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Mercury and Methylmercury Accumulation and Excretion in Prairie Voles (*Microtus ochrogaster*) Receiving Chronic Doses of Methylmercury

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Abstract. Methylmercury cation (MeHg) and divalent mercury (Hg^{++}) accumulation in liver, kidney, and brain were quantified in prairie voles (*Microtus ochrogaster*) at 0, 3, 6, and 12 weeks during chronic exposure to aqueous MeHg. Dose groups received deionized water or aqueous solutions containing 9, 103, or 920 ng MeHg/ml. Our study presents temporal patterns of Hg^{++} and MeHg concentrations in organ tissues and makes inter-tissue comparisons at each time point to illustrate the accumulation and distribution of Hg species during the study. MeHg was accumulated in tissues for 3 weeks and then concentrations plateaued. Mercury accumulated in brain, liver, and kidney to average concentrations of 510 ng/g, 180 ng/g, and 3400 ng/g, respectively. MeHg and Hg^{++} concentrations were roughly equivalent in liver, kidney, and urine. MeHg concentrations in brain tissue were 2 to 20 times the concentrations of Hg^{++}. Regression analysis was also used to demonstrate the utility of urinalysis as an indicator of Hg^{++} and MeHg concentrations in organ tissue ($p < 0.001$).

Introduction

MeHg Exposure and Effects

Methylmercury cation (MeHg) poses ecological and public health concerns globally (Johnson and Washington 2001) due to its ability to bioaccumulate, bioconcentrate, and biomagnify in food chains (USEPA 1997). This trophic transfer potential of MeHg results from a 90% absorption efficiency by mammalian gastrointestinal tract, whereas, 10 to 15% of divalent mercury (Hg^{++}) is absorbed (Goyer 1996). Although liver and kidney tissues are repositories for Hg^{++} and MeHg, the brain is more susceptible to MeHg toxicity, because a MeHg-cysteine complex, resembling methionione, can traverse the blood-brain barrier (Aschner and Clarkson 1987). MeHg in the brain may comprise 6% of the total accumulated Hg within vertebrates (Walsh 1982). In the brain, MeHg disrupts cell membrane integrity, inhibits protein synthesis, and reacts directly with neuromodulators in the peripheral nerves (Clarkson 1987; Mergler *et al*. 1998). It is widely thought that brain cells cannot be replaced, and brain damage is irreversible (Rabenstein 1978); therefore, MeHg exposure is frequently considered in risk assessments.

Evaluation of toxicant exposure and effect are required as part of current ecological risk assessment practices (USEPA 1997). Fish and wildlife health often drive ecological risk assessments at hazardous waste sites, increasing the need for procedures to evaluate mercury uptake and distribution in free-ranging species. To obtain effect data for risk assessments, laboratory studies of Hg^{++} and MeHg poisoning have often been performed using Hg concentrations in the 0.5 to 10 ppm range (Mitsumori *et al*. 1983; Greim *et al*. 1997; Sundberg *et al*. 1998; Pingree *et al*. 2001). Although these studies show extreme effects, such high concentrations of Hg^{++} and MeHg are rare in the environment. For refinement of risk assessments, Hg^{++} and MeHg distribution in organs and resultant effects must be documented following chronic low-dose exposure. Moreover, a sensitive non-lethal methodology is needed to assess exposure and effects in mammals exposed to heavy metals, such as Hg.

Past studies have proven that small terrestrial mammals are good indicators of heavy metal contamination and toxic effects (Wren 1986a, b; Talmage and Walton 1991). If wildlife being studied are not killed, a greater number of animals can be evaluated, and these individuals can be sampled repeatedly (Cobb and Hooper 1994; Cobb *et al*. 2003). Increased sample sizes also increase statistical power in exposure assessments. Using these two criteria, we selected a small model species that would provide a reasonable amount of urine daily for non-lethal assessment.

Evaluating MeHg Poisoning

Well-established, non-lethal methods of assessing metal exposure include analysis of urine, blood, or hair (Woods *et al*. 1991). Even though methods exist to quantify Hg^{++} and MeHg in a single analysis (Gelaude *et al*. 2002), there is currently a lack of controlled chronic Hg^{++} or MeHg exposure data that provide toxicant doses, uptake into tissues, and concomitant health effects endpoints. These data gaps preclude

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quantification of risks posed to wildlife by varying Hg^{++} and MeHg exposures (Meyer 1998). Our study quantified water consumption by laboratory rodents; MeHg and Hg^{++} in dosing solutions, urine, and target tissues (Moore 2002); and porphyrin profiles in allied studies (Rummel 2000).

Materials and Methods

Dosing Study

For the data in the reported experiment, 130 adult female voles *(Microtus ochrogaster)* were obtained from an established in-house colony and were housed individually in Nalgene cages with Aspen shavings and maintained at 21±1°C on a 12-hour light/dark cycle. Voles were fed Purina rat chow blocks supplemented with alfalfa cubes *ad libitum.* MeHg was administered via deionized drinking water in four dosing groups with targeted concentrations of 0, 0.01, 0.1, or 1 mg/L (as CH_3Hg^+), This water was provided *ad libitum.* Within each dose group, subgroups of voles were exposed for 0, 3, 6, or 12 weeks. Time-0 voles received no dose, and four dose groups were sampled at each of the 3 remaining time points. This design produced 13 dose groups (1+4×3) each containing 10 voles.

