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Dietary Mercury Has No Observable Effects on Thyroid-Mediated Processes and Fitness-Related Traits in Wood Frogs

Haruka Wada,[§,†,*] Christine M. Bergeron,[†] F.M. Anne McNabb,[‡] Brian D. Todd,[∥,†] and William A. Hopkins[†]

[†]Department of Fish and Wildlife Conservation, Virginia Tech, Blacksburg Virginia 24061, United States

[‡]Department of Biological Sciences, Virginia Tech, Blacksburg Virginia 24061, United States

S Supporting Information

ABSTRACT: Mercury (Hg) is a neurotoxicant known to cause developmental and behavioral abnormalities in vertebrates. Increasing evidence suggests that Hg can also disrupt endocrine functions and endocrine-dependent processes. For example, dietary Hg has been shown to delay tail resorption during metamorphic climax in amphibians, a process mediated by thyroid hormones. However, a direct link between Hg, hormone disruption, and developmental delays in amphibians has not been explored. Therefore, we examined the effects of dietary Hg (0.01, 2.5, and 10 μg/g total Hg, dry wt) on thyroid hormone concentrations, development, growth, performance, and survival of wood frogs (*Rana sylvatica*). Tadpoles accumulated Hg in a concentration-dependent manner; total Hg concentrations in tadpoles at the beginning of metamorphic climax (Gosner stage 42) were 0.03, 1.06, 3.54 μg/g, dry wt, for control, low, and high Hg diets, respectively. During metamorphic climax, tadpoles eliminated 35% of the inorganic Hg from their tissues but retained most of their accumulated methylmercury. Contrary to our predictions, we found no effect of Hg on the duration of tadpole development, size at metamorphosis, tail resorption time, or hopping performance. Consistent with the lack of effects on development, we also detected no differences in whole-body thyroid hormone concentrations among our dietary treatments. Our results, when compared with the effects of Hg on other amphibians, suggest that amphibian species may differ substantially in their sensitivity to dietary Hg, emphasizing the need for data on multiple species when establishing toxicity benchmarks.

INTRODUCTION

Mercury (Hg) is a contaminant of global concern due to its environmental ubiquity and toxicity to humans and wildlife. Research on the effects of mercury on human health and wildlife has historically centered around neurological and developmental abnormalities, in part due to tragic events involving Hg poisoning in Japan and Iraq.[1] In both cases, ingestion of methylmercury (MeHg)-tainted fish or grains led to neurological damage and death of both humans and wild animals. Increasing evidence suggests that Hg can act as an endocrine disruptor as well.[2−11] Mercury is known to accumulate in endocrine organs, prevent steroidogenesis, and lead to abnormal levels of sex hormones, glucocorticoids, and thyroid hormones.[12] However, the direct mechanistic link between Hg, its effects on hormone disruption, and any subsequent effects on development or survival has not been examined in depth.

The hypothalamic-pituitary-thyroid axis is one of the endocrine systems that can be affected by Hg exposure.[6] Thyroid hormones play important roles in various developmental processes, including development of the nervous system.[13] Metamorphosis in amphibians and fish is also controlled by thyroid hormones.[14] Two forms of thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4) are stored and released from the thyroid glands. Triiodothyronine is the more potent form of the hormone and most circulating T_3 is produced by deiodination of T_4 in peripheral tissues. Mercury has been shown to prolong tail resorption during metamorphosis in southern leopard frogs (*Rana sphenocephala*),[15] suggesting that Hg may interfere with circulating T_3 and/or T_4 concentrations that regulate this process.

In this study, we examined the effects of dietary Hg on thyroid hormone concentrations and fitness correlates, including development, growth, metamorphosis, locomotor performance, and survival in wood frogs (*Rana sylvatica*). Mercury is known to accumulate in aquatic systems where inorganic Hg from atmospheric deposition and point sources is methylated to the more bioavailable MeHg. Although amphibians are at risk of exposure to high levels of Hg in these aquatic environments, they have received less attention compared to mammals, birds, and fish. Amphibians are an ideal model for examining the effects of Hg on the thyroid hormone axis because the amount of time it takes for amphibians to complete tail resorption, one of the signature

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events of metamorphic climax, is directly related to circulating thyroid hormone levels.[14] Tail resorption is also ecologically significant because prolonged resorption time can increase predation risk and decrease chances of completing metamorphosis before ponds dry in summer.[16] We hypothesized that dietary Hg would slow amphibian development and metamorphosis via alterations in thyroid hormone levels. We also predicted that recently metamorphosed frogs that ingested Hg as tadpoles would exhibit reduced locomotor performance due to neurotoxic effects of Hg.

