

# TISSUE DISTRIBUTION AND MATERNAL TRANSFER OF MERCURY IN DIAMONDBACK TERRAPINS WITH IMPLICATIONS FOR HUMAN HEALTH

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**Abstract.** Mercury contamination, which has significant human health implications, impacts watersheds worldwide. Methylmercury, the mobile form of mercury, bioaccumulates at each trophic level and tends to biomagnify within predatory, long-lived organisms over time. Top aquatic predators--such as fish and turtles--are typically eaten by humans and can cause health problems associated with dietary mercury exposure. Diamondback terrapins (*Malaclemys terrapin*) are long-lived, top level predators and have been hunted for food throughout the Southeast for over a century. In addition, they are among the several U.S. species of turtles commonly sold in food markets in Asia. We present data from two studies of mercury contamination in diamondback terrapins and make recommendations for future research to further examine patterns of mercury contamination in South Carolina estuaries.

In the first study, adult female terrapins were collected from St. Helena Island and Charleston, SC, and Brunswick, GA. Total mercury was measured in scutes, unhatched eggs, and internal tissues from roadkilled Brunswick specimens. In the second study, six adult terrapins confiscated from the food trade were dissected and their tissues analyzed for total mercury concentrations.

These studies indicated that tissue mercury concentrations can vary significantly by region and that mercury can be maternally transferred to offspring in this species. These findings offer insight into the complexity of mercury transport within aquatic food webs and indicate that consumers who eat terrapin meat frequently may be at risk for mercury-related health problems. We suggest strategies for watershed managers to protect consumers and decrease consumption demand for diamondback terrapins by educating the public about environmental mercury contamination and the possible

health consequences of dietary mercury exposure.

## INTRODUCTION

Diamondback terrapins are the only native US turtles that exclusively inhabit estuaries. Facing threats from habitat loss, abandoned crab traps, and excessive harvesting for the food trade in Asia, they are listed as a species of concern by the US Fish and Wildlife Service (Dorcas et al., 2007). Prior studies have documented the potential for environmental mercury contamination to negatively affect wildlife, particularly in aquatic habitats (Linder and Grillitsch, 2000). Examining the biological impacts of mercury (Hg) contamination on diamondback terrapins will provide insight into how entire populations are affected by localized environmental mercury pollution. This information will be of interest to coastal fisheries managers, land use planners, and those influencing decisions about which and where industrial facilities will be given permits to build. It is also important for potential consumers, as diamondback terrapins are still harvested for consumption in some areas.

## BACKGROUND AND RELATED WORK

Environmental Hg contamination poses a significant threat to sensitive aquatic ecosystems and the organisms that inhabit them. Coal-fired power plants, chlor-alkali facilities, and cement factories are among the anthropogenic sources of atmospheric Hg emissions. Inorganic Hg deposits into lakes, rivers, and estuaries, where bacterial methylation converts it to methylmercury (MeHg). This organic form of Hg adheres to sediment particles, is taken up by bacteria and plankton, and biomagnifies at each trophic level. The abundance of sulfate-reducing bacteria in estuaries facilitates

methylation of inorganic Hg (Compeau and Bartha, 1985), making these wetlands particularly susceptible to MeHg contamination. Understanding the impacts that this contamination has on estuarine wildlife populations is crucial to the development of coastal management and remediation initiatives.

Methylmercury's tendency to bioaccumulate over time and biomagnify with trophic position places long-lived predatory species at highest risk for Hg exposure. The methyl groups of the compound bind readily to sulfhydryl (-SH) groups, causing MeHg to accumulate in protein-rich tissue such as liver, hair, feathers, and scutes (Linder and Grillistch, 2000). Dietary Hg exposure has been shown to affect reproductive success, neurologic function, and behavior in many species (Heinz, 1979; Webber and Haines, 2003).