Aqueous dosing solutions were prepared weekly with CH_3HgOH (Sigma Chemical Co., St. Louis, MO) and stored in high-density polyethylene containers. Water consumption was measured gravimetrically each week by determining the water weight lost from the reservoir for each cage. Cold vapor atomic absorption (CVAA, Varian Spectra AA-20) was used to verify MeHg and Hg^{++} concentrations in each solution before and after administration, to track the MeHg concentration and any transformation between preparations. The instrument was configured with a slit width of 0.5 nm and a wavelength of 253.7 nm. A linear, five-point calibration was used for all analyses.

Following dosing, urine was collected from each animal for 24 h to determine Hg^{++} and MeHg concentrations. Voles were euthanized by CO_2 asphyxiation. Kidney, liver, and brain were removed, flash frozen in liquid nitrogen, and stored frozen at −30°C.

Analysis

Hg speciation in tissue is based on selective reduction principles that have been utilized for half a century (Braman 1971). Our methods used modifications of more recently published methods (Oda and Ingle 1981; Rio Segade and Tyson 2003). Urine (∼1 ml), kidney (∼0.2 g), and liver (∼1.7 g) tissues were placed in 0.625 ml of 10 M KOH (EM Science, GR grade purity, CAS no. 1310-58-3) and added to a water bath at 95°±1°C, where they were swirled every 10 min for 30 min. After digests cooled, 1.6 ml of trace metals grade nitric acid (Fisher Chemical, CAS no. 7697-37-2) was added slowly with mixing until a pH of 7 ± 0.5 was achieved.

Brain tissue (∼0.6 g) required 3 ml of 10 M KOH and similar heating in a water bath. After cooling to room temperature, digests were neutralized (pH 7 ± 0.5) with 2.5 ml of concentrated hydrochloric acid, added slowly with swirling. After acidification to pH 2, the solution was filtered using Gelman Laboratory Acro-disc 25-mm syringe filters with l-μm glass membrane and quantitatively transferred to volumetric flasks. Then, 0.25 ml of 1% potassium dichromate (Fisher, CAS no. 7778-50-9) was added, and digests were diluted to volume (50 ml for urine, 25 ml for kidney, and 25 ml for liver) with deionized water. Hg^{++} and MeHg speciation required stannous ion and borohydride ion as the respective reductants (Oda and Ingle 1981). During analysis reductants were mixed with 3M HC1.

Each tissue type underwent a validation procedure in beef kidney, pig brain, and water (surrogate for urine). Each sample was injected with sufficient MeHg to produce a final concentration of 5 to 8 ng/ml of MeHg or Hg^{++} in the analysis solution and homogenized.

For each tissue validation, 60 samples were spiked and distributed over a 4-day analysis period. Fifteen samples each were spiked with Hg^{++}, MeHg, or both forms of mercury. Fifteen blank samples were not spiked. Analyses were randomized to obtain recovery data across 4 consecutive days. The practical detection limit was defined as 0.25 ng Hg^{++}/ml, which represents 1/2 the concentration of the lowest standard.

The analysis of urine and tissue digests was performed using a continuous flow injection system (Perkin Elmer FIMS-400). Calibration was performed daily using a blank and five concentrations ranging from 0.5 to 20 ng/g. Calibration was considered acceptable when two consecutive calibrations produced data within 10% of one another. After this condition was met, tissue digests were analyzed. Continuing calibration checks (CCCs) were performed after analysis of 20 digests. If instrument response was not within 10% of the previous calibrated response, the instrument was recalibrated, and all samples that were not flanked by acceptable full calibration or CCC samples were reanalyzed. The method produced good mean recoveries (73%–96%) for urine and kidney, but mean recoveries from brain tissue were more variable 66%–100% (Table 1). During the validation, matrix blanks for all sample types produced low responses of 0.3 ng/ml extract (CI95 = 0.006 to 0.38 ng/ml). This is near the practical detection limit of this method. Thus, no corrections were made to the analytical data generated using this method.

Data Analysis

Data were non-normal and were log transformed to produce data sets with homogeneity of variance, thereby allowing parametric analyses. Hg^{++} and MeHg concentrations in tissues from voles receiving different durations and concentrations of MeHg doses were compared using a two-way ANOVA (fixed model) with dose concentration and dose duration as the independent variables. This two-way ANOVA evaluated significant differences between dose and tissue type for each analyte (Hg^{++} and MeHg). Single-factor ANOVA was performed to test for significant differences in Hg^{++} and MeHg concentrations observed for each dose and time interval (Sokal and James 1997).

Regressions were established for Hg^{++} and MeHg concentrations in urine versus the respective Hg species in each tissue, as a possible forensic test for exposure assessments. ANOVAs were performed with SAS© (SAS Institute Inc. 1999) software and the regressions were performed and plotted with MiniTab© (SPSS Inc. 1986–l999).

Results

Water Samples

High-, medium-, and low-dose drinking water contained 920 ± 102 (mean ± SE), 103 ± 13, and 9 ± 1 ng MeHg/ml, respectively (Rummel 2000). During the 12-week dosing period, Hg^{++} in drinking water averaged less than 2% of total mercury, indicating minimal exposure of test animals to Hg^{++}. Concentrations of both mercury species were below the instrument detection limit in control water.

