Mercury Contamination in Turtles and Implications for Human Health

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Abstract Mercury contamination threatens many ecosystems worldwide. Methylmercury bioaccumulates at each trophic level, and biomagnifies within individuals over time. Long-lived turtles often occupy high trophic positions and are likely to accumulate mercury in contaminated habitats. Millions of turtles worldwide are sold in Asia for human consumption, and consumers may be at risk if turtles contain high levels of mercury. The authors dissected 71 turtles from 14 food trade species and analyzed their tissues (liver, kidneys, muscle, claws, and scutes) for total mercury content. Mercury was generally highest in carnivores, and lowest in herbivores. Liver and scutes had the highest concentrations. The authors compared mercury concentrations with consumption limits developed by the U.S. Environmental Protection Agency and Food and Drug Administration to evaluate mercury in fish tissue. Several samples exceeded the recommended 1,900 parts per billion (ppb) consumption threshold, indicating that consumers who eat certain turtle species frequently may be at risk for mercury-related health problems.

Introduction

More than 10 million turtles from around the world are sold annually in markets for consumption as food or medicine. This unsustainable trade has resulted in the dramatic decline of turtle populations worldwide, particularly in Asia. Turtles have been used for food and medicine in China for centuries, as it has been long believed that their consumption builds longevity and wisdom (McCord, 2000; Williams, 1999). Several native U.S. species are also being exported to Asian markets, including Common snapping turtles (*Chelydra serpentina*), Alligator snapping turtles (*Macrochelys temminckii*), softshell turtles (*Apalone spp.*), sliders (*Trachemys* spp.), Diamondback terrapins (*Malaclemys terrapin*), and others (Altherr & Freyer, 2000; Behler, 1997; Williams, 1999). Endangered species are also traded, but are usually hidden from view to avoid conflict with authorities. These species are commonly smuggled across international borders in packages labeled as seafood (Haitao, 2000; Williams, 1999).

Environmental Mercury Contamination in Southeast Asia

Mining, coal production, other industrial activities, and rapid urban development in Asia have resulted in contamination of ecological systems with mercury (Hg) and other pollutants. For example, the Guizhou Province in southern China is a major Hg and coal production center, responsible for 12% of total global Hg emissions. A 2002 study found elevated Hg levels in soil, rice, and fish in areas of the province near Hg or coal mines and industrial wastewater outputs (Finkelman, Belkin, & Zhang, 1999; Horvat et al., 2003).

Major river systems in Southeast Asia, particularly those near crowded cities, receive pollutant inputs from rapidly developing urban areas. Suckcharoen and coauthors (1978) reported that fish from the Chao Phraya estuary in Thailand had highly elevated Hg concentrations, ranging from 320 to 3,600 parts per billion (ppb) and averaging 1,480 ppb. Gold mining is another major industry in Southeast Asia that is well known to cause extensive regional Hg pollution. Residents in a region of the Philippines had elevated Hg levels from exposure to atmospheric Hg and ingesting contaminated fish and locally-grown grains (Drasch, Bose-O'Reilly, Beinhoff, Roider, & Maydl, 2001). Gold mines are also abundant throughout Malaysia, Papua New Guinea, and Indonesia. These Hg emissions are likely to contaminate wildlife in these areas as well, including turtles.

Dietary Mercury Exposure

Almost all Hg emitted into the atmosphere is in inorganic forms, and these are eventually deposited into lakes, rivers, estuaries, and other bodies of water. Bacterial methylation converts inorganic Hg to methylmercury (MeHg), which adheres to sediment particles and partitions into bacteria and plankton. From there it enters the food chain, where it biomagnifies at each successive trophic level and bioaccumulates within organisms over time (Wang, Kim, Dionysiou, Sorial, & Timberlake, 2004).

MeHg has a high affinity for sulfhydryl (-SH) groups, such as those present on some amino acids, and thus accumulates in protein-rich tissues like liver and muscle. Prior studies of Hg in turtles indicate that total Hg levels are usually highest in the liver (Burger, 2002; Golet & Haines, 2001; Gordon, Pople, & Ng, 1998; Linder & Grillitsch, 2000; Sakai et al., 2000b; Schneider, Belger, Burger, & Vogt, 2009; Storelli, Ceci, & Marcotrigiano, 1998a, 1998b). Keratin proteins, such as those present in scutes, are also rich in -SH functional groups and thus accumulate Hg. Since scutes consist of multiple layers of nonliving tissue deposited over time, they can accumulate high levels of Hg over the life of a turtle. Several studies have reported Hg concentrations in scutes that are much higher than those in liver and kidneys of the same animal (Blanvillain et al., 2007; Sakai, Ichihashi, Suganuma, & Tatsukawa, 1995; Sakai et al., 2000a).