The diamondback terrapin's unique life history puts this species at high risk for dietary Hg exposure. With a life span exceeding 40 years, they have the potential to bioaccumulate large amounts of MeHg and other organic contaminants over time. Atmospheric Hg deposition can be five to eight times greater in summer than in winter (Guentzal *et al.*, 2001), which coincides with the terrapin's highest period of activity. Terrapins also display high site fidelity (Gibbons *et al.*, 2001), so individuals inhabiting contaminated tidal creeks are unlikely to migrate to less polluted areas. This also suggests that terrapins may be useful sentinels for local contamination.

## EXPERIMENTAL DESIGN

The purpose of the first study was to determine if gravid terrapins maternally transfer Hg to their eggs, and if so, if mothers with higher scute Hg allocate more Hg to their eggs. The study sites, St. Helena Island and Charleston, SC, and Brunswick, GA, were chosen to represent a range of land uses and potential for contamination. Specimens were collected from St. Helena Island and Charleston Harbor on May 15-29, 2006, and transported to the Savannah River Ecology Laboratory in Aiken, SC. Scute and blood samples were collected from each individual, and oxytocin was administered to induce egg laying. Eggs were weighed and incubated at 80-83°C shortly after laying. Eggs that did not hatch were frozen at -10°C, homogenized, and analyzed for total Hg content.

Brunswick terrapins were collected May 28-June 26, 2006 as part of a separate project led by Dr. Terry Norton to salvage eggs from female terrapins killed on roads. One egg was removed from each clutch for Hg analysis, while the remainder was incubated at St. Catherine's Island Wildlife Center. Eggs that did not hatch were also made available for this study. Liver, kidneys, muscle, claw, scute, and egg follicles from deceased adults were analyzed for total Hg as well. St. Helena Island is a part of the ACE Basin, a largely undeveloped 134,000-acre

estuary. We hypothesized that terrapins from this site would have the lowest Hg concentrations. Since tidal marshes in the Brunswick area received discharges from industrial facilities for several decades (EPA, 2002), we hypothesized that turtles from this site would have the highest concentrations of tissue Hg.

The purpose of the second study was to determine if Hg concentrations in turtles sold in Asian food markets are high enough to pose a health risk to consumers. Seventy-one deceased turtles, including six diamondback terrapins, were chosen out of hundreds from a confiscated shipment destined for markets in Hong Kong (Hudson and Buhlmann, 2000). Specimens were dissected and their tissues (liver, kidney, muscle, scute, claw, and egg follicles) analyzed for total Hg. These Hg concentrations were then compared to risk-based consumption limits for mercury in fish developed by the EPA and FDA.

## METHODS

Specimens were collected by trammel net in the Ashley River (Charleston Harbor) and by road surveys along Highway 21 (St. Helena Island) and causeways in the Brunswick area (including Jekyll Island, Sea Island, and St. Simon's Island). Descriptions of induction, incubation, and hatchling care can be found in Green (2007).

### Sample Preparation

Scutes and eggs from terrapins collected in the field and internal tissues from deceased specimens were stored in sterile polyethylene Whirl-Pak® bags (NASCO) and frozen at -10°C. Samples were weighed, lyophilized (Labconco), reweighed to a constant dry weight, and homogenized in coffee grinders and/or a liquid nitrogen mill (Spex Sample Prep 6750 freezer mill, Metuchen, NJ, USA). Equipment was cleaned with a metal free detergent and 10% nitric acid between samples. Aliquots of processed tissues were then assayed for total Hg.

### Mercury Analysis

Tissues were analyzed for total Hg following EPA method 7473, using a DMA80 Direct Mercury Analyzer (Milestone, Inc., Monroe, CT, USA). Samples were analyzed in batches of ten, with each batch including a blank, a sample replicate, and a tissue standard certified for total Hg concentration (TORT-2, lobster hepatopancreas, National Research Council of Canada, Ottawa, Canada). For the first study, standard recovery ranged from 79% to 92% with an average of 84% (n=30). The average difference between sample replicates was 17% (n=13). Based on a 0.87 g sample and an average blank of 0.07 ng Hg (n=46), the method detection limit (MDL) was 0.184 ppb. For the second study, standard recovery ranged from 85% to 116% with an average of

101% (n=58). The average difference between sample replicates was 2% (n=57). Based on a 0.98 g sample and an average blank of 0.12 ng Hg (n=27), MDL was 0.65 ppb. All samples were above the MDL for both studies.