MATERIALS AND METHODS

Animal Husbandry. In late winter to early spring, adult wood frogs lay clutches of 1000 to 3000 eggs, which typically hatch in 10−30 days.[17] For this experiment, five clutches of recently laid wood frog eggs were collected from an isolated, forested wetland in Montgomery County, VA on February 15, 2009 and brought to our laboratory in Blacksburg, VA. All clutches began hatching on February 23 and 24, 2009. On February 28, when all tadpoles reached a free-swimming stage (Gosner stage 21−23), 60 morphologically normal tadpoles from each clutch (300 total) were collected into 1 bin, and 216 experimental tadpoles were then arbitrarily chosen and placed in individual polypropylene 2.2 L bins filled with dechloraminated water. The bins were randomly distributed among four racks of five shelves and each was assigned to one of three dietary treatments (described below). Because individual tadpoles could not be weighed without damage at this stage, 20 unused tadpoles from the initial 300 tadpoles were euthanized and weighed to estimate initial body size of experimental animals.

Animals were raised in a controlled environment with a 12 L:12D photoperiod and an ambient air temperature of 18.2 ± 0.02 °C. Every 2−3 days, 50% of the water in each bin was exchanged with fresh dechloraminated tap water. Before every third water exchange, water in a subset of selected bins was analyzed spectrophotometrically for $N\text{-}NH_4$, $N\text{-}NO_2$ and $N\text{-}NO_3$ using a HACH kit (Loveland, CO). Temperature, pH, and dissolved oxygen were measured using YSI 556 MPS probe (Yellow Springs, OH). To reduce possible variation in water temperature and light exposure among individuals, the position of bins on each rack was rotated weekly and treatments were interspersed randomly among the racks in such a manner that every shelf of each rack contained bins from every treatment. The protocol used in this study was approved by Virginia Tech Institutional Animal Care and Use Committee (IACUC).

Mercury Exposure. Tadpoles were distributed among three diet treatments: control, low Hg, and high Hg (n = 72/treatment). The target total Hg (THg) concentrations of the spiked diets were 2.5 μg/g dry wt (dw) with 2.75% of that as methylmercury (MeHg) for the low Hg diet, and 10 μg/g dw with 1.05% of that as MeHg for the high Hg diet. These concentrations were based on environmental Hg concentrations and speciation in periphyton from areas in the United States with low and high Hg contamination. The low concentration corresponds to approximately twice the Hg concentration found in gut of larval southern leopard frogs in areas with atmospheric deposition of Hg alone.[18] The high concentration corresponds to Hg concentrations found in areas highly contaminated by point sources.[19] Diets for the tadpoles were composed of a dry feed mix spiked with or without Hg, which was subsequently suspended in an agar-gelatin mixture similar to the diet formulated by Unrine and Jagoe[15] (see Supporting Information for details). Target THg concentrations in the diets were obtained by adding mercury(II) chloride and methylmercury(II) chloride in proportions that reflected those found in periphyton in nature and in Hg-contaminated mesocosms.[20]

Beginning on February 28, 2009, tadpoles were fed approximately 6% of their body weight per day on a wet-weight basis by placing a piece of agar- and gelatin-suspended diet at the bottom of each bin. Fresh diet was provided every 2−3 days after uneaten food was suctioned out and water was exchanged. The Hg-contaminated food and debris was disposed of in compliance with Virginia Tech's waste handling policies. Twenty-five percent of the tadpoles (18 tadpoles/treatment, 54 tadpoles total) were weighed every 8−9 days to determine the effects of Hg treatment on growth rate and to adjust diet portions to accommodate larval growth. The first tadpole reached metamorphic climax, defined as Gosner stage 42, on April 25, 2009 and the last animal completed metamorphosis (Gosner stage 46) on May 22, 2009.

Hopping Performance Trials. Within one day of completing metamorphosis, each frog was used in trials to quantify its hopping performance (modified from Goater et al.[21]). Each frog was moistened in dechloraminated tap water from its holding container and was placed in the center of a large wooden platform (2 × 3 m). The frog was then gently prodded from behind on its urostyle until the animal hopped at least four times, leaving dark, moist spots on the wooden platform at its initial starting point and each subsequent landing point. The distance of each of the first four hops was then measured to the nearest mm and averaged to provide an estimate of hopping performance. Each frog was returned to its holding container and was allowed to rest for one hour before repeating the procedure to obtain a second estimate of mean hop length. The average of the two trials was used in the statistical analysis. All procedures were conducted between 1000 and 1600 h.