Possible Risks to Human Consumers

The trade in wild turtles for food and medicine is not regulated by any government agency, and turtles are not monitored for contaminant levels above a threshold considered safe for human consumption. Thus, it seems reasonable to assume that turtles from Hg-contaminated habitats are sold on the market. As a result, people who consume turtles may be at risk of health consequences associated with elevated Hg exposure. Frequent consumers and pregnant women are expected to be at greatest risk.

Acute and chronic Hg exposure can cause a myriad of health problems in humans. In the U.S., consumption of contaminated fish is the major source of human exposure to Hg. The population at highest risk is children of women who ate large amounts of fish during pregnancy. The developing fetus is sensitive to mercury's adverse effects at much lower doses than in adults (Committee on the Toxicological Effects of Methylmercury, 2000; Linder & Grillitsch, 2000; Schober et al., 2003).

Mercury is destructive to the human nervous system, as it interferes with growth and migration of neurons, creating the potential for irreversible central nervous system damage. Chronic, low-level prenatal exposure to Hg in the maternal diet has been associated with subtle endpoints of neurotoxicity, such as poor performance on tests of attention, fine motor function, language, visual-spatial abilities, and verbal memory (Grandjean et al., 1997; Kjellström, Kennedy, Wallis, & Mantell, 1986; Kjellström et al., 1989).

Risk-Based Consumption Limits

As environmental mercury contamination has become a larger and more widespread problem in the U.S. in recent decades, the U.S. Environmental Protection Agency (U.S. EPA) and Food and Drug Administration (FDA) have collaborated to develop fish consumption advisories for the general public. Children and pregnant or nursing women are most susceptible to the harmful effects of dietary mercury exposure, and recommendations are that these groups completely avoid fish with tissue concentrations above 1,000 ppb (U.S. EPA, 2000). This is also the FDA threshold above which fish are ineligible for interstate commerce (U.S. EPA. 2000).

U.S. EPA has determined a reference dose for mercury of 1x10-4 mg/kg/day (equivalent to 0.1 ppb/day). A reference dose is defined as "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to a human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious health effects during a lifetime (U.S. EPA, 2000)." To avoid exceeding this reference dose, U.S. EPA has recommended that humans do not consume fish with tissue Hg concentrations above 1,900 ppb. Monthly consumption limits have been set for tissues with concentrations below the 1,900 ppb threshold and are based on standard estimates of meal size and consumer body weight.

Purpose of Study

The primary goal of our study was to measure concentrations of Hg found in a variety of turtle species sold for human consumption in Asian food markets and to compare Hg distribution and accumulation patterns in various tissues. We hypothesized that the highest Hg levels will be measured in turtles that are larger, older, and more carnivorous than smaller, herbivorous species. Our secondary goal was to compare tissue Hg levels with Hg thresholds and guidelines established by U.S. EPA and FDA to determine if Hg concentrations measured in some turtles were high enough to put consumers at risk for health problems related to dietary Hg exposure. We also considered that the possible threats to human health from eating Hg-contaminated turtles might also indirectly lead to needed conservation protection for turtles through government crackdowns on illegal trade in endangered species that heretofore continue to slide towards extinction as a result of unsustainable harvest.

Methods

Study Species

Turtle specimens were acquired from seized shipments of turtles destined for food or pet markets. Seventy-one deceased specimens, representing 14 species and six families from four continents, were dissected and their tissues analyzed for Hg as well as stable carbon and nitrogen isotopes (Green, 2007). All specimens were adults, except for Asian giant softshell turtles (*Pelochelys cantorii*), which were juveniles.

The 14 species used in this study include 10 species from Asia representing the families Geoemydidae (Bataguridae), Testudinidae, and Trionychidae. One Austral-Asian species (family Chelidae), one Central American species (family Geoemydidae), and two North American species (families Kinosternidae and Emydidae) were also included. Sample sizes for each species are in Table 1. Average body size, geographic range, IUCN Red List status, and distribution maps for each species can be found in Green (2007).

Turtle Acquisition

Malayan box turtles (*Cuora amboinensis*), Spiny turtles (*Heosemys spinosa*), and Black marsh turtles (*Siebenrockiella crassicollis*) were obtained from a shipment of about 10,000 turtles that likely originated in Malaysia and was destined for food markets. Customs officials in Hong Kong seized the shipment on December 11, 2001, and the Turtle Survival Alliance (TSA) handled the distribution of live specimens to zoos, conservation organizations, and private individuals (Hudson & Buhlmann, 2000). Sick animals that subsequently died were made available for this study. Other deceased specimens were obtained from food and pet markets.