### Risk-based Consumption Limits

To evaluate the health risks of consuming Hg-contaminated turtle meat, concentrations were compared with risk-based consumption limits developed by the EPA and FDA. EPA has recommended a Hg consumption threshold of 1900 ppb for the general public and 1000 ppb for children and pregnant or nursing women.

Monthly consumption limits have been suggested for tissues with concentrations below the 1900 ppb threshold. Reported Hg concentrations were from lyophilized samples and were converted to wet weights (based on the average moisture content for each tissue type) for comparison with EPA consumption limits.

## CONCLUSIONS

### Scutes

Mean Hg concentrations in adult scutes differed among sites (St. Helena Island: n= 10,  $\bar{x}$  = 18 ppb; Charleston: n=12,  $\bar{x}$  = 8 ppb; Brunswick: n= 13,  $\bar{x}$  = 490 ppb; p<0.0001). Terrapins from Brunswick had a much wider range of scute Hg than did those from ACE Basin and Charleston (Figure 1).

### Eggs

Brunswick eggs had the highest mean Hg content (St. Helena Island: n= 34,  $\bar{x}$  = 20.5 ppb; Charleston: n= 35,  $\bar{x}$  = 11.7 ppb; Brunswick: n= 28,  $\bar{x}$  = 26.2 ppb; p<0.0001). Egg Hg was also significantly different among females at each site (St. Helena Island: p<0.0001, Charleston: p=0.0337, Brunswick: p=0.0179).

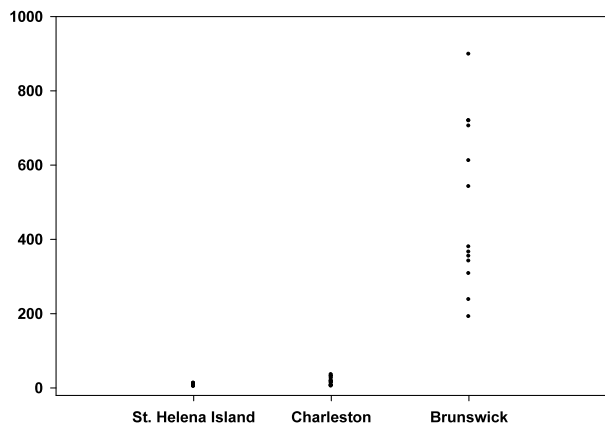


Figure 1: Scute mercury in gravid terrapins. Each point represents one individual.

There was no clear correlation between a female’s scute

Hg concentration and the mean Hg concentration of her eggs. Egg Hg varied very little within clutches and was not influenced by laying sequence.

### Tissues

Mean tissue Hg concentrations for Brunswick terrapins were:  $\bar{x}_{liver}$  = 2278 ppb,  $\bar{x}_{kidney}$  = 495 ppb,  $\bar{x}_{muscle}$  = 233 ppb,  $\bar{x}_{claw}$  = 393 ppb,  $\bar{x}_{scute}$  = 490 ppb. Mean tissue Hg concentrations for food trade terrapins were:  $\bar{x}_{liver}$  = 6319 ppb,  $\bar{x}_{kidney}$  = 486 ppb,  $\bar{x}_{muscle}$  = 263 ppb,  $\bar{x}_{claw}$  = 441 ppb,  $\bar{x}_{scute}$  = 647 ppb,  $\bar{x}_{egg\ follicle}$  = 545 ppb. For both groups, concentrations were highest in liver (Figure 2).

### Comparison to Risk-based Consumption Limits

All edible samples (liver, kidney, and muscle) were divided into seven categories based on the maximum monthly allowable consumption rate (EPA, 2000): Do Not Eat, 0.5 Meals/Month, 1 Meal/Month, 2 Meals/Month, 3 Meals/Month, 4 Meals/Month, and >4 Meals/Month (Figure 3). For both groups of terrapins, approximately 17% of samples had Hg levels within or above the range recommended for no more than one meal per month. One terrapin from the food trade had a liver Hg concentration that exceeded the 1900 ppb limit. One Brunswick and two food trade specimens had liver Hg concentrations above the 1000 ppb threshold for children and pregnant women.

