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Dimercaptopropane Sulfonate Chelation Affects In Vivo Hg and MeHg Distribution in Tissues and Urine of Prairie Voles (*Microtus ochrogaster*)

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Abstract Methyl mercury cation ($MeHg^+$) and divalent mercury (Hg^{2+}) were quantified in urine, liver, kidney, and brain of prairie voles (*Microtus ochrogaster*) during a 12 week exposure to aqueous $MeHg^+$ at concentrations of 10, 100, and 1000 ng $MeHg^+$/mL. Aqueous $MeHg^+$ exposures increased mercury accumulation in tissues of voles from each exposure group. Accumulation was greater within the higher two exposure groups. Similar [Hg^{2+}] and [$MeHg^+$] were determined within a given organ type before and after 2,3-dimercapto-1-propane sulfonate (DMPS) chelation. Similar correlations were seen for Hg^{2+} and $MeHg^+$ concentrations in pre and post chelation urine. Post chelation urine more reliably predicted mercury species concentrations in tissues than did urine collected before chelation. These data demonstrate the utility of DMPS in noninvasive assessment of wildlife exposure to mercury, which may have utility in evaluating meta-population level exposure to hazardous wastes.

Keywords Mercury · Nonlethal · Exposure assessment · Excretion · Accumulation

Mercury is a well documented toxicant, with the most severe risks arising from exposure to alkylated forms (Chumchal et al. 2011), particularly methylmercury cation ($MeHg^+$). Understanding the time course of mercury distributions among tissues at environmentally realistic exposures is important to determine the risk that wildlife experience from $MeHg^+$ exposure. While controlled chronic mercury exposure studies have determined toxicokinetic and teratogenic effects that relate to human health (Curtis et al. 2010; Lewandowski et al. 2002; Woods et al. 1991), few studies have provided measured concentrations of mercury in tissues together with consequential health affect endpoints for the protection of wildlife species. These data gaps have precluded prediction of risk to mercury-exposed wildlife (Meyer 1998).

Well-established non-lethal methods of assessing metal exposure include blood, urine, or hair analyses (Beyer et al. 1996; Cobb et al. 2007; Rainwater et al. 2009). However, these methods do not measure toxic metal concentrations in target organs. Safe and effective chelation can be achieved with 2,3-dimercapto-1-propane sulfonate (DMPS). Administration of DMPS, subsequent collection of urine, and analysis of depurated metals can improve estimates of toxic metal concentrations in tissues of mammalian species (Cherian et al. 1988; Aposhian 1998; Maiorino et al. 1996; Pingree et al. 2001a, b). An accurate non-lethal method for assessing environmentally relevant exposure in mammals exposed to metals would offer many benefits. Without the need to kill the subjects collected, many animals can be assessed repeatedly. Increasing the sample size and statistical power of analysis would increase certainty of relationships between contaminant exposure and effect endpoints.

Small mammals provide good estimates of metal contamination and toxic effects in the environment (Talmage and Walton 1991; Wren 1986). Voles, *Microtus ochrogaster*, are widespread (Beardsley et al. 1978), omnivorous, and

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semi-fossorial. As such, they are readily exposed to environmental contaminants and are easily captured (Talmage and Walton 1991). The primary objective of this study was to develop a non-lethal technique for assessing mercury exposure in a small mammal species. To accomplish this, we characterized the relationship between mercury concentrations in urine and those in tissues of prairie voles exposed to $MeHg^+$ during a study design that evaluated temporal and dose dependence of uptake and excretion. Exposure concentrations were chosen to bracket known mercury exposures in contaminated areas. Although typical mercury concentrations in freshwaters normally do not exceed 0.09 ng/mL (Leopold et al. 2010), concentrations in surface water from mining areas in Spain ranged from <0.1 to 23 ng/mL (Ordonez et al. 2013). In geologically active areas of Pakistan and mining regions of the USA, surface water concentrations reach 0.14–0.15 ng/mL (Gray et al. 2015), while leachate from mine wastes in China contained 2–3 ng/mL of mercury (Biber et al. 2015).