Dissection

Specimens were weighed, measured, thawed, and dissected. Portions of liver, kidney, pectoral muscle, hind leg muscle, scutes, and claws were removed for analysis. In addition, egg follicles were removed from female specimens when present. The dissected samples were stored individually in sterile polyethylene Whirl-Pak[®] bags (NASCO) and frozen at -10°C until processed. Tools were cleaned with 10% nitric acid between samples to prevent cross-contamination.

Sample Preparation

Samples were weighed, lyophilized (Labconco), reweighed to a constant dry weight, and then lipid extracted. Subsequent analysis for ¹³C/¹²C and ¹⁵N/¹⁴N ratios (Green, 2007) required removal of lipids (Post et al., 2007) through a 24-hour extraction within a 2:1 chloroform: methanol mixture followed by rinsing in methanol until the decanted liquid became clear. After air drying, the extracted tissue samples were homogenized in coffee grinders and/or a liquid nitrogen mill. Coffee grinders and freezer mill vials and stoppers were cleaned with a metal free detergent and 10% nitric acid between samples. Aliquots of lyophilized, homogenized, and lipid-extracted tissue were then assayed for total Hg.

Analyses—Total Mercury

Tissues were analyzed for Hg following U.S. EPA method 7473 (U.S. EPA, 1998), using a DMA80 Direct Mercury Analyzer. Samples were analyzed in batches of 10, with each batch including a blank, a sample replicate, and a tissue standard certified for Hg concentration (DORM-2, dogfish muscle, DOLT-2, dogfish liver; or TORT-2, lobster hepatopancreas, purchased from the National Research Council of Canada). Recovery of mercury from tissue standards 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), the method detection limit (MDL) was 0.65 ppb. All samples were determined to be above the MDL.

TABLE 1

Four-Letter Codes for Study Species

Species Code	Species	No.
CHPA	Chelodina parkeri	2
CHRE	Chinemys reevesi	4
CUAM	Cuora amboinensis	8
GEEL	Geochelone elegans	6
GESP	Geoemyda spengleri	4
HESP	Heosemys spinosa	6
KIFL	KIFL Kinosternon flavescens	
LEYU	LEYU Leucocephalon yuwonoi	
MATE	Malaclemys terrapin terrapin	6
PECA	PECA Pelochelys cantorii	
PESI	Pelodiscus sinensis	4
PYMO	PYMO Pyxidea (Cuora) mouhotii	
RHPM	RHPM Rhinoclemmys pulcherrima manni	
SICR	SICR Siebenrockiella crassicollis	

Calculation of Risk-Based Consumption Limits

Daily consumption limits are calculated using Equation 1 from U.S. EPA (2000):

$$CR_{lim} = (R_f D \times BW)/C_m$$
(1)

Where

CR_{lim} = maximum allowable consumption rate (kg/day)

 $R_f D$ = reference dose (1x10⁻⁴ mg/kg/day for Hg)

BW = consumer body weight (kg)

 C_m = Hg tissue concentration (mg/kg).

From this, the monthly consumption limit can be calculated using Equation 2:

$$CR_{mm} = (CR_{lim} \times T_{ap})/MS$$
 (2)

Where

CR_{mm} = maximum allowable consumption rate (meals/month)

T_{ap} = Time averaging period (365.25 days/ 12 months = 30.44 days/month)

MS = Meal size (kg).

Although these terms were developed for evaluating fish tissue, we used them to understand potential human health risks from the consumption of Hg-contaminated turtle meat.

To determine the maximum Hg tissue concentration that could be safely consumed in a given time period, Equation 2 was rearranged and calculated for a monthly consumption rate of 0.5, 1, 2, 3, and 4 meals per month as follows:

$$CR_{lim} = (CR_{mm} \times MS)/T_{an}$$
 (3)

This maximum allowable consumption rate was then substituted into Equation 1, which was rearranged as follows:

 $C_{m} = (R_{f} D \times BW) / C_{lim}$ (4)

This gives the maximum Hg tissue concentration that can be safely consumed in one meal every two months (1,877 ppb), one meal per month (938 ppb), two meals per month (470 ppb), three meals per month (313 ppb), and four meals per month (235 ppb). These limits were calculated using an average U.S. adult consumer body mass of 70 kg (154 lbs.) and an average meal size of 0.277 kg (8 oz.) (U.S. EPA, 2000). Table 2 lists the range of Hg concentrations associated with each consumption limit category. These limits are based on Hg concentrations measured in fresh tissue. Since all samples in our study were lyophilized, Hg concentrations have been reported on a dry weight basis. In order to compare our Hg concentrations to the U.S. EPA's risk-based consumption limits, we converted them to wet weights based on the average moisture content for each tissue type (liver: 76%; kidney: 82%; leg muscle: 78%; and pectoral muscle: 81%).



