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Mercury Concentrations in Bullfrogs (*Rana catesbeiana*) Collected from a Southern Nevada, USA, Wetland

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A considerable amount of time and effort has been spent determining concentrations of environmental contaminants in edible tissue from fish and wildlife species in the United States. The majority of these investigations are concerned with sport fish and migratory waterfowl. These species are known to accumulate both metals and organochlorine compounds which are transferred up the food chain and can result in exposure to the human population. However, one additional aquatic species that may be of concern is the bullfrog (*Rana catesbeiana*) because of its wide geographic distribution due to its human-mediated introduction into many aquatic ecosystems, and because the consumption of frog legs is considered a delicacy by certain populations. Bullfrogs are harvested and consumed by sportsmen across the United States, and management agencies often have extremely liberal, or no, restrictions on their harvest. In southern Nevada, for example, bullfrogs can be harvested year-round with no daily bag limit (Nevada Division of Wildlife 2001). Thus, certain human populations which consume bullfrogs on a regular basis may be exposed to elevated levels of environmental contaminants. Additionally, recent investigations have demonstrated that mercury may also have effects on survival, growth, development of amphibian populations (Briston and Threlkeld 1998; McCrary and Heagler 1997; Punzo 1993a; Punzo 1993b; Kanamadi and Saidapur 1992)

The State of Nevada has well established mining operations which target the extraction and processing of precious metals such as silver and gold. In the past, mercury was used as part of these processes and has been found in elevated amounts in areas such as the Carson River and Walker Lake, NV, with the prior being the only Superfund site in the State of Nevada (Federal Register 1990). The only contaminant listed at this Superfund site is mercury, and health advisories have been issued for this area. Another possible source of mercury is the burning of coal which may release elemental mercury (Hg^0), which is readily transported by the atmosphere resulting in the contamination of areas which are often distant from known point sources. Once deposited into the aquatic environment, Hg^O can be readily converted to methylmercury (MeHg) which is more toxic and readily bioaccumulates (Huckabee et al. 1978). The purpose of this investigation was to examine total mercury concentrations in bullfrog tissue harvested from a southern

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Nevada wetland, and determine if fish and wildlife consumption advisories need to be expanded to include bullfrogs.

MATERIALS AND METHODS

In April, 2001 twenty-eight bullfrogs were harvested by spear fishing techniques from a southern Nevada wetland located between the towns of Carp and Elgin, Nevada in an area locally known as the Meadow Valley Wash. Immediately following harvest, each frog was assigned a sample code, weighed (g), and the length determined using a measurement from the tip of the snout to the vent (cm). After these initial measurements were taken, sportsmen who donated the frogs removed the hind legs for consumption. The remaining carcass was placed on ice and taken to the laboratory where liver, brain, kidney and muscle tissue from the front legs were dissected for chemical analyses. Tissues were placed in whirlpack bags and frozen until analysis. Prior to analysis, tissues were thawed at room temperature, patted dry and weighed, and immediately analyzed. All concentrations reported herein are based on wet weight. One lobe of the liver ranging from 229 to 642 mg was analyzed from each frog. Muscle segments (front leg) between 256 and 722 mg were also analyzed, while the entire brain and kidney were used for analysis. Since no direct evidence could be found to support the theory that metal accumulation may differ between front- and hind-legs, we assumed metal accumulation was similar in these two tissues.

Mercury analysis was performed on an AMA 254 Atomic Absorption Mercury Analyzer from the Leco Corporation. This instrument allows analysis of total mercury and is matrix independent. Samples were analyzed in nickle sample boats with drying, decomposition (550° C), and waiting times of 120:300:45 seconds for all tissues and certified reference materials. Ultra pure oxygen was used as the carrier gas with an inlet pressure of 250 kPa and a flow rate of 200 ml/min. The AMA 254 has a detection limit of 0.01 ng Hg and a linear range from 0.05 to 40 ng. All samples analyzed were within the calibration range. QA/QC was performed with certified reference material (crm) from Health Canada Hair Mercury Quality Control Program. Approximately 5-10 mg of crm was weighed and analyzed after every ten tissue samples, followed by a blank run (empty boat) to ensure all mercury was removed.