Materials and Methods

Procedures for vole care, handling, and dosing are presented elsewhere (Cobb et al. 2007; Rummel 2000). Drinking water was fortified with aqueous MeHgOH (Sigma Chemical Co., St. Louis, MO, USA) at nominal concentrations of 10, 100, and 1000 ng/mL. Analytical verification of each test solution indicated the presence of 9, 103, or 920 ng $MeHg^+$/mL in the respective test solutions. These solutions represented the sole source of water (Cobb et al. 2007; Rummel 2000). Voles were randomly assigned to control and treatment groups and placed in metabolism cages. Urine was collected from control voles for 24 h at week 0. Urine was collected by removing the liquid from the liquid trap, below the metabolism cage. Ten control voles received an intraperitoneal (IP) injection of 1.0 mmol/kg DMPS. Urine was collected from both groups for 24 h. Controls were euthanized with CO_2 and dissected to remove tissues, which were frozen in liquid nitrogen. The remaining voles were distributed into four exposure groups that received $MeHg^+$ for 3, 6, or 12 weeks. After each interval, 10 voles from each group received IP DMPS. Urine was collected for 24 h after injection with DMPS, and voles were euthanized as described above.

Chemical analyses followed the method of Cobb et al. (2007). Briefly, samples were lyophilized in 10 M KOH at 95°C and after cooling were acidified to a pH of 2. Acidified digests were filtered (1 µm Teflon filters: Sigma-Aldrich) and volumetrically diluted. Mercury speciation used borohydride ion to liberate mercury species from extracts and stannous ion to selectively reduce mercuric ion into a flow injection cold vapor atomic absorption spectophotometer (FIMS-400, Perkin Elmer). This method demonstrated recoveries of 80.1 ± 5.0 for Hg^{2+} and 95.1 % ± 1.3 % for $MeHg^+$ in urine; 73.1 ± 7.4 for Hg^{2+} and 75.0 ± 7.7 for $MeHg^+$ in kidney; and 63.5 ± 12.5 for Hg^{2+} and 100.6 ± 7.0 for $MeHg^+$ in brain tissue.

All data were log transformed before statistical treatment. ANOVA with Bonferonni correction was performed to evaluate differences ($p \leq 0.05$) between mercury concentrations in each exposure group, tissue type, and DMPS treatment. Three way ANOVAs were performed to determine overall variance across all exposure groups, tissue types, and DMPS treatments. This was followed by a series of two way ANOVAs and one way ANOVAs.

Simple regressions of two types were performed using MiniTab©. First, mercury concentrations in each tissue type were treated as independent variables and mercury concentrations in urine as the dependent variable. Secondly, mercury in non-chelated tissue was treated as the independent variable and mercury concentration in DMPS-chelated tissue was treated as the dependent variable.

Results and Discussion

$[MeHg^+]$ in kidney and urine were similar in concentration, followed by liver then brain (Fig. 1). Urine had the highest $[Hg^{2+}]$, followed by kidney, brain, and liver. When considering all exposure groups as compared to control, significant increases ($p < 0.0001$) were observed in both $[MeHg^+]$ and $[Hg^{2+}]$. This finding justified further investigation of differences within and among these treatment groups. These more in depth comparisons showed $[MeHg^+]$ and $[Hg^{2+}]$ plateaued after week three in lower exposure groups (Table 1) but increased temporally in groups receiving higher exposures. This pattern held true for Hg^{2+}, but not $MeHg^+$, in urine. It should also be noted that exposure intervals shorter than 12 weeks were less likely to increase $[Hg^{2+}]$ (Table 2). $[MeHg^+]$ in tissues of control and low exposure voles were similar at all time points (Table 2, footnote). $[MeHg^+]$ and $[Hg^{2+}]$ in tissues also demonstrated interaction between exposure concentration and tissue type ($p < 0.036$).

These findings confirm work with voles that did not receive DMPS (Cobb et al. 2007), wherein $[Hg^{2+}]$ or $[MeHg^+]$ increased with exposure, and concentrations stabilized in tissues by week three (Cobb et al. 2007). In the absence of chelation, exposure concentration and duration increased $[Hg^{2+}]$ or $[MeHg^+]$ in tissues ($p < 0.001$). Kidney contained the highest $[MeHg^+]$ followed by urine, brain, and liver. $[MeHg^+]$ increased in urine of the low exposure cohort, until week six (Cobb et al. 2007).