Species Code	Consumption Category								
	Do Not Eat (>1900 ppb)	0.5 Meals/ Month (940–1900 ppb)	1 Meal/Month (470–940 ppb)	2 Meals/Month (310–470 ppb)	3 Meals/Month (230–310 ppb)	4 Meals/Month (120–230 ppb)	>4 Meals/ Month (<120 ppb)		
CHPA	14.3	28.6	0	0	0	14.3	42.9		
CHRE	14.3	0	14.3	0	7.1	7.1	57.1		
CUAM	0	0	4.1	0	8.3	12.5	75		
GEEL	0	0	0	7.1	0	0	92.9		
GESP	0	0	0	0	0	7.7	92.3		
HESP	4.3	4.3	8.7	0	8.7	4.3	69.6		
KIFL	5.6	0	5.6	11.1	0	0	77.8		
LEYU	0	0	0	0	0	4.8	95.2		
MATE	5.6	5.6	5.6	5.6	0	5.6	72.2		
PECA	0	25	0	12.5	0	25	37.5		
PESI	0	0	0	0	0	8.3	91.7		
PYMO	4.8	0	9.5	0	4.8	9.5	71.4		
RHPM	5.9	0	0	5.9	5.9	0	82.4		
SICR	10.5	5.3	0	10.5	10.5	10.5	52.6		

Percentage of Edible Tissues in Consumption Categories*

Consumption categories obtained from U.S. Environmental Protection Agency (2001).

Statistical Analysis

Data were entered into Excel spreadsheets and analyzed using SAS v.8.1 software. The number of turtles from an individual species varied from two to eight, with most species represented by four to six specimens. Data for individual species were generally non-normal by a Shapiro-Wilk test. Plots of Hg values showed that data were often highly skewed, and many outliers were present. Variance and range of data also varied considerably among species, and no single transformation was adequate to normalize the data and homogenize the variances. Data were analyzed using both parametric (ANOVA, Pearson's correlations) and nonparametric methods (Kruskal-Wallis tests, Spearman's correlations). Both types of analyses generally led to the same conclusions regarding relationships among variables and differences among tissues and species, suggesting that unmet assumptions about normality and homogeneity of variance were not important factors influencing the parametric analyses.

Results

Mercury

Tissue Distribution

Tissue distribution of Hg followed a general pattern in which liver Hg was the highest, followed by kidney and scutes. Muscle and follicles were generally lowest in Hg. Exceptions to this trend include Yellow mud turtles (Kinosternon flavescens), for which most scute samples were comparable to or higher than liver samples in Hg; and Blackbreasted leaf turtles (Geoemyda spengleri), for which scute Hg greatly exceeded liver Hg. Liver Hg concentrations were much more variable within each species than were scute Hg concentrations (Figure 1). Reeve's turtles (Chinemys reevesi) and Sulawesi forest turtles (Leucocephalon yuwonoi) both had greater amounts of Hg in egg follicles than in kidney samples. Diamondback terrapins and Reeve's turtles had scute Hg levels exceeding kidney Hg.

A total of 32 egg follicles at various stages of development was removed from 19 individuals representing 8 species. Mercury levels in follicle samples were generally low; most were below 400 ppb dry weight. Mercury in follicles from two different Diamondback terrapins measured 728 ppb and 831 ppb. Follicles from two Reeve's turtles had Hg concentrations of 1,716 ppb and 3,230 ppb. One Keeled box turtle (Cuora mouhotii) follicle contained 3,371 ppb Hg.

Differences in Tissue Hg Among Diet Groups

To examine the influence diet has on tissue Hg, species were divided into three groups: herbivores (Indian star tortoises, Spiny turtles, and Sulawesi forest turtles), omnivores (Central American wood turtles, Black marsh turtles, Reeve's turtles, Malayan box turtles, Black-breasted leaf turtles, and Keeled box turtles), and carnivores (Diamondback terrapins, Yellow mud turtles, Chinese softshell turtles, Asian giant softshell turtles, and Parker's snake-necked turtles). Mercury values in muscle, scute, and claw samples differed significantly among diet groups, and these tissues displayed the expected pattern of Hg in carnivores > Hg in omnivores > Hg in herbivores (Figure 2). Although omnivores seemed to have higher liver Hg values than carnivores and herbivores, no significant difference existed in liver or kidney Hg values among diet groups.