Linear regression was performed to examine the relationship between frog length, weight and mercury concentrations in various tissues. When an individual data point was missing for one variable, that point was eliminated from analysis. Additionally, ratios for mercury concentrations between brain, muscle, kidney and liver were determined. For ratio determination, frogs which did not have brain tissue present for analysis were assigned the average concentration of the remaining samples. Differences between the sexes were determined using a one-tailed, unpaired t-test after testing for homoscedesticity. When variances were equal, a pooled variance was used (Zar 1999). A significance level of $p=.05$ was used for all analyses.

RESULTS AND DISCUSSION

A total of 28 frogs were analyzed for mercury concentrations, including 14 males and 14 females with an average weight of 242.9 ± 95.9 g and an average length of 13.4 ± 1.9 cm. The male frogs had an average weight of 275.6 ± 95.3 g and an average length of 14.0 ± 1.7 cm; while the female frogs had an average weight of 210.2 ± 87.7 g and an average length of 12.7 ± 1.9 cm. The total mercury concentrations in muscle, brain, kidney and liver tissue for each frog are summarized in Table 1. The average concentrations of mercury were highest in the liver, followed by the kidney, leg muscle and brain, respectively. Mercury was detected in all liver, kidney and muscle tissues examined; however, several brain tissues (3 total) were below our limit of detection of 10 ppb (Table 1). Linear regression was then used to examine the relationship between tissue concentrations of mercury and length and weight. These data are summarized in Table 2.

In order to determine if mercury distribution was consistent between the four tissues examined, correlations between tissue types were calculated (Table 3). Mercury ratios in tissues were also examined by comparing muscle, kidney and liver tissue to brain tissue, resulting in the following average ratio of 1:2.80:4.52:7.65 ppm in brain, muscle, kidney and liver tissue, respectively.

Differences between mercury concentrations in the male and female frogs were also determined (summarized in Figure 1). Male frogs had a slightly higher concentration of mercury in all tissues compared to females. In general, as tissue concentrations increased so did the variability within the data; although statistical analysis using a t-test indicated only significant differences between liver tissue ($t=2.08$, $n=12$, $P=.03$)

Certified reference materials, including human hair, were incorporated into analytical runs every ten samples. All QA/QC samples had measured values within one standard deviation of the reported values, actual data are presented as Table 4.

Although studies examining the effects of mercury on embryo and tadpole development are quite numerous, very little data exists on the concentrations of mercury (or most other metals) in adult, wild populations of bullfrogs (Gillitsch & Chovanec 1995). This study is one of very few to examine the potential human health effects associated with consumption of amphibians on a regular basis, despite the wealth of information collected on other aquatic species such as fish.

Mercury concentrations in bullfrogs from the Meadow Valley Wash in southern Nevada all contained detectable levels of mercury in edible muscle tissue,

Table 1. Descriptive information for *Rana catesbeiana* and mercury concentrations in selected tissues.

Sex	Length (cm)	Weight (g)	Brain (ppm)	Muscle (ppm)	Kidney (ppm)	Liver (ppm)
F	12.2	159.2	n/a	0.041	n/a	0.135
F	16	434.2	n/a	0.117	0.15	0.241
F	13.4	242.1	0.008[1]	0.021	0.040	0.076
F	16	268.2	0.013	0.076	0.057	0.094
F	13.5	219.5	0.004[1]	0.012	n/a	0.046
F	13.9	232.3	0.022	0.057	n/a	n/a
F	13.2	283.4	0.017	0.049	0.102	0.121
F	11	159.7	0.024	0.086	0.092	0.147
F	10	124.0	0.025	0.060	0.143	0.161
F	13.2	247.1	0.019	0.049	0.047	0.077
F	10.2	110.6	0.024	0.082	0.136	0.190
F	10.4	116.1	0.018	0.054	0.056	0.104
F	11.3	127.6	0.022	0.055	0.076	0.152
F	13.5	218.1	0.014	0.026	0.058	0.086
Total	**177.8**	**2942.1**	**0.21**	**0.785**	**0.957**	**1.63**
Mean	**12.7**	**210.2**	**0.018**	**0.056**	**0.087**	**0.125**
SD	**1.9**	**87.7**	**0.007**	**0.028**	**0.040**	**0.053**
M	15.5	353.5	0.022	0.088	0.096	0.183
M	16	265.4	0.019	0.049	0.050	0.097
M	14.3	293.3	0.040	0.089	0.130	0.153
M	14.1	318.5	0.142	0.058	0.171	0.229
M	15.5	294.7	n/a	0.031	0.073	0.185
M	15.3	426.9	0.026	0.095	0.135	0.251
M	15.3	289.1	0.028	0.113	0.252	0.458
M	12.7	180.6	0.015	0.021	0.058	0.118
M	12.5	186.7	0.005[1]	0.013	0.036	0.067
M	11.5	143.0	n/a	0.015	n/a	n/a
M	15.4	432.3	0.022	0.066	0.124	0.155
M	13.2	299.1	0.025	0.058	0.072	0.22
M	10.7	112.6	0.031	0.087	0.143	0.179
M	14.1	263.3	0.012	0.051	0.061	0.099
Total	**196.1**	**3859**	**0.387**	**0.834**	**1.401**	**2.394**
Mean	**14.0**	**275.6**	**0.032**	**0.060**	**0.108**	**0.184**
SD	**1.7**	**95.3**	**0.036**	**0.032**	**0.060**	**0.099**

