BY SOME REPTILES

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Abstract—Some species of reptiles regularly ingest soil in the wild. Therefore, we evaluated the importance of soil ingestion as a route for the uptake of contaminants in lizards. We used sand as a substitute for soil during the present study. Different groups of leopard geckos (*Eublepharis macularius*) were provided with a control and five sodium selenite–spiked sand mixtures during a 28-d study. Twenty lizards were assigned to a control group and to each of five selenium-spiked sand mixtures that consisted of nominal selenium (Se) concentrations of 0.05, 0.46, 4.57, 11.41, and 22.83 mg Se/kg dry sand. Leopard geckos readily ingested the Se-spiked sand. We observed concentration-related effects in several endpoints. Overall growth in body mass was the most sensitive endpoint and was significantly ($p < 0.05$) lower in lizards that ingested the 4.57, 11.41, and 22.83 mg Se/kg sand mixtures compared to controls. Growth in snout-vent length, mean daily food ingestion, and food conversion efficiency were less sensitive and were significantly ($p < 0.05$) lower in lizards that ingested the 11.41 and 22.83 mg Se/kg sand mixtures compared to controls. Although our results are based on nominal amounts of Se ingested, leopard geckos appear to be as sensitive to sodium selenite as birds and mammals. The present study suggests that ingestion of soil could be an important potential route for the uptake of soil contaminants in some reptiles and should be evaluated in ecotoxicological studies and risk analyses of reptiles.

Keywords—Soil ingestion Selenium Contaminant uptake Lizard Growth

INTRODUCTION

Reptiles are the most diverse group of terrestrial vertebrates and differ from other terrestrial vertebrate groups (i.e., birds and mammals) in numerous ways that may influence the relative importance of the different routes by which contaminants enter organisms. In addition, the sensitivity of reptiles to contaminants may differ from that of birds and mammals. Metabolic rates are typically slower in ectothermic reptiles than in endothermic birds and mammals [1,2]. Thus, reptiles may respond differently than birds and mammals to some environmental contaminants because their metabolic rates may slow the elimination and detoxification of toxic substances. Conversely, their slow metabolic rates may reduce the toxicity of some chemicals that must be metabolized to attain full toxicity. Furthermore, the enzymes produced by reptiles may differ in the efficiency at which they degrade toxicants compared to those produced by birds and mammals [3]. Also, any contaminant that is more toxic at lower body temperatures may be more toxic to reptiles than to birds and mammals [4].

Reptiles can be exposed to environmental contaminants by several routes, including ingesting contaminated material, contact with skin, maternal transfer into eggs and embryos, and uptake from the nest site by incubating eggs. Although ingestion of contaminated food is probably one of the most important routes for contaminants to enter reptiles and other terrestrial vertebrates, ingestion of soil could also be an important route for the uptake of soil contaminants in some reptiles. For example, some species of birds and mammals are known to ingest contaminated substrate (reviewed in Beyer and Fries [5]), and it also is likely that contaminants enter reptiles when soil is ingested. As far as we could determine, nothing is known about the importance of soil ingestion as a source of contaminants in reptiles. However, soil ingestion has been documented for several species of reptiles under natural conditions [6–11], and research is needed to determine if soil ingestion could be an important uptake route for contaminants.

The purpose of our research was to determine if soil ingestion could serve as a possible route by which contaminants enter lizards. We used the leopard gecko (*Eublepharis macularius*) as our experimental animal because this species breeds well in captivity and individuals frequently consume substrate material (L. Talent, unpublished data).

Selenium (Se) was selected as the test contaminant because soil contaminated with Se is common in some areas due primarily to human activities, including agricultural discharge and the disposal of fly ash from coal-powered electric power plants [12]. In addition, considerable background information is available on the toxic effects of Se on wildlife (reviewed in Ohlendorf [13]). Although high tissue levels of Se have been reported in wild-caught reptiles [13–15], little is known about the biological effects of Se toxicity on reptiles, including factors related to growth [16–21]. Therefore, we evaluated Se concentration-related responses on endpoints associated with food ingestion, food conversion efficiency, and growth in mass and snout-vent length (SVL).

MATERIALS AND METHODS

Test organisms

Leopard geckos are indigenous to arid and semiarid habitat in Pakistan and India [22]. The juvenile female leopard geckos used for the present study were obtained from a captive breeding colony at Oklahoma State University. The specific origin of the founding population is unknown except that they were

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derived from 15 to 20 wild-type animals that were originally obtained from the Tulsa Zoo (Tulsa, OK, USA). Hence, all geckos were related.