Comparison of Hg Levels to Risk-Based Consumption Limits

All edible tissues (liver, kidney, and muscle) were divided into seven categories based on the CR_{mm}: do not eat, 0.5 meals/month, 1 meal/month, 2 meals/month, 3 meals/month, 4 meals/month, and >4 meals/month. Hg was highest in liver for all species, followed by kidney and muscle. Nine of 62 (14%) liver samples analyzed exceeded the 1,900 ppb consumption threshold recommended for all adults, including those of Reeve's turtles, Black marsh turtles, Diamondback terrapins, Spiny turtles, and Keeled box turtles. Twenty-one of 62 (34%) liver samples had Hg levels within or above the 470-940 ppb maximum range recommended for no more than one meal per month. The highest Hg concentrations measured in this study were in livers from a single Spiny turtle (4,768 ppb), a Central American wood turtle (5,017 ppb), and two Reeve's turtles (7,443 ppb and 16,561 ppb).

Nine of 42 (21%) kidney samples had levels of Hg above the range recommended for one meal per month. These were samples collected from an individual Spiny turtle, a Parker's snake-necked turtle, and two Keeled box turtles. One hundred nineteen of 125 (95%) muscle samples had Hg concentrations below the level recommended for four meals per month. Only six of 125 (5%) of muscle samples contained enough Hg to require consumption limitations, including muscles from Parker's snake-necked turtles, Reeve's turtles, Asian giant softshell turtles, and Keeled box turtles.

For many species that are popular in the food trade, a considerable proportion of edible samples were within ranges allowable for limited consumption (Table 2). Three of 18 (17%) Diamondback terrapin samples had Hg levels within or above the range recommended for no more than one meal per month, and one liver sample was above the 1,900 ppb consumption threshold. Four of 14 (28%)





Lower box boundaries represent 25th percentiles; upper boundaries represent 75th percentiles; horizontal line within box represents median Hg value; error bars indicate 10th and 90th percentiles; points outside of error bars represent outliers.

Reeve's turtle samples were within or above the range of Hg concentrations recommended for no more than one meal per month, and two liver samples were above the 1,900 ppb consumption threshold. Three of 19 (15%) of Black marsh turtle samples had Hg levels within or above the range recommended for no more than one meal every two months, and two liver samples were above the 1,900 ppb consumption threshold. Two of eight (25%) of Asian giant softshell turtle samples were within the range of Hg concentrations recommended for no more than one meal every two months (Figure 3). Three of seven (43%) of Parker's snake-necked turtle samples had Hg levels within or above the range recommended for no more than one meal per month.

Discussion

Mercury Distribution

The general pattern of tissue Hg distribution (liver > kidney \geq scutes > muscle) displayed by the majority of specimens is consistent with past studies of sea turtles (Anan, Kunito, Watanabe, Sakai, & Tanabe, 2001; Day, Christopher, Becker, & Whitaker, 2005; Sakai et al., 1995; Sakai et al., 2000a, 2000b), Common snapping turtles (Albers, Sileo, & Mulhern, 1986; Meyers-Schone, Shugart, Beauchamp, & Walton, 1993), Diamondback terrapins (Burger, 2002), and sliders (Meyers-Schone et al., 1993). Specimens with extremely high liver Hg had Hg levels in all other tissues that were comparable to those of specimens with relatively low liver Hg. This suggests that Hg accumulates in the liver, while Hg in other tissues remains relatively low. For Black-breasted leaf turtles, however, it seems as if this excess Hg is allocated to the scutes. Scute Hg for this species greatly exceeded Hg in all other tissues, including liver. This is displayed to a lesser extent in Yellow mud turtles, whose liver Hg nearly equaled scute Hg in most specimens. Blackbreasted leaf turtles may retain their scutes throughout their lifetime instead of shedding them, which would explain why this species had such large scute Hg concentrations. Scute Hg concentrations that exceed liver Hg concentrations have also been reported in Loggerhead sea turtles (Caretta caretta) (Day et al., 2005). Scutes are comprised of nonliving keratin. Unlike in other tissues, nutrients and other dietary components allocated to scutes do not return to the total body circulation and are metabolically unavailable. It is possible that the allocation of excess Hg to the scutes rather than the liver allows these species to fare better than others in contaminated environments.

Evaluation of Risk to Human Consumers

Comparisons Among Tissues

Of all turtle tissues sampled, liver is the riskiest to consume, as it had the highest proportion of samples in each of the four highest consumption limit categories (do not eat, 0.5 meals/month, 1 meal/month, and 2 meals/ month) and several samples exceeded the 1,900 ppb consumption threshold. A single 8 oz. meal of any of these samples will exceed the reference dose for Hg. Frequent consumption of turtles with liver Hg concentrations that are this high is likely to put consumers at risk for harmful health effects.

Kidneys are less risky to consume than liver, but nearly half (41%) of kidney samples contained levels of Hg requiring consumption limitations. Muscle samples from all species were substantially lower in Hg than liver and kidney samples. It is reasonable to assume that consumers who eat only muscle will be at lower risk of harmful health effects due to dietary Hg exposure. Turtles purchased in Asian markets, however, are often entirely consumed in soups and stews, so it is likely that most consumers will ingest several types of tissue in one meal.