[1]= below 10 ppb limit of detection

although concentrations were quite low. State health advisories regarding fish and game consumption usually restrict the number of meals that can be safely consumed when mercury concentrations approach 0.5 ppm, these regulations target women of child bearing age or infants. Federal regulations are slightly less stringent as they target the general public, with 1 ppm in edible tissue being the action levels established for the Environmental Protection Agency (EPA) and Food and Drug Administration (FDA). The highest mercury concentrations we found in frog leg muscle (0.117 ppm) are well below these limits. Liver and kidney tissues typically contained two or three times the amount of mercury than the muscle tissue, but were not consumed by the population in question. These tissues do, however, provide us with an additional monitoring tool to estimate mercury exposure in frog muscle when muscle tissue is unavailable.

Table 2. Coefficients of Determination and P values examining the relationship between tissue concentrations and bullfrog size, expressed as $R^2(P)$.

Tissue/Size	Brain	Muscle	Kidney	Liver
Length (All)	.005 (.76)	.054 (.233)	.007 (.697)	.051 (.269)
Females	.385 (.031*)	.009 (.749)	.031 (.603)	.017 (.674)
Males	.001 (.942)	.136 (.195)	.019 (.655)	.063 (.407)
Weight (All)	.043 (.342)	.145 (.045*)	.059 (.251)	.113 (.093)
Females	.318 (.056)	.009 (.749)	.031 (.603)	.017 (.674)
Males	.025 (.623)	.222 (.089)	.058 (.427)	.082 (.344)

* = significance at $p \leq .05$.

Length and weight were extremely poor predictors of mercury concentrations in frog tissue (Table 2). Since amphibians frequent both aquatic and terrestrial habitats, and are subject to both biotic and abiotic factors which influence bioaccumulation, it is not surprising that length and weight associations are weak

Direct uptake from water and ingestion of food (and soil) are the most probable routes of exposure to metals in amphibians (Linder and Grillitsch, 2000; Eisler 1987). Initial inspection of stomach contents from frogs in the present study resulted in the identification of numerous aquatic and terrestrial food sources such as dragonfly larvae, beetles, minnows, annelids and crawfish demonstrating the opportunistic nature of bullfrog feeding behavior (Cross and Gerstenberger, In Press).

Differences were noted in mercury concentrations between the sexes, with females having slightly lower mercury concentrations in all tissues (Figure 1). This finding is consistent with published data on various wildlife species, and is most likely attributable to the large amount of reproductive tissue expelled from

Table 3. Coefficients of determination and P values for mercury concentrations between tissue types, expressed as R^2(P).

TISSUE	Brain	Muscle	Kidney	Liver
Brain (All)	----			
Female	----			
Male	----			
Muscle (All)	.057 (.254)	----		
Female	.606 (.002*)	----		
Male	.016 (.670)	----		
Kidney (All)	235 (.021*)	.543 (.000*)	----	
Female	.568 (.011*)	.470 (.198)	----	
Male	.206 (.138)	.622 (.001*)	----	
Liver (All)	.139 (.079)	.503 (.000*)	.770 (.000*)	----
Female	.771 (.000*)	.701 (.000*)	.823 (.000*)	----
Male	.065 (.424)	.488 (.008*)	.760 (.000*)	----

* = significance at $p \leq .05$.