Water consumption for individual voles was not different between time and treatment groups, and averaged 99.4 ± 2.47

Table 1. Mercury recoveries from spiked tissues

Tissue matrix	Mercury species	Mercury concentration (ng/ml)	Reducing agent	Percent recovery[a]	Recovery range
Urine	Hg^{++}	5	$SnCl_2$	80.1 ± 5.0	87.1–72.9
Urine	Hg^{++}	5	$NaBH_4$	89.6 ± 6.6	100.9–82.5
Urine	CH_3Hg^+	5	$SnCl_2$	9.6 ± 1.2	11.5–7.6
Urine	CH_3Hg^+	5	$NaBH_4$	95.1 ± 1.3	97.3–93.4
Kidney	Hg^{++}	6	$SnCl_2$	73.1 ± 7.4	90.1–63.1
Kidney	Hg^{++}	6	$NaBH_4$	96.6 ± 3.3	101.8–91.9
Kidney	CH_3Hg^+	6	$SnCl_2$	11.0 ± 3.9	18.2–2.9
Kidney	CH_3Hg^+	6	$NaBH_4$	75.0 ± 7.7	87.9–61.8
Brain	Hg^{++}	8	$SnCl_2$	63.5 ± 12.5	86.8–47.4
Brain	Hg^{++}	8	$NaBH_4$	51.0 ± 10.8	65.7–31.3
Brain	CH_3Hg^+	8	$SnCl_2$	7.1 ± 2.7	13.2–3.7
Brain	CH_3Hg^+	8	$NaBH_4$	100.6 ± 7.0	108.9–88.5

$n = 15$ for each tisue type. Values represent mean ± standard deviation
[a] All percent recoveries for tissues spiked with CH_3Hg^+ reduced with $SnCl_2$ should theoretically be zero

Table 2. Organic mercury concentrations in tissues (ng/g) and urine (ng/ml) collected from prairie voles ($n = 10$/treatment) following exposure to aqueous methylmercury

		Control	10 ng/ml	100 ng/ml	1000 ng/ml
Tissue	Week	Average ± Std	Average ± Std	Average ± Std	Average ± Std
Brain	0	7.662 ± 7.743			
	3	8.220 ± 4.011	12.236 ± 3.909	56.395 ± 18.248	602.67 ± 220.31
	6	5.522 ± 3.710	11.439 ± 3.058	49.409 ± 21.829	450.35 ± 169.50
	12	6.977 ± 6.976	11.284 ± 5.656	45.134 ± 26.147	477.87 ± 318.89
Liver	0	2.955 ± 2.259			
	3	1.135 ± 1.924	3.171 ± 3.171	23.035 ± 11.718	296.98 ± 166.01
	6	1.656 ± 2.049	3.378 ± 2.919	13.216 ± 15.217	100.90 ± 68.526
	12	2.479 ± 2.900	4.153 ± 3.520	21.244 ± 11.375	242.86 ± 119.71
Kidney	0	79.873 ± 41.165			
	3	58.293 ± 45.617	109.324 ± 75.068	450.967 ± 260.89	3721.78 ± 2431.1
	6	40.648 ± 29.087	37.267 ± 48.786	293.798 ± 128.52	3104.16 ± 963.28
	12	72.579 ± 54.151	79.553 ± 36.572	394.463 ± 138.57	3400.27 ± 1677.5
Urine	0	89.552 ± 70.379			
	3	23.712 ± 27.039	12.330 ± 12.173	155.627 ± 70.61	415.62 ± 371.83
	6	44.046 ± 57.872	48.147 ± 37.769	93.362 ± 92.39	709.23 ± 741.07
	12	89.166 ± 45.010	45.857 ± 33.90	118.797 ± 105.20	682.10 ± 774.19

ml/week. Based on average weekly water consumption, Hg concentrations in dosing solution, and weekly body weights, MeHg exposure in treatment voles averaged 353 ± 20, 46 ± 5, and 4 ± 0.4 μg MeHg/kg body weight/day, for the high, medium, and low dose, respectively (Rummel 2000).

Tissue Samples

Hg^{++} and MeHg increased with increasing dose and reached a relatively stable concentration in each tissue by week 3 (Tables 2 and 3). Hg^{++} concentrations were generally similar for tissues from control and low-dose animals. Statistical evaluations described below quantify the temporal and dose-dependent changes in Hg^{++} and MeHg concentrations within each tissue.

The two-way ANOVA demonstrated differences in Hg^{++} and MeHg concentrations that were dependent on dose intensity and dose duration ($p < 0.001$). The two-way ANOVA for Hg^{++} ($n = 504$) and MeHg ($n = 496$) in tissue demonstrated a dose-dependent increase of Hg^{++} and MeHg occurrence in tissue ($p < 0.0001$). A significant interaction was found for dose administration and dose duration ($p < 0.001$).

Differences for MeHg accumulation among dose groups and among tissues were further evaluated using Duncan Lines analysis, which indicated that MeHg concentration increased with increasing dose (Table 4), and for all tissues at each time point at least three of the four doses were significantly different. When data were grouped by tissue, analyses indicated that kidney contained the highest MeHg concentration followed by urine, brain, and liver. For the medium- and high-dose groups, MeHg in kidney, liver, and urine at week zero

Table 3. Inorganic mercury concentrations in tissues (ng/g) and urine (ng/ml) collected from prairie voles (n = 10/treatment) following exposure to aqueous methylmercury