It is difficult to evaluate the safety of eating turtle eggs in general because only a small proportion of specimens contained egg follicles and Hg values differed greatly within and among individuals of the same species. Our study, however, demonstrates a capability for eggs to retain high Hg concentrations, and indicates potential danger from consuming turtle eggs.

Sampling both internal and external tissue types allowed for evaluation of the utility of nonlethal sampling data. No significant correlations existed between scute or claw Hg levels and those of internal tissues. Five of the six species in this study with scute Hg concentrations above 1,000 ppb, however, also had liver concentrations above the 1,900 ppb consumption threshold. So, while scute Hg concentrations may not be used reli-



ably to predict liver Hg concentrations, it is likely that high scute Hg levels indicate correspondingly high liver Hg levels. Mercury seems to accumulate in the liver at much higher concentrations than in other tissues. The decision to use lethal or nonlethal sampling methods depends on the scope and purpose of the study. In cases of rare or threatened species, nonlethal sampling should be used whenever possible.

Comparisons Among Species

For 10 species (Malayan box turtles, Indian star tortoises, Black-breasted leaf turtles, Spiny turtles, Yellow mud turtles, Sulawesi forest turtles, Diamondback terrapins, Chinese softshell turtles, Keeled box turtles, and Central American wood turtles), 70% of the edible samples (liver, kidney, and muscle) can be safely consumed more than four times per month. The remaining four species of higher risk include Reeve's turtles (58%), Black marsh turtles (52%), Parker's snakenecked turtles (43%), and Asian giant softshell turtles (37%).

Based on the range of Hg values measured, the proportion of edible samples in each monthly consumption limit category, and the popularity of certain turtle species in the food trade, it may be concluded that the turtles with the greatest potential to cause health risks to consumers include Black marsh turtles, Reeve's turtles, Asian giant softshell turtles, Parker's snake-necked turtles, and Diamondback terrapins. We note nevertheless that species such as Malayan box turtles are traded in the millions of individuals and in our small sample size we found high levels of mercury in 25% of Malayan box turtle samples.

Conclusion

Turtles in this study showed a similar pattern of tissue Hg distribution observed in many other species of turtles (liver > kidney \geq scutes > muscle). This supports findings of previous research studies that Hg accumulates in the liver and kidneys, but remains relatively low in muscle tissue.

As expected, species with more carnivorous diets had higher Hg in all tissues than more herbivorous species. One species in particular, Chinese softshell turtles, had relatively low tissue Hg despite being piscivorous. Many Chinese softshell turtles are now raised on farms to supply the food trade, where they are likely fed a diet containing little to no fish. It is possible that the Chinese softshell turtles in this study originated from farms. This may explain the substantial differences in tissue Hg between this species and Asian giant softshell turtles, another piscivorous species (Figure 3). Hg levels in Asian giant softshell turtles were several times higher than Chinese softshell turtles in all tissues. This, however, does not suggest that all farmed turtles are safe to consume. Mercury is present in the environment, and turtles raised on farms in areas contaminated with Hg are just as likely to accumulate dangerously high levels of mercury as wildcaught specimens.

Many samples in this study had enough Hg to require consumption limitations according to U.S. EPA standards. A small proportion of all samples contained levels of Hg that were several times higher than the 1,900 ppb consumption threshold recommended for most adults. A single 8 oz. meal of a turtle with Hg concentrations such as those measured in the livers of Black marsh turtles or Reeve's turtles, for example, would exceed the reference dose of 0.1 ppb Hg/day. The short- and long-term effects of consuming these high levels of Hg are uncertain, but it is clear that many of the individuals in this study should be consumed very infrequently or not at all. Although only a small proportion (~6%) of edible tissues sampled were above the recommended consumption threshold, our study's sample size of 71 specimens represents an extremely small percentage of the several million turtles sold for human consumption worldwide. Assuming that the turtles analyzed here are a representative sample of the total population of food trade turtles, several hundred thousand turtles on the market may have concentrations of Hg making them unfit for human consumption. This could signify a public health catastrophe.

A shortcoming of this study is that we don't know the locality of origin of these turtles with certainty, as many were confiscated in illegal trade, which precludes us from identifying potential source regions of Hg contamination. Yet the origins of animals sold in markets and restaurants are also often unknown to the consumer. Our point is that the potential to consume contaminated turtles exists and poses a clear threat to the health of human consumers. Future studies on turtle consumption patterns in markets and restaurants in Asia are needed to more accurately determine the severity of this threat.