Sample Collection. Amphibians were euthanized in 1% buffered tricaine methanesulfonate (MS-222) then their whole-body thyroid hormone and Hg concentrations were determined. Individuals were analyzed for thyroid hormone concentrations at three different developmental stages according to Gosner:[22] 36−37 (n = 18/treatment), 42 (front limb emergence; n = 18/treatment), and 46 (completion of tail resorption; n = 10/treatment). Individuals at Gosner stage 46 were euthanized within one day of testing hopping performance. Mercury concentrations were determined for individuals at Gosner stage 42 and 46 (n = 3/treatment/stage). Tadpoles at Gosner stage 36−37 were sampled by date, across two consecutive days (April 9−10, 45−46 days after hatching), whereas tadpoles at Gosner 42 and 46 were sampled individually when they reached the designated stage. All samples were frozen at ≤ −20 °C for further analyses.

In addition to Hg and hormone concentrations, we recorded mortality, growth, and time to reach Gosner stage 42 and 46. Tadpoles were checked once a day for mortality and onset of metamorphic climax (Gosner 42), and checked twice a day for completion of tail resorption (Gosner 46). When animals were euthanized for hormone or Hg analysis, they were weighed and their snout−vent-length (SVL) was measured with digital calipers.

Determination of Whole-Body Thyroid Hormone Concentrations. Thyroid hormones were extracted from whole-body samples using methanol and chloroform.[23] Prior to hormone analysis, we validated the extraction protocol by demonstrating parallelism of serially diluted sample extracts in the radioimmunoassay

for T_3 and T_4 (see Supporting Information for details). The extraction efficiencies were 62−63%. The extracts were kept at −20 °C until assayed.

Thyroid hormone concentrations of the whole-body extracts were determined using double-antibody radioimmunoassay following the method described by Wilson and McNabb.[23] Samples were haphazardly distributed across assays and analyzed in two assays run on consecutive days. Intra- and interassay variation was 10.5% and 9.2% for T_4 and 13.0% and 12.2% for T_3, respectively. See Supporting Information for details on extraction and radioimmunoassay methodology.

Determination of Mercury Concentrations. Tadpoles at Gosner stage 42 and metamorphs (stage 46) (n = 3/treatment/stage) as well as the diet (n = 3/treatment) were analyzed for inorganic Hg (II) and MeHg concentrations[24] by Quicksilver Scientific (Lafayette, CO) using acidic thiourea leaching and Hg-thiourea liquid chromatography coupled to cold vapor atomic fluorescence spectrometry (HgTu/LCCVAFS). All samples were freeze-dried and homogenized prior to analysis. Average moisture content for animals at Gosner stage 42 and 46 were 87.8% and 83.5%, respectively, and for diets was 58.6%. Detection limit of the analytical procedure was 25.0 pg Hg (see Supporting Information for details on methodology and quality control). Trophic transfer factor (TTF) describes the relationship between contaminant concentrations of an animal and its diet and was calculated as Hg concentration of tissue divided by those of diet.

Statistical Analyses. Statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, Illinois). Prior to analysis, assumptions of normality and equal variances were tested and corrected accordingly using transformations. Mercury concentrations in diet of controls and Hg treatments were compared using MANOVA, where inorganic, organic, and total Hg concentrations were analyzed in a single model. Mercury concentrations of tadpoles and metamorphs (log transformed) were analyzed using two-way MANOVA with Hg treatment and Gosner stage as main factors.

Effects of Hg on thyroid hormone concentrations as well as fitness-related measures, such as developmental variables, locomotor performance and survival, were analyzed as follows. Repeated-measures ANOVA was used to determine effects of Hg treatment on mass gain of the subset of animals that were repeatedly weighed throughout the experiment. Survival to Gosner stage 46 was compared among treatments using a chi-square contingency table analysis. Growth and developmental measures of tadpoles in each Gosner stage were analyzed separately. For tadpoles sacrificed at Gosner stage 36−37, ANCOVA was used to determine the effects of Hg treatment on larval mass, with the actual Gosner stage as a covariate. For tadpoles sacrificed at stage 42, mass, SVL, and days to reach stage 42 were analyzed using MANOVA. Similarly, the effects of Hg treatments on mass at Gosner stage 46, mass loss during metamorphic climax, SVL, and duration of tail resorption were examined using MANOVA. Whole-body concentrations of T_4 and T_3 (ng/g tissue) at three stages of development were analyzed using separate MANOVAs and MANCOVAs, using actual Gosner stage (stage 36−37) as a covariate. Relationships between whole-body thyroid hormone concentrations and tail resorption duration were determined using linear regression. Thyroxine concentrations of stage 36−37 and 46 were log transformed, whereas those of stage 42 were inversely transformed to approximate normality. Triiodothyronine concentrations of stage 42 were log transformed.