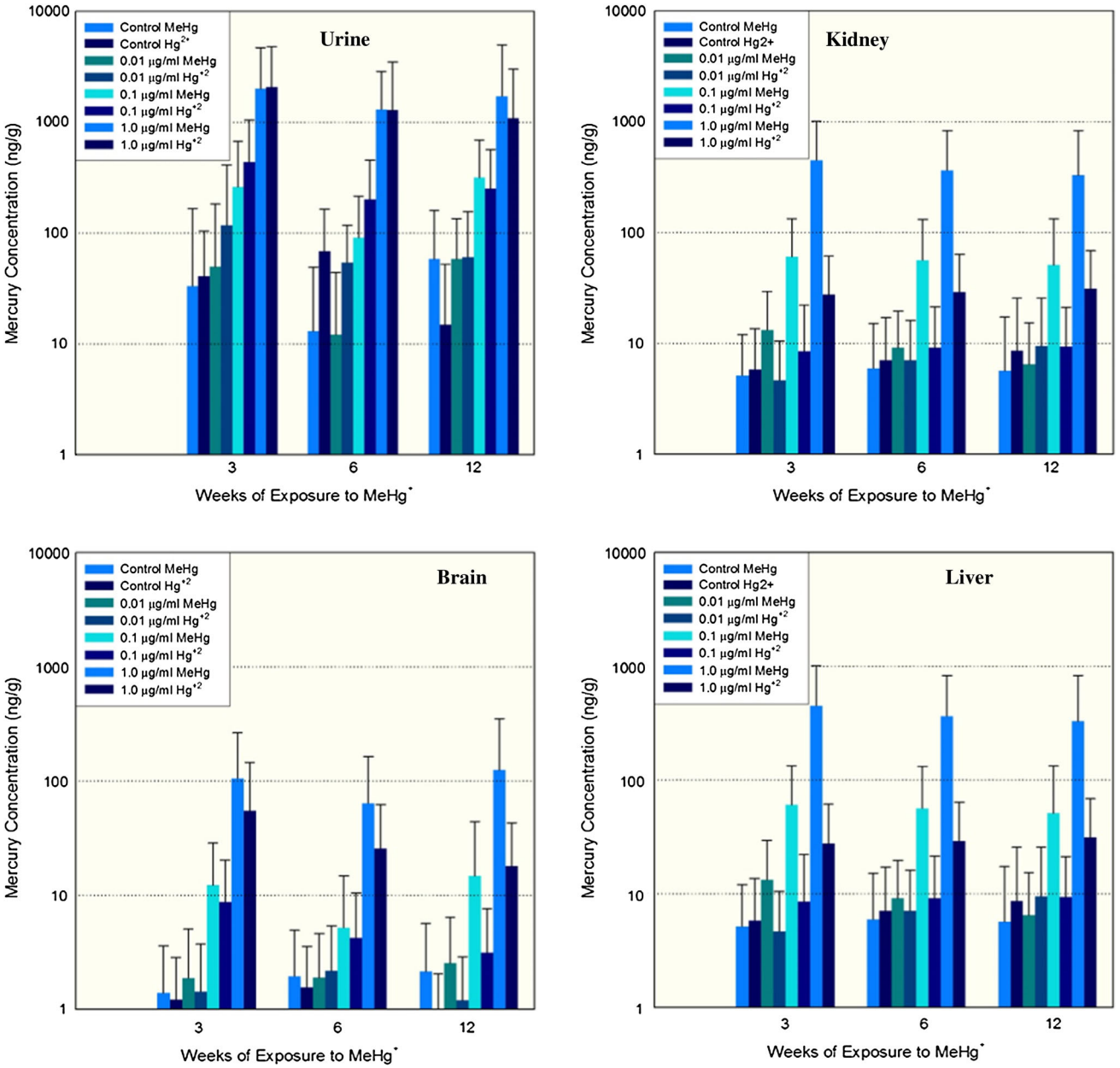

Fig. 1 Organic and inorganic mercury concentrations in tissue (ng/g) and urine (ng/mL) from prairie voles following exposure to aqueous $MeHg^{+}$ and post exposure chelation with 2,3-dimercapto-1-propane sulfonate

As compared to data from our previous work, chelation did not generally alter [Hg^{2+}] or [$MeHg^{+}$] from 10 and 100 ng/mL exposure groups. In the few cases where DMPS caused differences, concentrations decreased in tissues following treatment. Thus, DMPS treatment increased Hg^{2+} or $MeHg^{+}$ urinary excretion from higher exposure groups at weeks six and 12. Mercury concentrations in vole kidney and brain from higher exposures support the idea that Hg^{2+} and $MeHg^{+}$ are removed from tissues and excreted in urine. Post-chelation mercury was lower in the brain than non-chelated mercury for the 10 µg/L exposure at week six.