## DISCUSSION AND RECOMMENDATIONS

### Maternal Transfer and Tissue Distribution

As hypothesized, Brunswick terrapins had the highest egg and scute Hg concentrations.

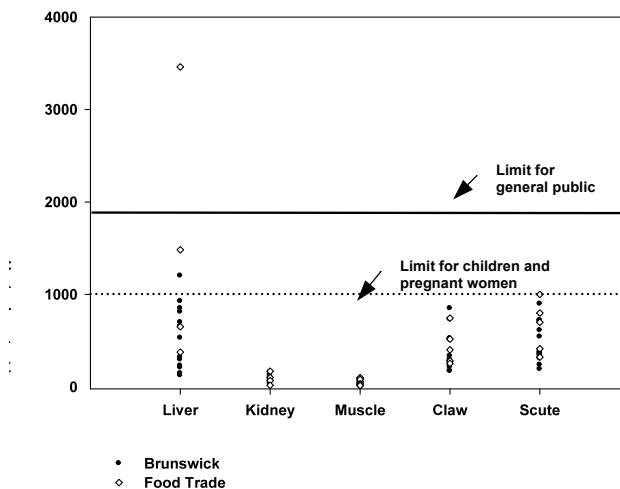


Figure 2: Tissue Hg in terrapins from Brunswick, GA and the Asian food trade. Each point represents one individual.

However, the small differences in egg Hg among sites indicate that only a minor amount of Hg is maternally transferred to eggs. Females with high scute Hg

transferred only slightly greater amounts of Hg to their eggs than did females with lower scute Hg.

Additionally, the general pattern of tissue Hg distribution (liver>scutes>muscle) displayed in Brunswick and food trade specimens is consistent with past studies of diamondback terrapins and other turtles (Burger, 2002). These findings support the idea that Hg accumulates most in liver and scutes, while remaining relatively low in tissues such as muscle and developing eggs.

### Risk to Consumers

A substantial proportion of edible tissues had enough Hg to require consumption limitations according to EPA recommendations. Although only a few samples were above the threshold considered safe for children and pregnant women, both studies used very small sample sizes. If specimens are representative of terrapin populations in general, thousands may have extremely high tissue Hg concentrations. This presents a substantial health risk to consumers, as terrapins are among the US turtle species most heavily exported for food (Behler, 1997). Public outreach and education about the possible health consequences of dietary Hg exposure may help to both protect human consumers and decrease consumption demand, relaxing pressure on threatened populations.

A long-term analysis of a diamondback terrapin population (including juveniles and adult males) is necessary to determine if maternal transfer leads to accumulation of Hg over several generations. A study examining Hg and stable isotopes in terrapins, fish, shellfish, plants, and sediment would lead to a better understanding of Hg transport in estuarine food webs.

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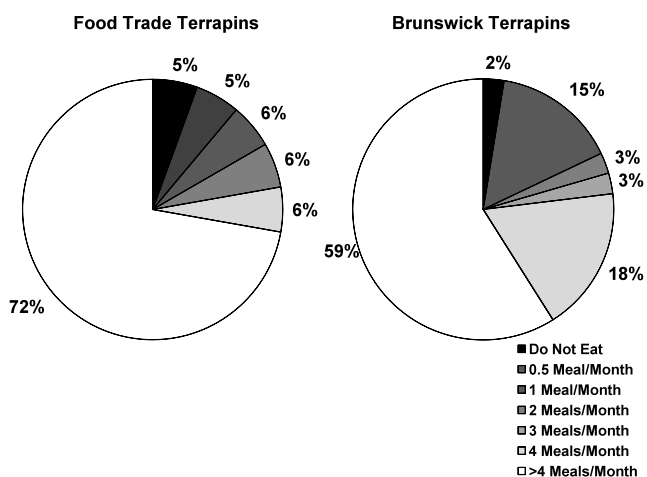


Figure 3: Percentage of edible tissues in each consumption limit category.

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