		Control	10 ng/ml	100 ng/ml	1000 ng/ml
Tissue	Week	Average ± Std	Average ± Std	Average ± Std	Average ± Std
Brain	0	6.555 ± 2.978			
	3	3.226 ± 1.711	4.421 ± 1.973	12.055 ± 16.687	30.883 ± 10.461
	6	3.226 ± 5.195	5.016 ± 2.065	10.213 ± 5.854	31.269 ± 9.193
	12	13.749 ± 13.748	6.490 ± 3.785	10.045 ± 3.952	30.172 ± 8.684
Liver	0	0.176 ± 0.494			
	3	0.687 ± 0.843	1.492 ± 2.848	8.315 ± 3.625	111.71 ± 96.057
	6	1.004 ± 0.655	1.658 ± 1.673	6.547 ± 3.470	54.551 ± 25.626
	12	0.057 ± 0.073	0.517 ± 1.467	3.514 ± 3.431	27.920 ± 16.658
Kidney	0	38.884 ± 35.828			
	3	99.538 ± 55.207	196.44 ± 161.82	533.19 ± 264.983	3373.4 ± 1791.9
	6	80.597 ± 54.556	109.38 ± 48.544	385.18 ± 165.828	2524.1 ± 1013.1
	12	88.727 ± 57.330	119.91 ± 65.517	330.50 ± 174.596	1832.6 ± 1058.6
Urine	0	9.689 ± 15.037			
	3	17.954 ± 31.993	15.350 ± 17.886	103.14 ± 141.179	807.00 ± 202.33
	6	58.364 ± 27.777	67.174 ± 57.981	100.86 ± 62.490	768.05 ± 555.19
	12	144.59 ± 107.88	73.254 ± 51.417	178.33 ± 92.852	672.25 ± 470.73

Table 4. Comparison of mercury concentrations among dose groups of prairie voles receiving aqueous MeHg exposure

	Inorganic				Organic			
Dose (d)	Week 0	Week 3	Week 6	Week 12	Week 0	Week 3	Week 6	Week 12
Kidney								
$d_C : d_L$	NA[a]	**0.047**	0.456	0.369	NA	**0.039**	0.843	0.682
$d_C : d_M$	NA	**<0.001**	**<0.001**	**<0.001**	NA	**0.003**	**0.003**	**<0.001**
$d_C : d_H$	NA	**<0.001**	**0.001**	**<0.001**	NA	**0.002**	**<0.001**	**<0.001**
$d_L : d_M$	NA	**0.005**	**0.004**	**0.001**	NA	**0.013**	**0.002**	**<0.001**
$d_L : d_H$	NA	**<0.001**	**<0.001**	**<0.001**	NA	**0.002**	**<0.001**	**<0.001**
$d_M : d_H$	NA	**0.001**	**<0.001**	**0.005**	NA	**0.005**	**<0.001**	**<0.001**
Liver								
$d_C : d_L$	NA	0.294	0.197	0.355	NA	0.086	0.059	0.110
$d_C : d_M$	NA	**<0.001**	**<0.001**	**0.016**	NA	**<0.001**	**0.050**	**0.001**
$d_C : d_H$	NA	**0.009**	**<0.001**	**<0.001**	NA	**<0.001**	**0.001**	**<0.001**
$d_L : d_M$	NA	**<0.001**	**0.001**	**0.017**	NA	**0.004**	0.089	**0.002**
$d_L : d_H$	NA	**0.009**	**<0.001**	**<0.001**	NA	**<0.001**	**0.002**	**<0.001**
$d_M : d_H$	NA	**0.013**	**<0.001**	**0.001**	NA	**<0.001**	**<0.001**	**<0.001**
Brain								
$d_C : d_L$	NA	0.216	0.335	0.289	NA	0.148	**0.016**	**0.021**
$d_C : d_M$	NA	0.167	0.150	0.086	NA	**<0.001**	**<0.001**	**<0.001**
$d_C : d_H$	NA	**<0.001**	**<0.001**	**0.003**	NA	**<0.001**	**<0.001**	**0.002**
$d_L : d_M$	NA	0.225	**<0.001**	**<0.001**	NA	**<0.001**	**<0.001**	**0.002**
$d_L : d_H$	NA	**<0.001**	**<0.001**	**0.001**	NA	**<0.001**	**<0.001**	**0.002**
$d_M : d_H$	NA	**0.031**	**<0.001**	**<0.001**	NA	**<0.001**	**<0.001**	**0.003**
Urine								
$d_C : d_L$	NA	0.652	0.544	0.058	NA	0.416	0.531	**0.010**
$d_C : d_M$	NA	0.063	0.178	0.332	NA	**0.045**	0.098	**0.010**
$d_C : d_H$	NA	**<0.001**	**0.004**	0.059	NA	**0.017**	**0.032**	**0.014**
$d_L : d_M$	NA	0.060	0.195	0.019	NA	0.030	0.155	0.069
$d_L : d_H$	NA	**<0.001**	**0.004**	**0.006**	NA	**0.017**	**0.025**	**0.037**
$d_M : d_H$	NA	**0.002**	**0.003**	**0.027**	NA	0.078	**0.043**	0.072

Values represent comparisons of concentrations among dose groups within a given tissue
Related values are p values from ANOVA
Values in bold represent comparisons that are significantly different with an alpha value of 0.05
[a] NA = not applicable

Table 5. Comparison of mercury concentration among time points for prairie voles receiving aqueous MeHg exposure