Although there is still much to be learned about Hg levels in food trade turtles, greater public awareness and education about the Hg content of turtles and the possible health consequences of dietary Hg exposure may help to both protect human consumers and decrease consumption demand for some turtle species. This may stimulate greater public awareness of the problem of environmental Hg contamination and encourage citizens to demand greater restrictions on Hg emissions. Even if government authorities seem reluctant to truly crack down on the illegal wildlife trade, and many IUCN Red List species and CITES Appendix I and II species still appear regularly in the Asian turtle trade, governments may be more receptive to regulating the turtle trade if they are made aware that there could be significant health risks for their human populations who consume these turtles. This may also help to better regulate and restrict harvest of turtles from wild populations that are suffering from extensive harvesting for the food trade.

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References

- Albers, P.H., Sileo, L., & Mulhern, B.M. (1986). Effects of environmental contaminants on snapping turtles of a tidal wetland. *Archives of Environmental Contamination and Toxicology*, 15(1), 39–49.
- Altherr, S., & Freyer, D. (2000). Asian turtles are threatened by extinction. *Turtle and Tortoise Newsletter*, 1, 7–11.
- Anan, Y., Kunito, T., Watanabe, I., Sakai, H., & Tanabe, S. (2001). Trace element accumulation in hawksbill turtles (*Eretmochelys imbricata*) and green turtles (*Chelonia mydas*) from Yaeyama Islands, Japan. *Environmental Toxicology and Chemistry*, 20(12), 2802–2814.
- Behler, J.L. (1997, July). *Troubled times for turtles*. Paper presented at the International Conference on Conservation, Restoration, and Management of Turtles and Tortoises, New York Turtle and Tortoise Society, Purchase, NY. Retrieved November 21, 2006, from http://www.nytts.org/proceedings/behler.htm
- Blanvillain, G., Schwenter, J.A., Day, R.D., Point, D., Christopher, S.J., Roumillat, W.A., & Owens, D.W. (2007). Diamondback terrapins, *Malaclemys terrapin*, as a sentinel species for monitoring mercury pollution of estuarine ecosystems in South Carolina and Georgia, USA. Environmental Toxicology and Chemistry, 26(7), 1441–1450.
- Burger, J. (2002). Metals in tissues of diamondback terrapin from New Jersey. Environmental Monitoring and Assessment, 77(3), 255–263.
- Committee on the Toxicological Effects of Methylmercury, Board on Environmental Studies and Toxicology, National Research Council. (2000). *Toxicological effects of methylmercury*. Washington, DC: National Academy Press.
- Day, R.D., Christopher, S.J., Becker, P.R., & Whitaker, D.W. (2005). Monitoring mercury in the loggerhead sea turtle, *Caretta caretta*. *Environmental Science and Technology*, *39*(2), 437–446.

continued on page 22

References continued from page 21

- Drasch, G., Bose-O'Reilly, S., Beinhoff, C., Roider, G., & Maydl, S. (2001). The Mt. Diwata study on the Philippines 1999—assessing mercury intoxication of the population by small scale gold mining. *Science of the Total Environment*, 267(1–3), 151–168.
- Finkelman, R.B., Belkin, H.E., & Zhang, B. (1999). Health impacts of domestic coal use in China. Proceedings from the National Academy of Sciences colloquium on Geology, Minerology, and Human Welfare, 96, 3427–3431.
- Golet, W.A., & Haines, T.A. (2001). Snapping turtles (*Chelydra* serpentina) as monitors for mercury contamination of aquatic environments. *Environmental Monitoring and Assessment*, 71(3), 211–220.
- Gordon, A.N., Pople, A.R., & Ng, J. (1998). Trace metal concentrations in livers and kidneys of sea turtles from south-eastern Queensland, Australia. *Marine and Freshwater Research*, 49, 409–414.
- Grandjean, P., Weihe, P., White, R.F., Debes, F., Araki, S., Yokoyama, K., Murata, K., Sorensen, N., Dahl, R., & Jorgensen, P. (1997). Cognitive deficit in 7-year old children with prenatal exposure to methylmercury. *Neurotoxicology and Teratology*, 19(6), 417–428.
- Green, A.D. (2007). *Mercury in turtles from the Asian food trade*. Unpublished master's thesis, Athens, University of Georgia.
- Haitao, S. (2000). Results of turtle market surveys in Chengdu and Kunming. *Turtle and Tortoise Newsletter*, *6*, 15b–16.
- Horvat, M., Nolde, N., Fajon, V., Jereb, V., Logar, M., Lojen, S., Jacimovic, R., Falnoga, I., Liya, Q., Faganeli, J., & Drobne, D. (2003).
 Total mercury, methylmercury, and selenium in mercury polluted areas in the province Guizhou, China. *Science of the Total Environment*, 304(1–3), 231–256.
- Hudson, R., & Buhlmann, K. (2000). Turtle rescue—turtle survival alliance executive summary. *Turtle and Tortoise Newsletter*, *6*, 6–12.
- Kjellström, T., Kennedy, P., Wallis, S., & Mantell, C. (1986). Physical and mental development of children with prenatal exposure to mercury from fish. Stage 1: Preliminary tests at age 4. Stockholm: National Swedish Environmental Protection Board.
- Kjellström, T., Kennedy, P., Wallis, S., Stewart, A., Friberg, L., Lind, B., Witherspoon, P., & Mantell, C. (1989). Physical and mental development of children with prenatal exposure to mercury from fish. Stage 2: Interviews and psychological tests at age 6. Stockholm: National Swedish Environmental Protection Board.
- Linder, G., & Grillitsch, B. (2000). Ecotoxicology of metals. In D.W. Sparling, C.A. Bishop, & G. Linder (Eds.), *Ecotoxicology of amphibians and reptiles* (pp. 325–460). Pensacola, FL: SETAC Press.
- McCord, W. (2000). Current review on China food market/turtle crisis. *Turtle and Tortoise Newsletter*, 1, 13–14.
- Meyers-Schone, L., Shugart, L.R., Beauchamp, J.J., & Walton, B.T. (1993). Comparison of two freshwater turtle species as monitors of radionuclide and chemical contamination: DNA damage and residue analysis. *Environmental Toxicology and Chemistry*, 12, 1487–1496.
- Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., & Montaña, C.G. (2007). Getting to the fat of the mat-