Regressions of mercury concentrations in DMPS urine and organ tissues suggested that post-DMPS urine provided a better estimation of mercury species in organ tissues than did untreated urine. For voles receiving saline in our previously published work, highly significant and moderately powerful regressions ($0.32 < r^2 < 0.40$, $p < 0.001$) were found for [$MeHg^{+}$] in urine versus renal, hepatic, or brain tissue (Table 3). Less powerful regressions were obtained

Table 1 *p* values comparing post-chelation mercury concentration in prairie voles as a function of aqueous $MeHg^+$ exposure concentration and duration

Tissue	Week (t)[a]	Inorganic (ng/mL)			Organic (ng/mL)		
		10	100	1000	10	100	1000
Kidney	t_0:t_3	**0.012**	**0.002**	**<0.001**	0.688	**<0.001**	**<0.001**
	t_0:t_6	0.104	**0.001**	**<0.001**	**<0.001**	0.357	**<0.001**
	t_0:t_{12}	0.072	**0.001**	**<0.001**	0.288	**<0.001**	**<0.001**
	t_3:t_6	0.070	**0.031**	**0.048**	0.068	0.147	**0.038**
	t_3:t_{12}	**0.047**	0.064	**0.019**	0.858	0.701	0.736
	t_6:t_{12}	0.911	0.640	0.933	0.085	0.167	**0.038**
Liver	t_0:t_3	0.347	**<0.001**	**<0.001**	0.259	**0.007**	**0.001**
	t_0:t_6	0.032	**0.022**	**<0.001**	0.134	0.097	**0.014**
	t_0:t_{12}	0.815	**0.001**	**0.009**	0.876	**0.025**	**0.009**
	t_3:t_6	0.112	0.217	**0.009**	0.972	0.123	**0.001**
	t_3:t_{12}	0.347	**0.002**	**0.004**	0.253	0.263	0.076
	t_6:t_{12}	**0.018**	0.148	0.458	0.086	0.077	**0.007**
Brain[b]	t_0:t_3	**0.030**	0.288	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	t_0:t_6	0.212	0.283	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	t_0:t_{12}	0.197	0.785	**<0.001**	0.085	**0.001**	**<0.001**
	t_3:t_6	**0.030**	0.743	0.165	0.827	**0.001**	0.368
Urine[b]	t_0:t_3	0.381	**0.011**	**0.012**	**0.035**	**0.012**	**0.022**
	t_0:t_6	**<0.001**	**0.005**	**0.006**	0.771	0.051	**0.014**
	t_0:t_{12}	**0.016**	**0.004**	**0.032**	0.231	0.060	0.072
	t_3:t_6	**0.002**	0.198	0.755	0.137	0.436	0.365
	t_3:t_{12}	**0.021**	0.447	0.641	0.110	0.413	0.738

Bold values are statistically significant ($p < 0.05$)

[a] t_0 = week 0; t_3 = week 3; t_6 = week 6; t_{12} = week 12

[b] No differences were seen for brain (t3:t12, t6:t12) or urine (t6:t12)

for Hg^{2+} in urine versus tissue ($0.31 < r^2 < 0.341$, $p < 0.001$).

Significant regressions were obtained ($p < 0.0001$) for both [Hg^{2+}] and [$MeHg^+$], when tissues of voles that received DMPS chelation were compared to non-DMPS treatments. Correlations were high for [$MeHg^+$] ($r^2 > 0.957$) and [Hg^{2+}] ($r^2 > 0.909$) in hepatic tissues (Table 4). Comparisons of mercury concentration in urine before and after chelation could be made on an individual basis. [Hg^{2+}] in urine with and without chelation showed a significant regression ($y = 0.927x + 0.300$, $p < 0.0001$); however, the correlation ($r^2 = 0.535$) was lower than for regressions using average [Hg^{2+}] in urine. The [$MeHg^+$] in urine responded similarly with a significant regression ($y = 0.971x + 0.411$, $p < 0.0001$) and a lower correlation ($r^2 = 0.501$). When data were pooled by treatment group, post-DMPS chelated urine versus post saline treatment urine data demonstrated strong correlation, and significant regression ($p < 0.0001$) for $MeHg^+$ and Hg^{2+} ($y = 1.1602x - 0.112$, $r^2 = 0.8954$; and $y = 1.112x - 0.0638$, $r^2 = 0.9133$, respectively).