	Inorganic				Organic			
Week (t)	Control	0.01 μg/ml	0.1 μg/ml	1.0 μg/ml	Control	0.01 μg/ml	0.1 μg/ml	1.0 μg/ml
Kidney								
$t_0 : t_3$	0.056	**0.021**	**<0.001**	**<0.001**	0.475	0.230	**<0.001**	**0.002**
$t_0 : t_6$	0.092	**0.001**	**<0.001**	**<0.001**	0.138	0.161	**0.002**	**<0.001**
$t_0 : t_{12}$	**0.023**	**0.012**	**<0.001**	**<0.001**	0.708	0.767	**<0.001**	**<0.001**
$t_3 : t_6$	0.118	0.177	0.015	0.291	0.631	**0.015**	0.116	0.491
$t_3 : t_{12}$	0.835	0.117	0.064	0.125	0.483	0.396	0.520	0.909
$t_6 : t_{12}$	0.757	0.544	0.808	0.149	0.288	0.160	0.112	0.258
Liver								
$t_0 : t_3$	0.291	0.197	**<0.001**	**0.008**	0.056	0.523	**0.001**	**<0.001**
$t_0 : t_6$	**0.004**	**0.016**	**<0.001**	**<0.001**	**0.009**	0.392	**0.045**	**0.001**
$t_0 : t_{12}$	0.485	0.521	**0.021**	**<0.001**	0.491	0.212	**<0.001**	**<0.001**
$t_3 : t_6$	0.213	0.897	0.558	0.187	0.438	0.658	**0.026**	**<0.001**
$t_3 : t_{12}$	0.086	0.351	0.054	**0.039**	0.239	0.490	0.758	0.310
$t_6 : t_{12}$	**0.001**	0.195	**0.007**	**0.010**	0.079	0.510	0.116	**<0.001**
Brain								
$t_0 : t_3$	**0.016**	0.064	0.939	**<0.001**	0.151	**0.003**	**<0.001**	**<0.001**
$t_0 : t_6$	0.582	0.336	**0.019**	**<0.001**	0.192	**<0.001**	**<0.001**	**<0.001**
$t_0 : t_{12}$	0.350	0.934	0.070	**<0.001**	0.451	**0.028**	**0.003**	**0.002**
$t_3 : t_6$	0.079	0.243	**0.043**	0.688	0.734	0.878	0.503	0.432
$t_3 : t_{12}$	0.064	0.199	0.173	0.051	0.796	0.937	0.404	0.289
$t_6 : t_{12}$	0.520	0.499	0.626	0.509	0.414	0.929	0.728	0.225
Urine								
$t_0 : t_3$	0.507	0.630	0.056	**<0.001**	0.074	**0.012**	0.196	0.060
$t_0 : t_6$	**0.009**	**0.015**	**0.005**	**0.003**	**0.032**	0.140	0.785	**0.032**
$t_0 : t_{12}$	**0.022**	**0.007**	**<0.001**	**0.006**	0.828	0.140	0.421	0.069
$t_3 : t_6$	**0.019**	**0.045**	0.646	0.833	0.846	**0.023**	0.378	0.226
$t_3 : t_{12}$	**0.020**	**0.006**	0.477	0.463	**0.021**	**0.015**	0.589	0.419
$t_6 : t_{12}$	0.106	0.748	0.150	0.180	**0.023**	0.842	0.629	0.867

Values represent comparisons of concentrations among dose groups within a given tissue.

was lower than concentrations observed at weeks 3, 6, or 12 (Table 5). A plateau was reached at week 3 and maintained thereafter. MeHg concentrations in control and low doses were similar for tissues through all time points, but MeHg excretion in urine increased until week 3 for the low-dose group. MeHg concentrations also increased through week 6 for kidney from the low dose ($p = 0.015$) and liver from medium and high doses ($p = 0.026$). Differences were also seen for comparisons involving liver from weeks 6 and 12 in the high dose ($p < 0.001$). Duncan Lines analysis of Hg^{++} showed that the high and medium dose differed from low and control doses, but the low and control groups were similar to one another. Duncan Lines also separated all four tissues into four different groups with kidney being the highest followed by urine, brain, and liver as was observed for methylmercury.

Urinary Hg as a Predictor of Hg in Organ Tissues

Total MeHg data reported in concentration units were related to total MeHg mass excreted, as shown by linear regression ($p < 0.001$, $r^2 = 0.88$; Fig. 1). This allowed data to be reported in ng Hg/ml urine/24 h.

The regression of urinary MeHg concentrations versus renal MeHg demonstrated a highly significant yet marginally strong correlation ($r^2 = 0.342$, $p < 0.001$; Fig. 2). A slightly better regression was obtained for Hg^{++} in kidney and urine ($r^2 = 0.399$, $p < 0.001$; Fig. 3). MeHg concentrations from urine were regressed with hepatic MeHg concentrations and displayed a weak yet significant regression ($r^2 = 0.326$, $p < 0.001$; Fig. 4), which was similar to results for Hg^{++} in hepatic tissue and urine ($r^2 = 0.341$, $p < 0.001$; Fig. 5). Speciated mercury in urine was similarly predictive of MeHg and Hg^{++} in brain tissue ($r^2 = 0.359$, $p < 0.001$; Fig. 6; $r^2 = 0.318$, $p < 0.001$; Fig. 7, respectively).

Discussion

At each time point, the dose-dependent increase in MeHg within a tissue demonstrates doses that exceed clearance capacities of the voles. For liver, increases in MeHg and Hg^{++} accumulation approximate a log progression just as does the dosage. Conversely, in kidney and brain, Hg^{++} retention increased by factors of 1.5–4 and 2.5–6, respectively. MeHg accumulation in these tissues increased by factors ranging from 4–8× between the lower two doses and 7–13× between the higher two doses. Intertissue differences in MeHg are logical since the liver is the first organ that the blood reaches after leaving the stomach, affording an opportunity for MeHg to accumulate there, in direct proportionality to concentrations in the oral dose. Kidney and brain receive blood that has passed through the liver, but during the time frames evaluated,