Table 4. QA/QC data for mercury determinations in frog tissues.

Sample Code	Date Analyzed	Measured Concentration	Known Concentration	% Recovery	Within 1 SD
98-2-2	4/20/01	10.80	10.2 ± 2.1	94.4	Yes
98-2-2	4/20/01	10.16	10.2 ± 2.1	99.6	Yes
98-2-2	4/24/01	11.01	10.2 ± 2.1	92.1	Yes
98-2-2	4/24/01	11.75	10.2 ± 2.1	84.8	Yes
98-2-2	4/24/01	9.56	10.2 ± 2.1	93.7	Yes
98-1-3	5/3/01	13.67	13.7 ± 3.0	99.8	Yes
98-1-3	5/3/01	12.14	13.7 ± 3.0	88.6	Yes
98-1-3	5/3/01	12.59	13.7 ± 3.0	91.9	Yes
98-1-3	5/3/01	13.95	13.7 ± 3.0	98.2	Yes

females during reproduction. Mercury has a high affinity for ligands containing sulfur, and is known to be contained in membranes from cellular organelles (Goyer 1991).

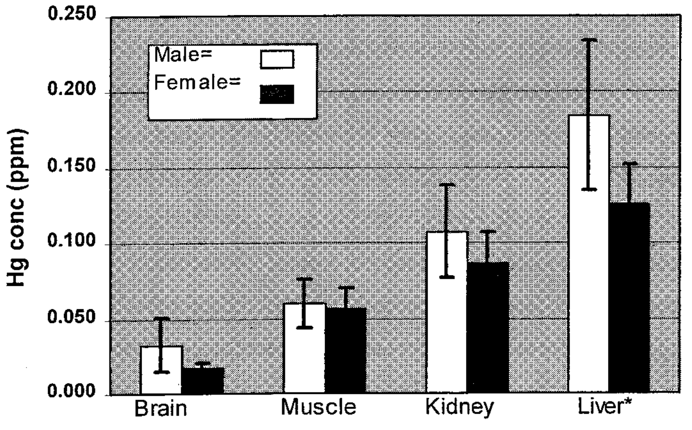

Figure 1. Average mercury concentrations in male and female bullfrogs. n=14 for all tissues, except where tissues were unavailable (see Table 2 for details). * = significance at $p \leq .05$.

This may contribute to an enhanced ability to eliminate mercury in female bullfrogs. Pharmacokinetic models could be created to estimate seasonal changes in mercury distribution due to reproductive status.

The effect of age on bioaccumulation was not determined in this study, but should be pursued. Age-related increases in metal concentrations have been demonstrated in other species, and the ability to biotransform environmental contaminants is known to vary with age in amphibian populations (Gillitsch and Chovanec 1995). Although biologically interesting, age is much more difficult to determine than length or weight in frogs. Due to field difficulties, age studies would have to be performed in the laboratory and would have limited field applications. Thus, age is limited in its usefulness to act as an indirect measure of metal bioaccumulation, especially when it is used to set consumption guidelines regarding human health.

Based on our data, the consumption of frogs from the Meadow Valley Wash should not be restricted, as mercury concentrations appear to be quite low. Similar investigations of mercury in frog muscle tissue should be completed near the one Nevada Superfund site on the Carson River, and in other Nevada locations with known mercury contamination problems (e.g. Lake Mead and Walker Lake). Bullfrogs are an under-recognized food source with little regulatory oversight which could potentially contain concentrations of mercury that meet or exceed

State and Federal guidelines. This, combined with the wide geographic distribution of bullfrogs may be contributing to elevated body burdens of mercury that are not being adequately examined. Routine monitoring programs for contaminants in fish, shellfish and waterfowl are in effect for local, regional and international arenas but bullfrogs have somehow eluded many of these programs. Amphibians are indicators of ecosystem health and should be included in future assessment plans and contaminant monitoring protocols.

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