Table 1. Mercury Concentrations (μg/g, dry weight) of Diet and Wood Frogs (*Rana sylvatica*) Fed Control, Low Mercury (Hg), and High Hg Diets until the Completion of Metamorphosis. [a]

	mercury species	treatments: control	low Hg	high Hg	F	P
diets	THg	0.01	2.5	10.13	1521.9	<0.001
	HgII	0.01	2.42	10.03	1537.1	<0.001
	MeHg	0.006	0.08	0.11	819.0	<0.001
tadpoles	THg, Gosner 42	0.03	1.06	3.54		
	THg, Gosner 46	0.05	0.85	2.57	498.3	<0.001
	HgII, Gosner 42	0.01	0.81	3.17		
	HgII, Gosner 46	0.02	0.54	2.04	368.5	<0.001
	MeHg, Gosner 42	0.02	0.24	0.37		
	MeHg, Gosner 46	0.03	0.31	0.52	219.7	<0.001

[a] Statistics in the table show effects of treatment on total Hg (THg), inorganic Hg (HgII), and methylmercury (MeHg) concentrations of diets and tadpoles.

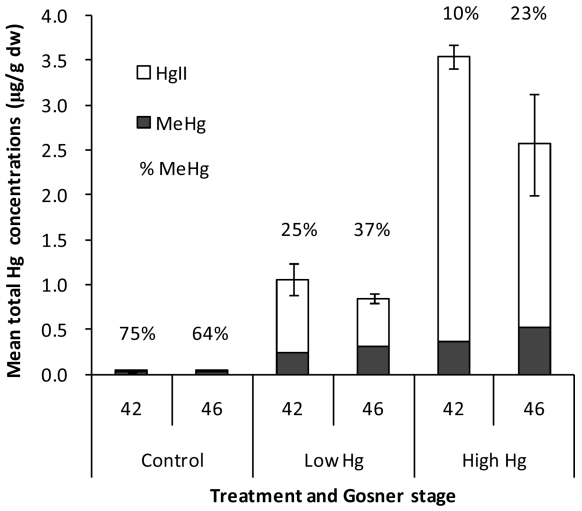

Figure 1. Mean whole-body mercury (Hg) concentrations of wood frogs (*Rana sylvatica*) that were fed control, low Hg, and high Hg diets during the entire larval period. Individuals were sampled for Hg at Gosner stage 42 (front limb emergence) and 46 (completion of metamorphosis). Closed bar represents MeHg concentrations and open bar represents HgII concentration. Percentages of MeHg are indicated on the top of each bar (MeHg = methylmercury; HgII = inorganic mercury).

Because body size is known to influence locomotor performance, hopping performance was analyzed using ANCOVA, with SVL as a covariate. Results were considered significant at $\alpha \leq 0.05$ level. Covariates that did not influence dependent variables significantly at the $\alpha \leq 0.15$ were removed from statistical models. Data are presented as mean ±1 standard error.

RESULTS

Mercury Concentrations in Diets and Tadpoles. Actual Hg concentrations in diets differed significantly among treatments

and matched the target concentrations (Table 1). Percent of MeHg in diets decreased as THg concentrations increased, from 57% in controls, 3.2% in low Hg, to 1.0% in high Hg treatments.

Dietary Hg concentrations strongly influenced whole-body Hg concentrations of wood frogs (Table 1, Figure 1). However, the effect of Hg treatment on tissue Hg concentrations depended on Gosner stage and Hg species (THg: stage: $F = 0.023$, $p = 0.883$; treatment × stage: $F = 7.0$, $p = 0.01$; MeHg: stage: $F = 23.3$, $p < 0.001$; treatment × stage: $F = 6.6$, $p = 0.012$; HgII: stage: $F = 0.002$, $p = 0.961$; treatment × stage: $F = 7.8$, $p = 0.007$). During larval development, wood frog tadpoles preferentially accumulated MeHg compared to HgII, indicated by higher TTFs for MeHg compared to HgII (Figure 2). Trophic transfer factors