Leopard geckos were housed individually in plastic cages (28 × 17.5 × 12.5 cm) that were covered with a 3-mm steel mesh lid. A 40-W incandescent light bulb was positioned over one corner of each cage to permit thermoregulation across a temperature gradient of approximately 27 to 35°C. Room temperature was maintained at approximately 27°C, and a 14:10 h (light:dark) cycle was provided.

One hundred twenty juvenile lizards of similar mass (mean ± standard error of the mean) (13.12 ± 0.40 g), SVL (80.72 ± 0.78 mm), and age (six weeks) were randomly assigned to five selenium-spiked sand treatment groups and a control group, which resulted in 20 animals per group. At the beginning of the study, mass of each lizard was determined to the nearest 0.01 g, and SVL was determined to the nearest mm. Thereafter, lizards were measured at weekly intervals during a 28-d study.

Selenium-spiked sand

We used selenium-spiked sand as simulated soil during the present study. A series of sodium selenite (Na_2SeO_3; Sigma, St. Louis, MO, USA) concentrations were used in a range-finding study to determine a range of sublethal concentrations of sodium selenite–spiked sand that would be ingested by leopard geckos. Subsequently, experimental sodium selenite–spiked sand mixtures were prepared by dissolving sodium selenite in reagent grade water (18 MΩ) and saturating fine silica sand with the solution. Nominal sand concentrations of 0.1, 1, 10, 25, and 50 mg Na_2SeO_3/kg dry sand were prepared. These concentrations of Na_2SeO_3 resulted in nominal Se concentrations of 0.05, 0.46, 4.57, 11.41, and 22.83 mg Se/kg dry sand. The control was prepared by saturating sand with an equivalent volume of reagent-grade water.

The saturated sand mixtures were oven dried at 60°C for 48 h and then tumbled for 2 h at approximately 30 rpm in a rotary mixer. To both facilitate determining the mass of sand ingested for each of the five Se-spiked sand mixtures and the control and also to minimize the potential for percutaneous uptake of Se due to contact with the skin of lizards, cage bottoms were not covered with the sand mixtures. Instead, a preweighed spiked sand mix or control mix was provided ad libitum in a petri dish in each animal cage because a preliminary study demonstrated that the leopard geckos would seek out and consume sand from a dish (L. Talent, unpublished data). As the Se mix was consumed, additional preweighed Se-spiked sand was added. At weekly intervals, the sand mixture remaining in the petri dish and any of the mix that had been spilled into the cage were removed and weighed. Weekly ingestion of the Se-spiked sand mixtures was calculated by subtracting the amount of sand remaining from the amount that was placed in the cage. Daily sand consumption was estimated each week of the study by determining the mass of sand ingested during the week and dividing by 7 to get the mean amount of sand consumed each day during weeks 1 through 4. Sand consumption was expressed as percentage of body mass ingested in sand per day.

After the completion of the study, samples of the control and each Se-spiked sand mix were analyzed for total selenium by the Oklahoma State University Water Quality Research Laboratory. However, because of analytical problems encountered in these determinations, measured values of total selenium were not considered reliable. Additional samples were not available for repeat determinations. Therefore, we relied on nominal Se concentrations (0.05, 0.46, 4.57, 11.41, and 22.83 mg Se/kg dry sand) and the amount of sand ingested to estimate the Se ingestion of each lizard. Nominal daily Se ingestion was estimated by multiplying the amount of sand ingested by each lizard during the 28-d study by the nominal Se concentration in the sand mix and dividing by 28. Nominal Se ingestion values were expressed as mg Se ingested/kg body mass/d.

Food ingestion

Lizards were fed mealworms (*Tenebrio molitor*) and tap water ad libitum. Mealworms and water were not considered as sources of Se in the 28-d study. Prior to being placed in escape-proof dishes in lizard cages, mealworms were dusted with Nekton-Rep® (Günter Enderle, Pforzheim, Germany), a multivitamin and mineral mix. The mass of mealworms consumed daily was determined by multiplying the number of mealworms eaten by their mean mass. The mean daily mass of mealworms ingested by each lizard was determined by dividing the total mass of mealworms consumed during the study by 28. Food intake values are expressed as percentage of lizard body mass consumed in food per day. Gross food conversion efficiency was determined at the end of the study by dividing the increase in body mass (g) by the mass (g) of food consumed during the study.

