Supporting Information for:

Dietary mercury has no observable effects on thyroid-mediated processes and fitness-related traits in wood frogs

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Diet preparation

Dry components (218g Aquamax Grower 600, PMI Nutrition International; 218g Rabbit chow, LM Animal Farms; 32g TetraMin, Tetra; 32g Sera Micron, Sera) were ground and combined, and Hg dissolved in 95% ethanol was added to the mix. In the control diet, Hg-free ethanol was added. The diet was mixed until homogenized and allowed to dry under a fume hood overnight. The next day, 750mL nanopure water, 20g agar, and 14g gelatin were stirred and heated to ~70ºC on a hot plate, then added to the dry feed mixture. The diet was mixed until homogenized, cooled to allow the mixture to congeal, then stored in -80ºC freezer until use.

Determination of whole-body thyroid hormone concentrations

Thyroid hormones were extracted from whole-body samples using methanol and chloroform [1]. Prior to hormone analysis, we tested two extraction methods: methanol and chloroform methods [1] and methanol, chloroform, and chromatographic separation [2]. Both showed parallelism of serially diluted sample extracts in the radioimmunoassay for T_3 and T_4, therefore we chose the former method with higher extraction efficiencies.

Whole-body samples were homogenized with methanol containing 1mM propylthiouracil (MeOH-PTU; 3.5mL for Gosner stage 36-37 and 42, and 1.8mL for Gosner stage 46). The homogenizer tip was rinsed with additional 1mL MeOH-PTU. After adding the rinse into the rest of the homogenate, ~2000 cpm of labeled T_4 was added to all tubes to trace hormone recovery efficiency and the tubes were left at 4^oC until extraction. On the day of extraction, all tubes were shaken then centrifuged at 1300 x g for 15 min at 4^oC. Supernatants, in addition to the supernatants from resuspended

tissue pellet, received 5mL of chloroform and 0.5mL of ammonium hydroxide (2N NH_4OH), centrifuged, and the aqueous layer was collected. The chloroform-containing phase of the first supernatant was extracted two more times with 0.5mL 2N NH_4OH. The combined aqueous layer was then extracted with an equal amount of chloroform, and the resulting aqueous layer was dried with a stream of air and resuspended with 300µL of 75% ethanol. To determine the extraction efficiency, each extract was counted for 10 min. The extracts were kept at -20°C until the assay.

Thyroid hormone concentrations of the whole-body extracts were determined using double-antibody radioimmunoassay following the method described by Wilson & McNabb [1]. The radioimmunoassay were validated for wood frog whole-body samples. Assay standards were prepared in the same ethanol concentration as was present in the whole-body extracts.

Determination of mercury concentrations

Mercury concentrations in the diet and tissue samples were determined by separating organic monomethyl mercury (CH_3Hg^+) from inorganic mercuric Hg^{2+} mercury using the charges on their respective thiourea complexes. Online cold-vapor generation follows separation with an absolute instrument detection limit of 4 pg/g for MeHg and 7 pg/g Hg (II) (for a 100mg sample) [3]. Mean percent recoveries for matrix spikes were 92.2% for MeHg and 98.0% for HgII (n=3). Mean percent recoveries of the standard reference materials were 94.7 and 97.1% (BCR-463; tuna fish from Institute for reference materials and measurements, Geel, Belgium), and 92.6 and 93.4% (DOLT-3; dogfish liver from National Research Council Canada, Ottawa, Canada) for MeHg and HgII, respectively. Percent difference between duplicate samples was 3.1% for MeHg and

6.3% for HgII (n=3 samples).

References

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3. Shade, C. W., Automated Simultaneous Analysis of Monomethyl and Mercuric Hg in Biotic Samples by Hg-Thiourea Complex Liquid Chromatography Following Acidic Thiourea Leaching. Environmental Science & Technology **2008,** 42, (17), 6604-6610.