Regressions of mercury in tissues from DMPS treated voles versus saline treated voles produced slopes that ranged from 0.867 to 1.02 demonstrating the consistency of mercury species in tissues before and after chelation treatment. For each tissue evaluated, mercury in urine as a function of mercury in tissue (Cobb et al. 2007) produced highly significant regressions ($p < 0.001$). Stronger regressions for mercury concentrations were obtained when DMPS chelation was employed ($0.486 < r^2 < 0.677$) as compared to regressions using data from non-chelated cohort ($0.318 < r^2 < 0.399$).

Chronic exposure of a representative wildlife species to low mercury concentrations (<1 μg/mL) in drinking water allowed the development of a framework for estimating mercury accumulation in tissues by quantifying mercury excretion in urine. Throughout the study, [Hg^{2+}] and [$MeHg^+$] were higher in tissues of exposed voles. The predominant form of mercury found in the liver and brain was $MeHg^+$. In the highest exposure group, DMPS decreased mean total mercury concentrations in liver and kidney, but not in brain. This was observed with a complementary four to fivefold increase in total urinary mercury excretion. Differences between post DMPS total mercury concentrations and non-DMPS total mercury concentrations in soft tissues represented approximately half of the difference observed in

Table 2 *p* values comparing post-chelation mercury concentration in prairie voles as a function of aqueous $MeHg^+$ concentrations

	Dose (d)[a]	Inorganic (μg/mL)			Organic (μg/mL)		
		Week 3	Week 6	Week 12	Week 3	Week 6	Week 12
Kidney[b]	d_C:d_M	**0.017**	**0.004**	**<0.001**	**0.006**	0.100	**<0.001**
	d_C:d_H	**0.002**	**0.002**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	d_L:d_M	0.071	**0.006**	**0.001**	**0.018**	0.083	**<0.001**
	d_L:d_H	**0.003**	**0.001**	**<0.001**	**<0.001**	**0.001**	**<0.001**
	d_M:d_H	**0.002**	**0.006**	**<0.001**	**<0.001**	**0.002**	**<0.001**
Liver[b]	d_C:d_M	**<0.001**	**0.030**	**<0.001**	**0.003**	**0.048**	**0.024**
	d_C:d_H	**<0.001**	**0.001**	**0.008**	**0.001**	**0.013**	**0.009**
	d_L:d_M	**<0.001**	0.066	**<0.001**	**0.006**	0.061	**0.026**
	d_L:d_H	**<0.001**	**0.001**	**0.008**	**0.001**	**0.013**	**0.009**
	d_M:d_H	**<0.001**	**0.005**	**0.014**	**0.002**	**0.019**	**0.008**
Brain	d_C:d_L	0.228	**0.013**	0.293	**0.025**	**0.025**	0.124
	d_C:d_M	0.192	0.057	0.468	**<0.001**	**<0.001**	**<0.001**
	d_C:d_H	**0.001**	**<0.001**	**0.005**	**<0.001**	**<0.001**	**0.009**
	d_L:d_M	**0.019**	0.326	0.987	**<0.001**	**<0.001**	**0.001**
	d_L:d_H	**<0.001**	**<0.001**	**0.002**	**<0.001**	**<0.001**	**0.010**
	d_M:d_H	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.008**
Urine[b]	d_C:d_M	**0.044**	**0.018**	**0.040**	**0.004**	**0.034**	**0.027**
	d_C:d_H	**0.014**	**0.006**	**0.048**	**0.018**	**0.013**	**0.036**
	d_L:d_M	**0.006**	**0.030**	**0.012**	**0.007**	**0.036**	**0.025**
	d_L:d_H	**0.011**	**0.008**	**0.039**	**0.018**	**0.013**	**0.036**
	d_M:d_H	**0.031**	**0.026**	**0.036**	**0.028**	**0.026**	**0.045**

Bold values are statistically significant ($p < 0.05$)