Data analysis

Prior to statistical analysis, we tested the data for normality and homogeneity of variance. Subsequently, one-way analysis of variance (ANOVA) was conducted followed by a Holm–Sidak post hoc multiple-comparisons test that compared Se concentration groups to controls. If data were not normal or were of unequal variance, they were analyzed with a Kruskal–Wallis nonparametric analysis of variance on the ranks followed by a Dunn's test to compare Se concentration groups to each other or the control. We tested for the effects of selenium concentration in sand mixes on ingestion of the sand mixtures, food consumption, food conversion efficiency, and growth in mass and SVL. Results are presented as means ± standard error of the mean. A level of $p < 0.05$ was considered to be statistically significant. Tests were conducted using SigmaStat® Version 3.1 (Systat, San Jose, CA, USA).

RESULTS

Ingestion of sand and selenium

Leopard geckos readily consumed the sodium selenite–spiked sand mixtures, but the nominal amount of Se in the mixes did affect consumption (ANOVA [F] = 13.68; degrees of freedom [df] = 5, 114; $p < 0.001$). The mean daily percentage of body mass ingested was approximately 4 to 5% in the controls and three lower Se concentrations throughout the study. However, lizards that received the 11.41- and 22.83-mg Se/kg sand mixtures consumed less than 4% of their body mass during weeks 2 through 4 (Table 1). The mean daily percentage of body mass consumed by these two groups was significantly ($p < 0.05$) higher during week 1 than during the remainder of the study apparently because sand mixtures that contained higher Se levels were partially avoided.

Although the amount of Se ingested by each lizard was mathematically calculated on the basis of the nominal value and must be interpreted with caution, the means of these values

Table 1. Mean percentage (mean ± standard error of the mean) of body mass ingested in sand per day by leopard geckos (*Eublepharis macularius*) that were exposed to different nominal concentrations of Se-spiked sand mixes during a 28-d study[a]

Selenium concn. (mg Se/kg sand)	Week 1	Week 2	Week 3	Week 4
Control	5.33 ± 0.28	5.18 ± 0.26	4.89 ± 0.27	5.21 ± 0.39
0.05	4.61 ± 0.23	4.56 ± 0.29	4.35 ± 0.33	4.58 ± 0.33
0.46	5.38 ± 0.33	5.24 ± 0.25	5.27 ± 0.30	5.15 ± 0.41
4.57	4.80 ± 0.31	5.13 ± 0.24	4.90 ± 0.19	4.63 ± 0.29
11.41	5.23 ± 0.30	3.92 ± 0.34[b]	3.29 ± 0.22[b]	3.38 ± 0.23[b]
22.83	4.05 ± 0.16[b]	1.67 ± 0.17[b]	1.52 ± 0.12[b]	1.44 ± 0.12[b]

[a] $n = 20$ for each Se concentration at each week.
[b] Indicates value is significantly ($p < 0.05$) different from control.

differed among treatment groups (Kruskal–Wallis analysis [H] = 89.48; $df = 4$; $p < 0.001$). The estimated mean daily Se ingested ranged from a low of 0.0018 ± 0.0001 mg Se/kg body mass/d in the 0.05-mg Se/kg sand mixture group to a high of 0.5231 ± 0.0216 mg Se/kg body mass/d in the 22.83-mg Se/kg sand mixture group (Fig. 1).

Food ingestion and food conversion efficiency

A significant relationship was seen between the nominal concentration of Se in sand mixes and food ingestion ($F = 62.09$; $df = 5, 114$; $p < 0.001$). The mean daily percentage of body mass ingested in mealworms was significantly lower than the control ($p < 0.05$) in lizards that received the 11.41- and 22.83-mg Se/kg sand mixtures (Fig. 2). Mean daily percentage of body mass ingested ranged from a high of 1.93 ± 0.06% for control lizards to a low of 0.38 ± 0.06% for lizards in the 22.83-mg Se/kg sand mixture group.