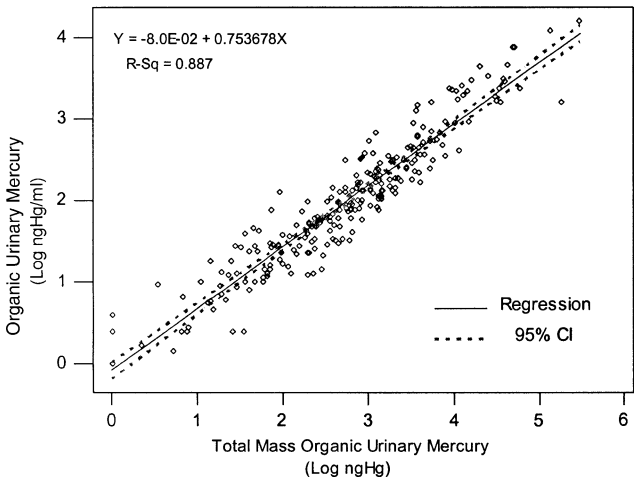

Fig. 1. Correlation of total mass organic mercury in urine excreted from prairie vole individuals as a function of organic urinary mercury concentration. Total mass mercury in urine is in ngHg where urinary mercury concentration is in ngHg/ml urine

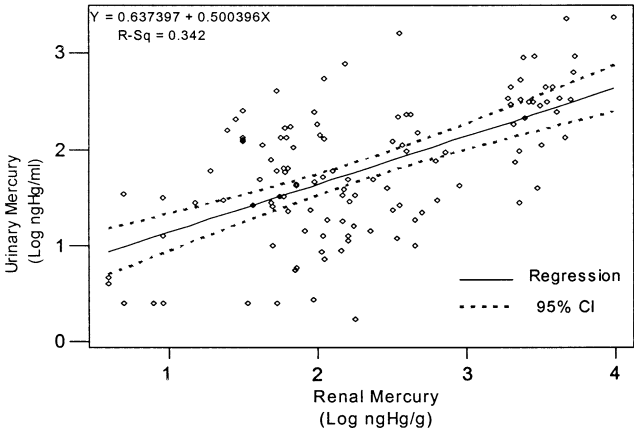

Fig. 2. Comparison of organic mercury in urine as a function of organic mercury in renal tissue. Data represent each individual concentration transformed into log units

these organs were more efficient than liver in retaining MeHg, by factors of 2.5 and 20, respectively. This is important when evaluating the time that a dose must be experienced until MeHg reaches a steady state (i.e., equilibrium concentration) in the body.

Hg^{++} accumulation in brain tissues was 2–20× lower than that for MeHg, with MeHg/Hg^{++} ratios in brain increasing in a dose-dependent fashion at all time points studied. Hg^{++} accumulation in liver showed no apparent temporal response at 10 ng/ml and followed an inverse relation to dose at 100 ng/ml and 1000 ng/ml. These observations can be explained by increased detoxifying processes in the liver at higher doses and induction of sequestering proteins in the kidney (Jernelov *et al.* 1976; Berndt *et al.* 1985). These data also support the increased ratio of Hg^{++} to MeHg found in urine after 3 weeks of exposure (Fig. 8).

Relationship to Previous Dosing Studies

Our study builds upon previous knowledge of mercury behavior in rodents following high doses of various mercury species. The biological half-life of MeHg in Sprague-Dawley rats is considered to be 20 days when exposures are at mg/kg concentrations (Mitsumoro *et al.* 1983). If exposures occur for 4–5 half-lives, a steady-state should be reached. Thus, a steady state should be reached in 80–120 days (12–15 weeks). Sundberg *et al.* (1998) determined a terminal half-life of total Hg to be 7.1 days following single intravenous injection of MeHg, providing a steady-state estimate of 28 to 36 days (4 to 5 weeks). It should be noted that one week after a single intramuscular injection, Hg^{++} concentrations in organs and blood were 25–50% of that found in mice receiving an oral dose (Harry *et al.* 2004). This fact could impact the apparent half-life in studies where mercury was delivered by routes other than oral. When mice (strains C57b1/6, DBA/2, B10.D2, A.SW, and B10.S) received subcutaneous injections of 0.5 mg Hg^{++}/kg three times weekly (214.3 μg/kg/d) for 12 weeks, Hg plateaued in blood and kidney after 4 weeks at 40 to 90 μg/L (Greim *et al.* 1997). We observed a plateau in Hg^{++} and MeHg concentrations across the time frame of 3–12 weeks. Since we did not analyze whole bodies, it is difficult to say if steady state was reached in the whole body, but we did note significant concentration increases in urine until week 6 at lower concentrations and insignificant increases until week 12 for all doses. These temporal excretion patterns suggest that 12 weeks was the soonest that Hg^{++} and MeHg could have reached true equilibrium.

Although our study and many others propose urine as a non-lethal indicator of Hg exposure, our data may have significant implications for toxicokinetic modeling. There are studies that show primary organic Hg excretion is through fecal material in rodents (Komsta Szumska *et al.* 1983; Gregus and Klaassen l986; Ishihara 2000) and in pigs (Gyrd-Hansen 1981), with as much as 16× more MeHg being excreted in the feces.

This excretion route cannot be discounted in studies proposing mass balances of mercury within organisms. In fact, current models of MeHg and Hg^{++} toxicokinetics (Carrier *et al.* 2001a, b) are based on minimal excretion of MeHg via the urine. Conversely, our data show mercury excretion in urine that is 13×, 2.9×, and 1.3× that of the measured doses of 9, 103, and 940 ng/ml. So, current model assumptions of minimal MeHg excretion via urine may be in error. The extent of this error is difficult to estimate since voles were selected for the high volume of urine they release daily. Also, toxicokinetic model descriptions reference only one study with MeHg and Hg^{++} speciation was found. We have shown that MeHg represented a high percentage of total urinary Hg: 55 to 65% at week 3, 52 to 54% at week 6, 54 to 61% at week 12.