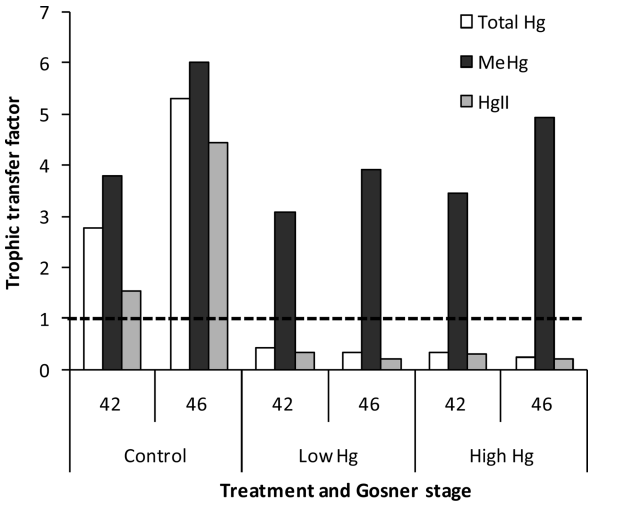

Figure 2. Trophic transfer factor (TTF) for wood frogs (*Rana sylvatica*) fed control, low mercury (Hg), and high Hg diets during the entire larval period. TTF was calculated as Hg concentrations in amphibians divided by Hg concentrations in diet. Dotted horizontal line represents a 1:1 relationship between amphibian tissue and diet. Individuals were sampled for Hg at Gosner stage 42 (front limb emergence) and 46 (completion of metamorphosis) (MeHg = methylmercury; HgII = inorganic mercury).

of MeHg were 9.1 (low Hg) and 10.9 (high Hg) times that of inorganic Hg at Gosner stage 42. During metamorphic climax (tail resorption) when tadpoles cease feeding, tadpoles retained more MeHg than HgII in their tissues. During this period, tissue HgII concentrations decreased by approximately 35% in both Hg treatments. In contrast, tissue MeHg increased by 28% and 43% in low and high Hg treatments, respectively.

Fitness-Related Measures. Survival, metamorphic success, growth, development, and locomotor performance of wood frogs did not differ among control and Hg treatments (Table 2). Overall survival to Gosner stage 42 in all treatments was ≥97%. All tadpoles that survived to Gosner stage 42 also successfully completed metamorphosis except for one individual; thus, metamorphic success was 94.4%, 88.2%, and 100% in control, low Hg, and high Hg treatments, respectively. Tadpoles gained body mass over time in a sigmoid fashion regardless of treatment (time: $F = 1154.1$, $p < 0.001$; treatment: $F = 0.17$, $p = 0.85$; time × treatment: $F = 0.26$, $p = 0.99$). Similarly, Hg treatment did not affect mass or SVL of tadpoles that were sacrificed at Gosner stage 36−37, 42, and 46, nor mass loss during metamorphic climax (Table 2). Developmental rates, such as days to reach stage 42 and duration of tail resorption, were also not influenced by Hg treatments. On average, tadpoles reached the onset of metamorphic climax 66 days after hatching and took 11 additional days to fully resorb their tails. The SVL of metamorphs was significantly correlated with hopping performance ($F = 9.01$, $p = 0.005$), with larger individuals being capable of hopping greater distances on average. However, locomotor performance was not affected by dietary Hg ($F = 0.71$, $p = 0.50$; Table 2).

Thyroid Hormone Concentrations. Consistent with the lack of effects on growth and development, Hg treatments did not alter the whole-body thyroid hormone concentrations in tadpoles at any of the developmental stages we sampled (Figure 3a and b). At 45−46 days of development, most tadpoles were either Gosner stage 36 or 37. When thyroid hormone concentrations of tadpoles in Gosner stage 36 and 37 were compared, Gosner stage significantly affected whole-body T_3 concentrations ($F = 6.80$, $p = 0.012$) but not T_4 concentrations ($F = 0.02$, $p = 0.89$) of tadpoles. Specifically, individuals at Gosner stage 37 had 44% higher T_3 concentrations compared to individuals at