A significant Se concentration–dependent response also occurred in the conversion of ingested food into body mass ($H = 62.08$; $df = 5$; $p < 0.001$). The conversion efficiency of controls was 0.54 ± 0.02, but the mean conversion efficiencies of all other groups were lower, and the 11.41- and 22.83-mg Se/kg sand mixture groups were significantly ($p < 0.05$) lower. The food conversion efficiency of lizards that received the 4.57-mg Se/mg sand mixture was reduced to 0.45 ± 0.03, but they still increased in mass during the study. However, most lizards that received the 11.41- and 22.83-mg Se/kg sand mixtures did not ingest enough food to maintain their body mass during the study.

Growth

A clear Se concentration–dependent response was observed relative to total growth in mass during the study ($F = 59.31$; $df = 5, 114$; $p < 0.001$) (Fig. 3). Growth in mass was significantly ($p < 0.05$) lower than controls in geckos that received the 4.57-, 11.41-, and 22.83-mg Se/kg sand mixtures. Controls gained 5.50 ± 0.37 g during the study, but lizards in the 4.57- and 11.41-mg Se/kg sand mixture groups gained only 3.90 ± 0.36 and 0.81 ± 0.51 g, respectively, whereas lizards in the 22.83-mg Se/kg sand mixture group lost 1.70 ± 0.20 g of mass.

Similar to the effects seen on mass, there was also a Se concentration–dependent relationship relative to growth in SVL ($H = 64.27$; $df = 5$; $p < 0.001$). Growth was significantly ($p < 0.05$) less than controls in lizards that received the 11.41- and 22.83-mg Se/kg sand mixtures. During the study, control lizards grew 8.35 ± 0.66 mm in SVL, but lizards in the 4.57-, 11.41-, and 22. 83-mg Se/kg sand mixture groups grew 6.60 ± 0.38, 2.80 ± 0.61, and 1.30 ± 0.36 mm, respectively.

Based on growth in mass, which was the most sensitive endpoint, the mathematically derived no-observed-adverse-effects level (NOAEL) and lowest-observed-adverse-effects level of nominal Se ingestion were 0.022 and 0.206 mg Se ingested/kg body mass/d, respectively.

DISCUSSION

Soil ingestion as a route for contaminant exposure

The results of the present study demonstrated that, under captive conditions, leopard geckos readily ingested sand in

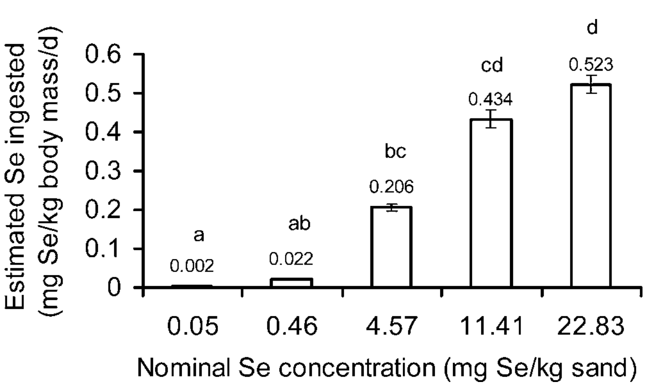

Fig. 1. Mathematically calculated estimates of mean mass (mg Se/kg body mass) of selenium ingested daily by leopard geckos (*Eublepharis macularius*) that were exposed to different nominal concentrations of selenium-spiked sand mixtures. Values above means are the calculated values. Bars are standard errors of the mean. Means with different lowercase letters are different at $p < 0.05$. $n = 20$ for all groups.

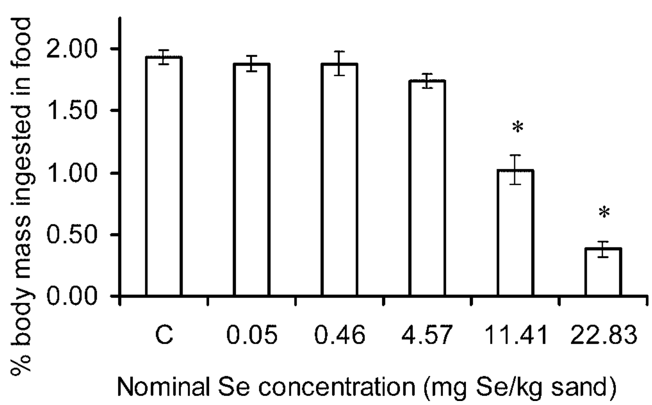

Fig. 2. Mean percentage of body mass ingested in food per day by leopard geckos (*Eublepharis macularius*) that were exposed to different nominal concentrations of selenium-spiked sand mixtures. Bars are standard errors of the mean. Means with an asterisk are different from the control at $p < 0.05$. $n = 20$ for all groups.