Possibly of more importance is the fact that from week 3 to week 12, the fraction of divalent mercury present in the urine was drastically altered (Fig. 8). At week 3, Hg^{++} excretion in urine increased in a somewhat dose-dependent fashion. At week 6, the fraction of Hg^{++} is constant and low across doses. At week 12, the fraction of Hg^{++} rebounds at lower doses but decreases at the 1000-ppb dose. This decrease in Hg^{++} excretion at the high dose and may be due to increased cleavage of MeHg-glutathione conjugate to MeHg-

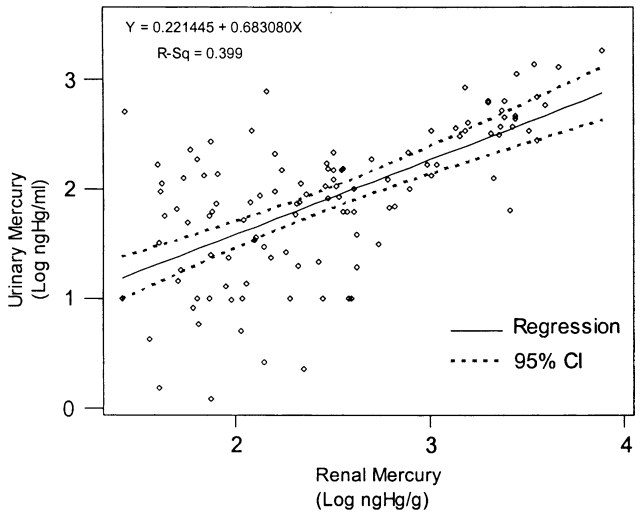

Fig. 3. Comparison of inorganic mercury in urine as a function of inorganic mercury in renal tissue. Data points represent each individual concentration transformed into log units

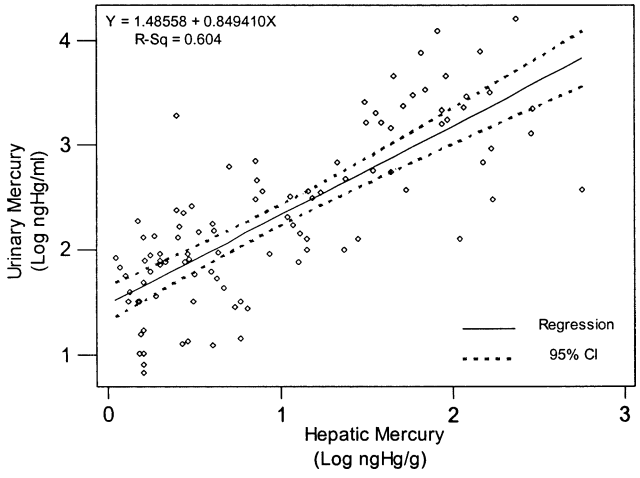

Fig. 4. Comparison of organic mercury in urine as a function of organic mercury in hepatic tissue. Data represent each individual concentration transformed into log units

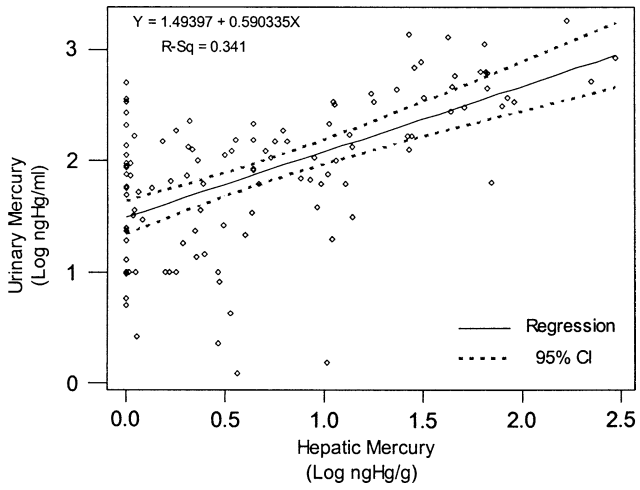

Fig. 5. Comparison of inorganic mercury in urine as a function of inorganic mercury in hepatic tissue. Data points represent each individual concentration transformed into log units

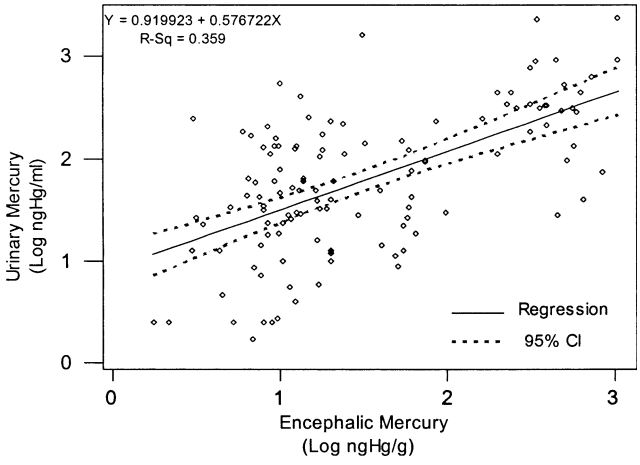

Fig. 6. Comparison of organic mercury in urine as a function of organic mercury in brain tissue. Data represent each individual concentration transformed into log units

cysteine (Yasutake *et al.* 1989), which has been shown to be the dominant form of MeHg excretion. This would increase the rate of MeHg removal from the kidney into urine, thereby decreasing the fraction of Hg^{++} in urine. Inhibition of synthesis of sulfur-rich biomolecules, such as glutathione, in the kidney has been shown to diminish MeHg sequestration, thereby increasing excretion. Temporal Hg distributions observed in our study could alter time courses computed for deputation via the urinary route.