Table 2. Fitness-Related Measures and Hopping Performance of Wood Frogs (*Rana sylvatica*) Fed Control, Low Mercury (Hg), and High Hg Diets until the Completion of Metamorphosis

			mean (1 SE)				
response		Gosner stage	control	low Hg	high Hg	*F*	*P*
survival (%)		46	94.4	88.2	100.0	1.01	0.31
growth		36−37	938(18)	922 (27)	960 (22)	2.55	0.09
	body mass (mg)	42	830 (25)	840 (8)	832 (22)	0.09	0.92
		46	463 (21)	476 (24)	462 (22)	0.12	0.89
	mass loss during tail resorption (mg)	42−46	331 (18)	363 (27)	333 (17)	0.73	0.49
	SVL (mm)	42	17.9 (0.3)	17.7 (0.2)	17.9 (0.2)	0.41	0.66
		46	16.4 (0.3)	16.5 (0.3)	16.5 (0.2)	0.59	0.56
development	days to reach stage 42		65.5 (0.4)	66.4 (0.4)	65.6 (0.3)	1.86	0.16
	duration of tail resorption (days)	42−46	10.8 (0.4)	11.3(0.6)	9.9 (0.5)	2.25	0.12
hopping performance	hopping distance (mm)[a]	46	20.4(1.5)	22.3(1.5)	19.9(1.4)	0.71	0.50

[a] LS means, corrected for body size.

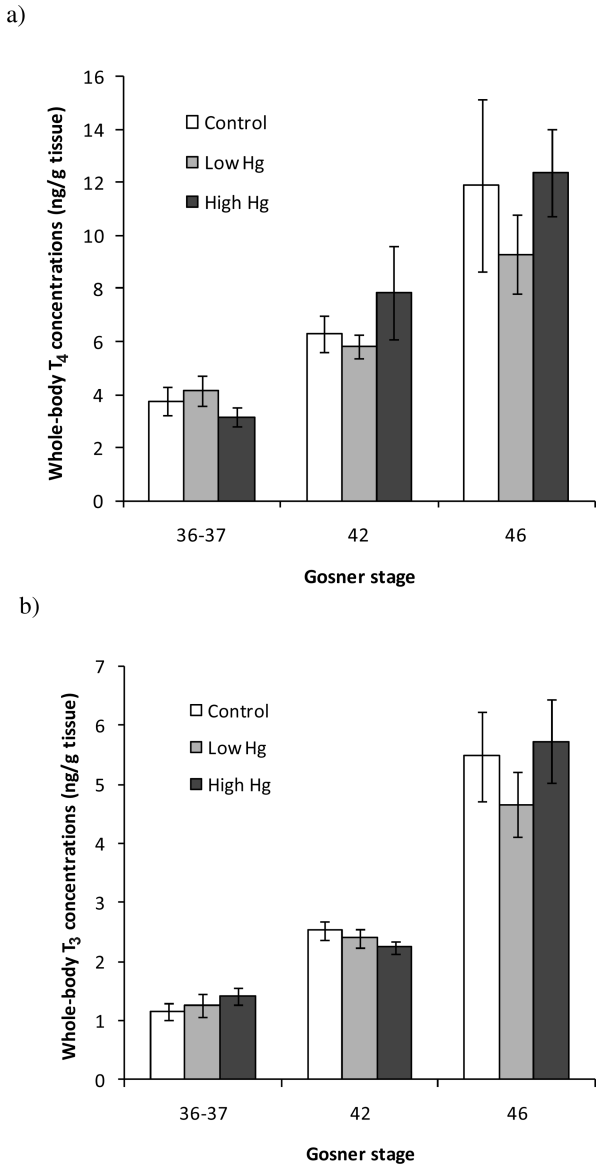

Figure 3. Mean whole-body thyroid hormone concentrations of wood frogs (*Rana sylvatica*) fed control, low mercury (Hg), and high Hg diets during the entire larval period. Whole-body thyroxine (T_4, 3a) and triiodothyronine (T_3, 3b) concentrations were measured at Gosner stage 36−37, 42, and 46.

Gosner stage 36. There was no effect of dietary Hg on either T_4 or T_3 concentrations (T_4: $F = 1.09$, $p = 0.34$; T_3: $F = 1.14$, $p = 0.33$). Similarly, Hg treatment had no effect on the whole-body thyroid concentrations at the beginning or end of metamorphic climax (stage 42: T_4: $F = 0.21$, $p = 0.81$; T_3: $F = 0.85$, $p = 0.44$; stage 46: T_4: $F = 0.84$, $p = 0.44$; T_3: $F = 0.69$, $p = 0.51$). Neither T_4 nor T_3 concentrations after completion of metamorphosis were significantly correlated with the tail resorption duration (T_4: $r^2 = 0.083$, $p = 0.12$; T_3: $r^2 = 0.067$, $p = 0.17$).

DISCUSSION

We fed wood frog tadpoles ecologically relevant concentrations of dietary Hg from hatching to completion of metamorphosis to examine how dietary Hg affects survival, growth, development, performance, and thyroid hormone concentrations. Despite high concentrations of Hg in tadpoles and metamorphs, we observed no adverse effects of Hg on fitness-related traits or whole-body thyroid hormone concentrations. This suggests that wood frogs may be relatively insensitive to dietary Hg compared to the three other amphibian species studied to date (southern leopard frogs;[15] American toads, *Bufo americanus*;[25] western clawed frogs, *Silurana tropicalis* [26]).

