Adverse effects of environmentally relevant dietary mercury exposure in larvae of the southern leopard frog, *Rana sphenocephala*

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Abstract: We exposed larvae of the southern leopard frog (*Rana sphenocephala*) to diets supplemented with *aufwuchs* (periphyton and associate organisms) from control and mercury (Hg) enriched mesocosms combined in proportions intended to mimic mercury concentrations and speciation in *aufwuchs* observed from aquatic systems contaminated by atmospheric deposition. Actual total mercury (THg) concentrations in the diets were 54, 423, 1409, and 3298 ng Hg/g DW 22 %, 3.4 %, 1.9 % and 1.5 % present as methylmercury (MMHg) for control, low, medium and high treatments, respectively. Rates of mortality, malformation, larval growth and development were observed over the entire larval period (60-254 d). Increased incidence of mortality, malformation, and changes in growth and development were observed at concentrations that reflect the highest concentrations expected in the amphibian diet from atmospheric deposition. The results of this study are probably more ecologically realistic than results obtained from previous studies of aqueous Hg toxicity and suggest that dietary Hg exposure in habitats primarily contaminated by atmospheric deposition has the potential to cause adverse effects in amphibian larvae. This is the first study to demonstrate the potential for adverse effects due environmentally realistic Hg exposure in amphibians.

Key words: Amphibian, Diet, Growth, Development, Malformation

INTRODUCTION

Declines in amphibian populations over the last 50 years have been documented worldwide and pollutants, such as Hg, are hypothesized to be among many possible contributing factors in some of these declines\(^1\)\(^-\)\(^2\). Hg from atmospheric deposition tends to accumulate in biota inhabiting wetlands that are critical breeding sites for amphibians\(^3\), yet little is known about Hg toxicity in amphibians. Although the teratogenicity and lethality of aqueous mercuric chloride and methylmercuric chloride on developing amphibian embryos and larvae has been well studied\(^4\), the toxicity of dietary Hg has not. The objective of this study was to determine if environmentally realistic dietary Hg exposure has the potential to cause changes in endpoints with demonstrated or theoretical impacts on amphibian population dynamics (timing and size at metamorphosis, growth and development rates, malformations, mortality). For a description of what constitutes environmentally relevant Hg exposure see Unrine and Jagoe in this abstract book.
RESULTS AND DISCUSSION

Malformation rate monotonically increased with treatment and was well explained \( (r^2 = 0.9945) \) by a log-logistic concentration response model \( (F_{1, 6} = 179.29, p = 0.0475) \). Malformation rates were 5.9\%, 5.6\%, 11.1\%, and 27.8\% in control, low, medium and high treatments, respectively (Figure 1). The control and low concentration treatments each had one tadpole with very mild scoliosis. Two of the medium concentration and five of the high concentration tadpoles displayed pronounced scoliosis, with a concentration-dependent increase in severity. We observed the appearance of microptalmia and micromelia in one tadpole fed the medium Hg diet and ectromelia of the tibiale and fibulare in both hind limbs of a tadpole fed the high Hg diet. Curvature in the tadpoles’ notochord persisted as curvature of the spine in metamorphs for tadpoles with severe scoliosis. Metamorphic success rates (total number of metamorphs – number that died or had arrested development) were 82.4 \%, 100 \%, 66.7 \% and 72.2 \% for control, low, medium, and high Hg diets, respectively (Figure 1). The percentage of normally developed individuals completing metamorphosis was 77 \%, 94 \%, 61 \% and 50 \% in control, low, medium and high Hg diets respectively and was significantly reduced for medium and high Hg diets (Figure 1).

Repeated measures ANOVA of mass yielded a significant treatment by stage interaction \( (F_{9, 147} = 2.56, p = 0.0238) \) for tadpoles that completed metamorphosis (Fig. 2A). Univariate ANOVA of mass measurements at each stage indicated that treatment effect of mass at 55 DPH (Days Post Hatching) \( (F_{3, 49} = 3.20, p = 0.0345) \) was the source of this interaction. Tadpoles fed high Hg diets were 39\% larger than control tadpoles at 55 DPH. We found no significant differences for mass at completion of hind limb development (HL) \( (F_{3, 49} = 0.16, p = 0.5222) \), completion of forelimb development (FL) \( (F_{3, 49} = 1.00, p = 0.4015) \), or completion of tail resorption (TR) \( (F_{3, 49} = 0.52, p = 0.6677) \) (Figure 2A). Completion of tail resorption marks completion of metamorphosis.

Figure 1. Developmental outcomes and mortality for southern leopard frog larvae exposed to Hg via the diet.
We observed concentration-dependent decreases in mean DPH to reach each developmental landmark with the exception of HL in the low treatment group. Log-rank tests yielded statistically significant differences for HL ($X^2 = 9.0094, p = 0.0292, df = 3$) and FL ($X^2 = 8.6365, p = 0.0345, df = 3$) but not TR ($X^2 = 3.7859, p = 0.2855, df = 3$) (Figure 2B). Comparisons of each treatment against control yielded a significant difference only for the high Hg treatment versus control for HL ($X^2 = 4.7294, p = 0.0297, df = 1$) and FL ($X^2 = 4.2336, p = 0.0396, df = 1$).

**Figure 2.** Mean mass at developmental stage for survivors by treatment (A) and mean days post hatching (DPH) at developmental stage (B) by Hg treatment. HL = complete hind limb development, FL = complete forelimb development, TR = complete tail resorption, 55 DPH = 55 days post hatching.

Time between completion of forelimb development and completion of tail resorption is defined as the tail resorption time and is controlled by thyroid hormones\(^5\). There was a concentration dependent increase tail resorption time, which became significant for tadpoles fed the medium and high Hg diets. This suggests that Hg may inhibit the thyroid axis as has been observed in fish\(^6\) and mammals\(^7\).
CONCLUSIONS

Dietary exposure in sites receiving Hg primarily through atmospheric deposition may be sufficient to cause adverse effects on amphibian development and decrease survival through metamorphosis. While approximately 10-15 % of tadpoles were either mildly malformed or died in the control and low Hg treatments, approximately 40-50 % of tadpoles were either severely malformed or died in the medium and high Hg treatments. Furthermore, many Hg exposed individuals metamorphosed precociously and had increased tail resorption times. These combined effects have potential to decrease the number and quality of offspring recruited to the terrestrial environment, ultimately decreasing the number of individuals with future potential for reproduction. Effects of dietary Hg exposure at low levels could also be exacerbated by other factors such as habitat destruction, disease, and global climate change. That we were able to detect malformations, decreased survival, and changes in normal growth and developmental rates at dietary Hg concentrations relevant to habitats contaminated solely by atmospheric Hg suggests that Hg pollution from atmospheric deposition has the potential to adversely impact amphibian populations. Further study of the effects of Hg pollution on amphibians is warranted and should focus on dietary Hg exposure.

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REFERENCES