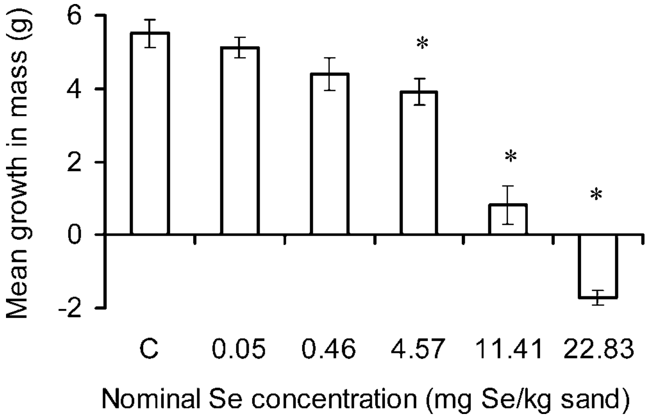

Fig. 3. Mean growth in mass by leopard geckos (*Eublepharis macularius*) that were exposed to different nominal concentrations of selenium-spiked sand mixtures during a 28-d study. Bars are standard errors of the mean. Means with an asterisk are different from the control at $p < 0.05$. $n = 20$ for all groups.

sufficient amounts to suggest that soil ingestion may be an important route for uptake of soil contaminants under natural conditions. However, there is a paucity of information regarding the amount of soil ingested either intentionally or unintentionally by reptiles in the wild, and ingestion rates may differ from those of captive reptiles. Furthermore, as far as we could determine, no information is available on the effects of different types of soil on ingestion rates of either captive or wild reptiles. Hence, the present study raised a number of questions that need to be investigated before the significance of soil ingestion as an exposure route for contaminants can be adequately evaluated. Nevertheless, our results demonstrated that leopard geckos exposed to graded concentrations of Se-spiked sand exhibited concentration-dependent responses relative to food ingestion and growth. These data support the hypothesis that soil ingestion may be a relevant route of exposure to soil contaminants in species of reptiles that regularly ingest soil either incidentally along with their food or intentionally to obtain minerals or for some other purpose.

Although environmental contaminants will probably enter an organism by multiple routes, in heavily contaminated areas, soil ingestion alone may result in the uptake of toxic amounts of a contaminant. For example, up to 8 mg Se/kg soil has been recorded from the top 15 cm of soil in the San Joaquin Valley (CA, USA), in restored upland grassland habitats near the site of the Kesterson reservoir [23]. This concentration of Se is higher than the concentration (4.57 mg Se/kg) in the present study that resulted in reduced growth in mass of leopard geckos.

For predatory lizards such as leopard geckos, ingestion of contaminated prey will likely be the major route for the uptake of contaminants. Additional contaminant intake could occur through either intentional or incidental soil ingestion. Therefore, the potential exists for some reptiles to accumulate higher levels of contaminants than predicted by risk assessments that consider only trophic transfer of contaminants through the food chain. We think that our data support the contention that soil ingestion should be evaluated as an exposure route in ecotoxicological studies and risk analyses of reptiles.

Selenium toxicity

Because our results are based on nominal Se values, the actual amount of Se ingested per day that resulted in adverse effects is unknown. However, the leopard geckos exposed to Se-spiked sand mixtures showed concentration-dependent responses in food consumption, food conversion efficiency, and growth in mass and SVL. Growth in mass was the most sensitive endpoint evaluated and was significantly ($p < 0.05$) reduced in lizards that were exposed to the 4.57-mg Se/kg sand mixture. Food ingestion, food conversion efficiency, and growth in SVL were also reduced in geckos exposed to Se-spiked sand mixtures, but these endpoints were significantly reduced ($p < 0.05$) only in the 11.41- and 22.83-mg Se/kg sand mixture groups. It should be noted that all endpoints examined were interrelated because both food consumption and conversion efficiency affect growth in mass and SVL.