ter: Models, methods, and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*, *152*(1), 179–189.

- Sakai, H., Ichihashi, H., Suganuma, H., & Tatsukawa, R. (1995). Heavy metal monitoring in sea turtles using eggs. *Marine Pollution Bulletin*, 30(5), 347–353.
- Sakai, H., Saeki, K., Ichihashi, H., Kamezaki, N., Tanabe, S., & Tatsukawa, R. (2000a). Growth-related changes in heavy metal accumulation in green turtle (*Chelonia mydas*) from Yaeyama Islands, Okinawa, Japan. Archives of Environmental Contamination and Toxicology, 39(3), 378–385.
- Sakai, H., Saeki, K., Ichihashi, H., Suganuma, H., Tanabe, S., & Tatsukawa, R. (2000b). Species-specific distribution of heavy metals in tissues and organs of loggerhead turtle (*Caretta caretta*) and green turtle (*Chelonia mydas*) from Japanese coastal waters. *Marine Pollution Bulletin*, 40(8), 701–709.
- Schneider, L., Belger, L., Burger, J., & Vogt, R.C. (2009). Mercury bioaccumulation in four tissues of *Podocnemis erythrocephala* (Podocnemididae: Testudines) as a function of water parameters. *Science of the Total Environment*, 407(3), 1048–1054.
- Schober, S.E., Sinks, T.H., Jones, R.L., Bolger, P.M., McDowell, M., Osterloh, J., Garrett, E.S., Canady, R.A., Dillon, C.F., Sun Y., Joseph, C.B., & Mahaffey, K.R. (2003). Blood mercury levels in US children and women of childbearing age, 1999–2000. *Journal of the American Medical Association*, 289(13), 1667–1674.
- Storelli, M.M., Ceci, E., & Marcotrigiano, G.O. (1998a). Comparison of total mercury, methylmercury, and selenium in muscle tissues and in the liver of *Stenella coeruleoalba* (Meyen) and *Caretta caretta* (Linnaeus). Bulletin of Environmental Contamination and Toxicology, 61(4), 541–547.
- Storelli, M.M., Ceci, E., & Marcotrigiano, G.O. (1998b). Distribution of heavy metal residues in some tissues of *Caretta caretta* (Linnaeus) specimen beached along the Adriatic Sea (Italy). Bulletin of Environmental Contamination and Toxicology, 60(4), 546–552.
- Suckcharoen, S., Nuorteva, P., & Hasanen, E. (1978). Alarming signs of mercury pollution in a freshwater area of Thailand. *Ambio*, 7(3), 113–116.
- U.S. Environmental Protection Agency. (1998). EPA method 7473: Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry. Retrieved June 22, 2008, from http://www.epa.gov/SW-846/pdfs/7473/pdf
- U.S. Environmental Protection Agency. (2000). *Guidance for assessing chemical contaminant data for use in fish advisories, volume 2: Risk assessment and fish consumption limits.* Retrieved June 22, 2008, from http://www.epa.gov/waterscience/fish/advice/volume2/index.html
- Wang, Q., Kim, D., Dionysiou, D.D., Sorial, G.A., & Timberlake, D. (2004). Sources and remediation for mercury contamination in aquatic systems–A literature review. *Environmental Pollution*, 131, 323–336.
- Williams, W. (1999). The deadly shell game. Animals, 132(3), 16-22.

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