Differential Assimilation and Retention of Mercury in Wood Frog Tadpoles. The TTF (the ratio of contaminant concentrations between an animal and its diet) for MeHg at Gosner stage 42 was ∼10 times higher than that of HgII, indicating that wood frog tadpoles preferentially accumulated MeHg over HgII. Previous studies have shown that Hg, especially MeHg, is readily assimilated into biological tissues leading to bioaccumulation and biomagnification. Assimilation efficiency of MeHg may be higher than HgII due to low elimination and high retention of MeHg. For instance, killifish (*Fundulus heteroclitus*), sweetlips (*Plectorhinchus gibbosus*) and crayfish (*Orconectes virilis*) retained >90% of ingested MeHg even weeks after a brief exposure [27−29] but retained less than 30% of HgII.[29] As a result, TTF for MeHg is often higher than 1, and can be as high as 8 in killifish.[27,30,31] High assimilation efficiencies of MeHg in predatory organisms may also be due to partitioning of MeHg in prey or their diet. For instance, zooplankton can assimilate four times as much as MeHg compared to HgII due to the high proportion of MeHg in cytoplasm of planktonic prey.[32] Similarly, subcellular partitioning of Hg may also depend on Hg species in algae, leading to higher TTF for MeHg than HgII. Southern leopard frog tadpoles fed Hg-contaminated aufwuchs had 5.6 times higher TTF for MeHg than HgII (determined from the difference in MeHg:HgII ratio in tadpoles to diet).[20] The even higher ratio of TTF between MeHg and HgII in our study compared to previous studies indicates that (1) the bioavailability of Hg in diet which was directly spiked with Hg is higher than that of Hg incorporated into prey or algae, and (2) wood frogs may also contribute to biomagnification of MeHg in food webs.

Wood frogs retained a large proportion of MeHg in their tissues during metamorphic climax. Tissue MeHg concentrations increased by 28 and 43% in low and high Hg treatments, whereas tissue HgII decreased 34 and 36% during the same period. Since tadpoles do not feed during climax, this observation is likely due to a combination of slower elimination of MeHg compared to HgII and rapid loss of body mass during this period. On average, tadpoles lost 42% of their body mass during metamorphic climax. When this body mass loss is taken into account (i.e., Hg concentrations at Gosner stage 46 multiplied by 0.58), tadpoles eliminated approximately 62% of HgII, whereas they lost only 22% of MeHg during this 11 day period. Previous studies suggested that while some elements, such as copper, arsenic, and lead, are eliminated significantly through metamorphosis,[33] other elements like Hg are not.[18,20] One possible mechanism for variation in elimination rates is the uneven distribution of these elements among tissues. When they are bound to gut epithelia, they can be eliminated during intestinal remodeling. In fish, dietary inorganic Hg was concentrated in the gastrointestinal tract[34], whereas MeHg was distributed more evenly throughout the body.[27,34] If tissue distribution of Hg species in amphibians is similar to fish, then a higher proportion of inorganic Hg would be shed during climax. Regardless of the mechanism, retention of MeHg after metamorphosis suggests that amphibian tadpoles may be important in trophic transfer of Hg in both aquatic and terrestrial food webs,[35] much like emerging insects.[36,37] The

biomass of amphibians is particularly high compared to other vertebrates[38,39] and a variety of birds, reptiles, and mammals prey upon larval and metamorphosed amphibians. This implies that amphibians play an important role in the fate and transport of Hg in food webs.

Effects of Mercury on Fitness-Related Measures and Thyroid Hormone Concentrations. Previous studies on mammals, birds, and fish have shown that Hg can affect neurological and reproductive function. The Environmental Protection Agency determined the lowest observed adverse effect level (LOAEL) for humans to be 1.1 μg/g MeHg wet wt (ww) in diet.[40] In birds, the preliminary LOAEL is estimated to be 0.4 μg/g MeHg ww in diet for loon chicks, *Gavia immer*.[41] Based on laboratory studies, the latter concentration is associated with damaged immune function and decreased activity levels.[42,43] The MeHg concentrations of 0.033 and 0.044 μg/g ww in our low and high Hg diets (calculated from dw, 58.6% moisture) were well below the LOAEL for mammals, preliminary LOAEL for loon chicks, and concentrations that triggered mortality, lethargic behavior, and low metamorphic rates in western clawed frogs (0.27 μg/g ww [26]), however they were higher than MeHg concentrations that led to increased mortality and decreased development and tail resorption rates in southern leopard frogs (0.013 μg/g ww[15]).