[a] d_C = control, d_L = low dose, d_M = medium dose, d_H = high dose

[b] No differences between control and low exposure for Kidney, Liver, or Urine

Table 3 Linear regression statistics describing mercury species concentrations in urine versus tissues of prairie voles before and after receiving injections of DMPS

Tissue	Organic mercury before chelation[a]			Inorganic mercury before chelation[b]		
	Slope	Intercept	r^2	Slope	Intercept	r^2
Kidney[c]	0.500	0.637	0.342	0.683	0.221	0.399
Liver[c]	0.524	1.222	0.326	0.590	1.494	0.341
Brain[ac]	0.577	0.920	0.359	1.048	0.846	0.318
Tissue	Organic mercury after chelation[a]			Inorganic mercury after chelation[b]		
	Slope	Intercept	r^2	Slope	Intercept	r^2
Kidney[c]	0.825	0.502	0.599	0.824	0.251	0.486
Liver[c]	0.849	1.490	0.604	0.912	1.602	0.539
Brain[c]	0.940	0.832	0.677	1.707	0.428	0.500

[a] n = 12 treatment groups

[b] All regressions were significant with $p < 0.001$

[c] n = 126

urine. This suggests mercury removal from other reservoirs in the body. In our study, DMPS most effectively decreased total body burden of mercury in voles exposed to either 1.0 or 0.1 ng/mL $MeHg^+$.

$MeHg^+$ and Hg^{2+} were found in almost equal portions in both kidney and urine. The predominant form of mercury found in the liver and brain was $MeHg^+$. These results were expected since ingested $MeHg^+$ is more likely to reach the brain or liver before encountering the kidney. A 1:1 ratio of renal $MeHg^+$ and Hg^{2+} mercury can be explained by metabolism in organs before mercury reached the kidney and by urinary clearance through the kidney. The time required to

Table 4 Linear regression statistics describing mercury species concentrations in tissues and urine of prairie voles before and after receiving injections of DMPS

Tissue	Organic mercury			Inorganic mercury		
	Slope	Intercept	r^2	Slope	Intercept	r^2
Kidney[a,b]	0.883	0.136	0.957	1.024	−0.230	0.942
Liver[a,b]	0.932	0.095	0.978	0.899	0.026	0.990
Brain[a,b]	0.997	0.033	0.978	0.867	0.139	0.909
Urine[b,c]	0.971	0.411	0.501	0.927	0.300	0.535

[a] n = 12 treatment groups

[b] All regressions were significant with $p < 0.001$

[c] n = 126

reach the 1:1 ratio occurred sooner and at far lower concentrations than previously reported. Rats exposed to either 5 or 10-ppm $MeHg^+$ in drinking water did not produce this 1:1 ratio until renal mercury concentrations were 60 μg/mL (Woods et al. 1991). Our study used a maximum exposure of 1 μg/mL $MeHg^+$ and achieved a 1:1 ratio before mercury in renal tissues reached 1 μg/g. This suggests demethylation and/or excretion of $MeHg^+$ proceeds even when exposures occur at low concentration.

Neurological damage and dysfunction caused by low [$MeHg^+$] in brain tissue (Mergler et al. 1998), posed concerns regarding whether DMPS redistributes $MeHg^+$ to the brain. Our study showed no increase in [$MeHg^+$] or [Hg^{2+}] within the brain. Also, a slight but insignificant decrease in total mercury concentration was seen in brain tissue.

Urinalysis after chelation has been an effective non-invasive technique for estimating body burdens of a metal (Gonzalez-Ramirez et al. 1995; Kosnett 2010). Previously, we demonstrated that without chelation, strong relationships existed between urinary and organ derived mercury concentrations (Cobb et al. 2007). Our current work demonstrates stronger relationships ($p < 0.001$) for mercury species in tissues and urine following DMPS chelation, thus establishing a bridge from mercury concentrations in non-chelated tissues and post chelation urine. The relationship between the post-chelated urine and tissues is the key to non-lethal and non-invasive estimates of mercury concentrations in soft tissues. These relationships provide a powerful basis for models to estimate mercury concentrations in target tissues and could allow multiple assessments of rodents during long term exposure scenarios, including field studies. This technique could be used with larger wildlife species to better identify hazardous sites. Studies could include mercury accumulation or bioconcentration in predators of small mammals, such as mink, otters, or Florida panthers.

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