The calculated NOAEL and lowest-observed-adverse-effects level values for Se ingestion of 0.022 and 0.206 mg Se ingested/kg body mass/d, respectively, relative to growth in mass are not measured values. Instead, they are mathematical approximations based on nominal Se concentrations and include uncertainties associated with nominal concentrations. However, even without analytical verification of total Se concentrations in the sand mixes, the concentration-dependent effects observed on food ingestion and growth of leopard geckos indicate that soil ingestion was a relevant route of exposure to soil contaminants in the present study. Furthermore, using nominal concentrations was adequate to show that leopard geckos consumed sufficient Se-spiked soil to exhibit good concentration-dependent effects on endpoints related to food consumption and food conversion efficiency, which affected growth.

Compared to other terrestrial vertebrates, little information is available on the biological effects of Se on reptiles. Roe et al. [16] determined that alligators (*Alligator mississippiensis*) that nested downstream from a coal-burning power plant transferred Se into their eggs. In addition, Hopkins et al. [17–20] conducted studies on snakes exposed to dietary Se and found that they exhibited normal food consumption and growth compared to controls. Moreover, Hopkins et al. [21] studied the transfer of Se from selenomethionine-enriched crickets (*Acheta domesticus*) to western fence lizards (*Sceloporus occidentalis*) in a simulated food chain and found that lizards exhibited normal food consumption and growth. However, the lizards accumulated Se in tissues at levels that approached the thresholds for reproductive toxicity in oviparous vertebrates. Although the snake and lizard studies provided information on the Se levels in prey items and the resultant tissue levels of Se after they had ingested the prey, no information was reported on the daily amounts of Se ingested. Therefore, the present study appears to be the first attempt to determine the biological effects of ingesting estimated nominal amounts of Se in reptiles and the first study to observe a concentration-dependent response in reptiles relative to food consumption and growth.

A limited amount of information is available on the amount of Se ingested as sodium selenite in spiked food or water that adversely affected some birds and mammals. Similar to leopard geckos, birds and mammals often reduce food intake after ingesting excessive Se [24,25]. In a 20-d study using domestic chickens (*Gallus gallus*), Nash et al. [26] found that a mean daily Se intake lower than 0.94 mg/kg body mass did not produce significant reductions in feed intake and weight gain. Beems and Van Beek [27] reported a NOAEL of 0.7 mg Se/kg body mass/d in a six-week growth study of hamsters (*Mesocricetus auratus*). In addition, Abdo [28] reported that the estimated NOAEL in laboratory mice (*Mus musculus*) and rats (*Rattus norvegicus*) were 0.90 and 0.40 mg Se/kg body mass/d, respectively, for a number of endpoints, including weight depression.

We were not able to determine the true amount of Se ingested by lizards in the present study. Furthermore, we did not analyze the lizard food and water for Se. However, based on mathematical estimates of Se ingested derived from nominal concentration of Se in sand mixtures, lizards that ingested an estimated 0.21 mg Se/kg body mass/d had reduced growth in mass, and lizards that ingested an estimated 0.43 mg Se/kg body mass/d had reduced daily food ingestion, food conversion efficiency, and growth in mass and SVL. Therefore, our data suggest that leopard geckos may be at least as sensitive to Se as birds and mammals, and further research is needed to fully evaluate the effects of Se on geckos and other reptiles.

One factor that should be considered when comparing the sensitivity of juvenile reptiles to juvenile endothermic vertebrates is the percentage of the growth cycle that an animal is exposed to a contaminant. For example, the present study was designed to cover approximately the length of time typically covered in sensitivity tests for birds and mammals. This design resulted in studying only 28 d in the growth cycle of a lizard that may take up to 12 months to reach full adult size under laboratory conditions. Domestic chickens, hamsters, rats, and mice reach adult size in six months or less. Therefore, to accurately compare the effect of Se or any other contaminant on growth of reptiles to that of birds and mammals, a study should probably cover an equivalent percentage of the growth cycle in all species studied. Such a study would require that leopard geckos be exposed for two to three months, and the additional days of exposure may indicate that they are relatively more sensitive to Se during their growth cycle than the results of our 28-d study indicate. Moreover, in order to realistically compare the sensitivity of reptiles to that of birds and mammals, it would be useful to determine selenium toxicity threshold values for reptiles similar to those derived for birds by Fairbrother et al. [29] and Adams et al. [30]. Reptiles are a diverse group, and the effects of Se need to be evaluated on several species to evaluate and compare the sensitivity of reptiles in general. The leopard gecko appears to have potential as a lizard laboratory model for future Se sensitivity studies and may be representative of a large group of nocturnal lizards.

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