Wood frog tadpoles in our experiment accumulated equivalent or higher MeHg concentrations than those suggested or associated with adverse effects in fish and other amphibians. Tadpoles that were fed low and high Hg diet accumulated 0.029 and 0.044 μg/g MeHg ww (0.24 and 0.37 μg/g dw) by the start of metamorphic climax and 0.053 and 0.089 μg/g MeHg ww (0.31 and 0.52 μg/g dw) by the end of metamorphosis. In fish, 0.2 μg/g MeHg ww (or 1.0 μg/g dw, assuming 80% moisture) is the whole-body threshold level for adverse effects in adults and juveniles, calculated based on LOAEL and no observed adverse effect level (NOAEL) derived from measures of reproduction, survival, and behavior.[44] Because of a higher vulnerability of young fish to Hg exposure, Wiener and Spry[45] suggested that 1 to 10% of adult threshold levels should be used for egg and larval stages. This translates to 0.002−0.02 μg/g MeHg ww as a whole-body threshold level for fish during early development. The existing work on toxicity thresholds for amphibians is limited. However, Unrine et al.[15] observed lower survival, decreased tail resorption rate, and decreased metamorphic success when whole-body MeHg concentration of leopard frogs was ∼0.003 ug/g ww, a level below that observed in our low Hg treatment. In a separate concurrent study in our laboratory wherein American toad tadpoles were fed the same diets used in this study, metamorphs reared on the high Hg diet had whole-body MeHg concentrations of ∼0.051 μg/g ww and suffered impaired growth compared to ones fed on control diets.[25]

Contrary to our prediction, we did not observe any effects of dietary Hg on development, whole-body thyroid hormone concentrations, survival, or performance despite the fact that whole-body concentrations in wood frogs were higher than the preliminary toxicity threshold for fish[45] and amphibians.[15] Tail resorption duration and hopping distance in our experiment were similar to the values previously reported for wood frogs.[46,47] Similarly, whole-body concentrations of T_4 and T_3 were comparable to other amphibian species,[48,49] however thyroid hormone concentrations at Gosner stage 46 did not correlate with the duration of tail resorption. It is possible that other factors, such as dietary selenium concentrations, could alter Hg toxicity,[50] giving rise to differences between previous studies and ours. It is also possible that procedural differences in experimental conditions among studies may affect Hg accumulation and effects, leading to perceived differences in species sensitivity that are actually attributable to differences in methodology. However, a concurrent, parallel study in our laboratory using the same diet formulation and husbandry methods clearly showed that American toads were more sensitive than wood frogs, suggesting that some differences in species sensitivity are likely real.[25] Differences in sensitivity between American toads and wood frogs could theoretically arise from differences in length of the larval period, which would create differences in exposure duration. However, our data do not support this possibility because the larval period of wood frogs in our experiment was ∼77 days whereas that of toads was ∼65 days.[25] Thus, inherent physiological differences among species most likely contribute to observed differences in species sensitivity, but future studies evaluating additional species and multiple populations of the same species are needed to fully address the factors that give rise to differences in sensitivity of amphibians.

In summary, to better understand the link between Hg, endocrine disruption, and developmental delays in amphibians, wood frog tadpoles were fed Hg-spiked diet from hatching to completion of metamorphosis. Despite the fact that TTF for MeHg in wood frogs was high and whole-body Hg concentrations exceeded those associated with increased mortality, malformation, and/or delayed development in two other amphibian species, we did not observe any adverse effects of Hg on development, survival, performance, or whole-body thyroid hormone concentrations in wood frogs. Comparison of these results with the limited data available for Hg effects on other amphibian species suggests that there may be large interspecies variation in Hg sensitivity. These factors underscore the need for future work examining the effects of Hg on amphibians, in particular species and population variation in Hg sensitivity and the role of amphibians in transporting Hg in the environment.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Additional information is provided of experimental diet formulation, determination of whole-body thyroid hormone and mercury concentrations. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 519-661-2111, x84669; fax: 519-850-2923; e-mail: hwada@uwo.ca

Present Addresses

[§]Department of Psychology, University of Western Ontario, London Ontario, N6A 5C2, Canada

[‖]Department of Wildlife, Fish, and Conservation Biology, University of California, Davis, Davis California, 95616, United States.

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