Mercury excretion has been measured in the urine and tissues of rats exposed to mercuric chloride, elemental Hg (Cherian *et al.* 1988), and MeHg (Pingree *et al.* 2001), albeit at higher concentrations than in our study. The latter of these is the more important as the same toxicant was used and the urine was collected 9 weeks into the exposure, a time frame in which we observed a plateau in mercury concentrations within organ tissues. When Pingree *et al.* (2001) dosed rats with 10 mg/L MeHg in water, approximately 2000 ng/ml total mercury was excreted in urine in approximately equal proportions of inorganic mercury and organic mercury. In our study, 1280–1470 ng/ml of total mercury was excreted in urine from dose groups receiving 1000 ngHg/ml for 3 to 12 weeks. The Hg in our system was also equally distributed as Hg^{++} and MeHg. Pingree *et al.* (2001) also found a linear relationship between Hg^{++} concentrations in kidney and in urine following therapeutic chelation with DMPS. Although data for chelated mercury are not reported herein, we found significant linear relationships between mercury concentrations in urine and those in liver and kidney. An associated study within our research group will evaluate the improvements in regressions after chelation, which will allow comparison to existing studies.

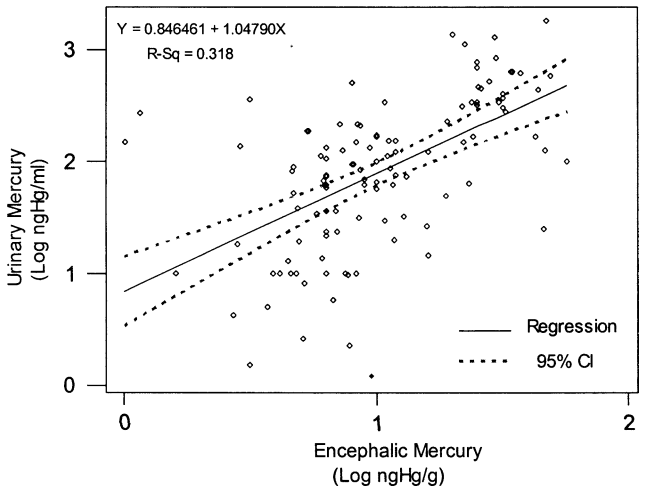

Fig. 7. Comparison of inorganic mercury in urine as a function of inorganic mercury in brain tissue. Data points represent each individual concentration transformed into log units

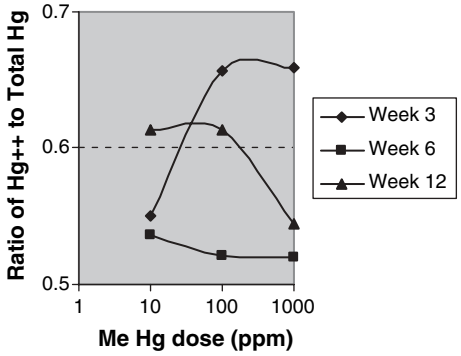

Fig. 8. Temporal change in divalent mercury to total mercury ratio in urine samples of voles receiving chronic doses of methylmercury

Significance of Our Work to Ecological Risk Estimation

There is a current need for a field application to quantify low levels of Hg exposure in wildlife. Low MeHg concentrations (0.01, 0.1, and 1 μg/ml) in drinking water consumed by voles over 12 weeks allowed development of linear models of Hg^{++} and MeHg excretion from renal, hepatic, or brain tissues into urine. The 95% confidence limits of these regressions allow predictions within one half log dose (a factor of 3.16). We realize that our figures have mercury species concentrations in urine as the dependent variable and that in field conditions urine would be used as the independent variable, but in the experiment we can state with confidence that mercury species concentrations in urine originate from organ tissues. With these empirical data, estimations of mercury species in organs and estimations of mercury doses can be determined from non-lethally collected urine samples. Furthermore, collection of either total MeHg mass or the concentration in urine could be used without loss of accuracy. The latter finding should allow field researchers to collect urine and retain 1 to 2 ml of the sample, thereby minimizing transport issues in the field.

Throughout the study, Hg^{++} concentrations increased within tissues for 3 weeks of dosing and with few exceptions plateaued thereafter. Although the 3-week time point is unlikely to represent a true equilibrium (see above), this plateau suggests that following 3 weeks of exposure in the range of 4 μg/day $\leq x \leq$ 350 μg/day. Significant changes in Hg^{++} and MeHg concentration would not be expected within kidney, brain, and urine, but some increase in Hg^{++} and MeHg concentrations would be expected in liver tissue. This is to assume all other parameters are similar to our study, such as environmental conditions, exposure to no other significant metal or contaminant, and health of individuals. It is interesting to note that the relative mercury concentrations in liver and kidney are used to assess the duration of wildlife exposure to mercury and other heavy metal toxicants. Since our earliest time point was 3 weeks, these data represent chronic exposures. Hg^{++} in kidney and liver from all time points in the three MeHg-exposed groups produced ratios (10 to 30) that indicate chronic exposure (Eisler 1987).

Conclusions

Dose-dependent increases were observed for MeHg and Hg^{++} in tissues and urine of prairie voles receiving chronic, low-dose exposure to methylmercury, the temporal aspect of exposures did not consistently affect MeHg or Hg^{++} concentrations in tissues. At all doses and time points, reliable MeHg concentration ratios were observed for each tissue type and among urine and individual tissues. This suggests a method for evaluating toxicant concentrations in target tissues by sampling urine and using the concentrations therein to estimate concentrations in tissues. Ratios of MeHg to Hg^{++} in urine were altered across dosing groups and throughout the study duration, suggesting a change in toxicant binding or excretion at these doses and durations.

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