

CHALLENGE TESTS FOR MERCURY DOES THE “CHALLENGE TEST” REALLY SHOW YOU THE “BODY BURDEN” OF MERCURY??

For over two decades now, many clinical metals toxicologists have been relying on “challenge tests,” also called provocation tests, to diagnose mercury and other metal toxicities. The diagnostic premise of the testing is that it shows the “body burden” of the individual – that pool of deeply held metals that represents our lifetime accumulation of unexcreted metals.

The literature examining the challenge tests ranges from the years 1991 through 2001 and has thus far failed to find any evidence of the challenge tests revealing any more than recent exposures, and in some instances (Frumkin et al, 2001) failing to see exposures made clear by ambient testing. Recently, challenge tests have come under fire from federal authorities as a diagnostic tool. The problem is not really that the challenge tests have no use (especially in the case of lead, where EDTA challenge testing is documented to have slightly better correlations with bone lead than a do blood lead measurements, or the case of gadolinium where levels in blood and urine are undetectable without EDTA provocation); the problem is instead the way they are generally used and interpreted. There are many practitioners who use the data from challenge tests in scientifically and clinically valid ways, but in general use the challenge test has **three main flaws**:

1. The propagation of the myth of a special relevance of the pool identified by the challenge (i.e. “body burden”) and the yes/no interpretation (i.e. “I found mercury in the patient”)
2. The use of a non challenged reference range to compare the challenged test to; this is probably the biggest problem from a regulatory standpoint since there is such obvious potential for over-treatment
3. The lack of standardization of the challenge conditions
 - a. DMPS has very different strength and

specificity than DMSA

b. IV vs. oral administration has vastly different pharmacokinetics

c. Use of adjuncts such as EDTA, glutathione, and glycine vastly changes the dynamics of the test and its output.

The measurement of mercury in the body and extrapolation to body burden and toxic conditions is a very complicated field, requiring acute clinical discernment, including integration of patient history, current exposures, symptomology, and effect of co-morbidities. The simplification and deification of the challenge test is no longer serving the evolution of the field of clinical metals toxicology, and it is now time for the adoption of better tools. At Quicksilver Scientific, we have develop advanced mercury testing that 1) identifies different sources of mercury by measuring the relative amounts of the two main forms of mercury in the body, methylmercury and inorganic mercury, and 2) quantifies excretion capabilities for those two forms. Unfortunately, instead of being welcomed by the community, there has been quite a bit of angry backlash and accusation, born mostly of a stubborn refusal to move forward. **So to the question, “Does the challenge test really show you ‘body burden’ of mercury?”; let’s see what the scientific literature says...**

Diagnostic Chelation Challenge with DMSA: A Biomarker of Long-Term Mercury Exposure?

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Environmental Health Perspectives • VOLUME 109 | NUMBER 2 | February 2001

Chelation challenge testing has been used to assess the body burden of various metals. The best-known example is EDTA challenge in lead-exposed individuals. This study assessed diagnostic chelation challenge with dimercaptosuccinic acid (DMSA) as a measure of mercury body burden among mercury-exposed workers. Former employees at a chloralkali plant, for whom detailed exposure histories were available ($n = 119$), and unexposed controls ($n = 101$) completed 24-hr urine collections before and after the administration of two doses of DMSA, 10 mg/kg. The urinary response to DMSA was measured as both the absolute change and the relative change in mercury excretion. The average 24-hr mercury excretion was 4.3 $\mu\text{g}/24 \text{ hr}$ before chelation, and 7.8 $\mu\text{g}/24 \text{ hr}$ after chelation. There was no association between past occupational mercury exposure and the urinary excretion of mercury either before or after DMSA administration. There was also no association between urinary mercury excretion and the number of dental amalgam surfaces, in contrast to recent published results. We believe the most likely reason that DMSA chelation challenge failed to reflect past mercury exposure was the elapsed time (several years) since the exposure had ended. These results provide normative values for urinary mercury excretion both before and after DMSA challenge, and suggest that DMSA chelation challenge is not useful as a biomarker of past mercury exposure. **Key words:** biomarkers, chelation, chloralkali, DMSA, environmental diseases, mercury, neurotoxicity, occupational diseases, renal toxicity, succimer.

Environ Health Perspect 109:167–171 (2001). [Online 25 January 2001]
<http://ehpnet1.niehs.nih.gov/docs/2001/109p167-171frumkin/abstract.html>

Table 1. Mercury excretion before and after DMSA chelation.

Values	Exposed ($n = 119$)	Unexposed ($n = 101$)	<i>p</i> -Value for difference
Baseline values			
Urinary Hg concentration, uncorrected ($\mu\text{g Hg/L}$)			
Group mean \pm SD	3.37 \pm 2.51	2.89 \pm 2.18	0.13
95% value	9.0	6.5	
Maximum value	18.2	12.8	
Urinary Hg concentration, corrected ($\mu\text{g Hg/g creatinine}$)			
Group mean \pm SD	2.74 \pm 2.05	2.26 \pm 1.92	0.08
95% value	7.00	5.62	
Maximum value	11.75	11.82	
24-hr Hg excretion ($\mu\text{g}/24 \text{ hr}$)			
Group mean \pm SD	4.61 \pm 3.85	3.94 \pm 3.43	0.17
Maximum value	21.84	22.4	
Postchelation values			
24-hr Hg excretion ($\mu\text{g}/24 \text{ hr}$)			
Group mean \pm SD	7.87 \pm 5.85	7.73 \pm 5.58	0.87
Maximum value	46.81	27.94	
Change in 24-hr Hg excretion (post-DMSA–baseline, $\mu\text{g}/24 \text{ hr}$)			
Group mean \pm SD	3.25 \pm 5.96	3.80 \pm 5.53	0.48
Range	–14.59, 39.66	–10.70, 25.39	
Ratio of post-DMSA Hg excretion to baseline mercury excretion ^a			
Group mean \pm SD	2.40 \pm 2.25	2.77 \pm 2.58	0.27
Range	0.23, 16.66	0.26, 18.29	

^aExcludes one unexposed subject whose baseline Hg excretion was 0.

Studies of DMPS show that there is a difference in urinary excretion between exposed and unexposed groups. In the Frumkin study, there was a difference between in excretion PRIOR to DMSA treatment (*p* values); however following treatment, there was none. Ambient levels were actually a BETTER predictor of past exposure than challenged levels. Also, in this study, there was no signal from amalgam surfaces during DMSA treatment. However, such a signal is clearly evident in studies with DMPS treatment.

We attempted to validate a potential biomarker of long-term occupational mercury exposure, the DMSA chelation challenge response, by studying the association of this biomarker with quantitative estimates of exposure in a cohort of exposed and unexposed individuals. The biomarker could not distinguish exposed and unexposed subjects, and it was not associated with the magnitude of exposure. We conclude that DMSA chelation challenge, according to the protocol described here, is not useful in retrospective exposure assessment among mercury workers.

Discussion: Two main points come out of this study. One is the inability of the challenge test to show historical exposure, and this is with a group that was industrially exposed to extreme levels of mercury. The second point is the inequality of DMSA and DMPS. Though many people know that DMPS is stronger than DMSA, we have seen with mercury speciation analysis that *DMSA biases toward methylmercury and DMPS biases toward inorganic mercury*.

Mobilized mercury in subjects with varying exposure to elemental mercury vapour

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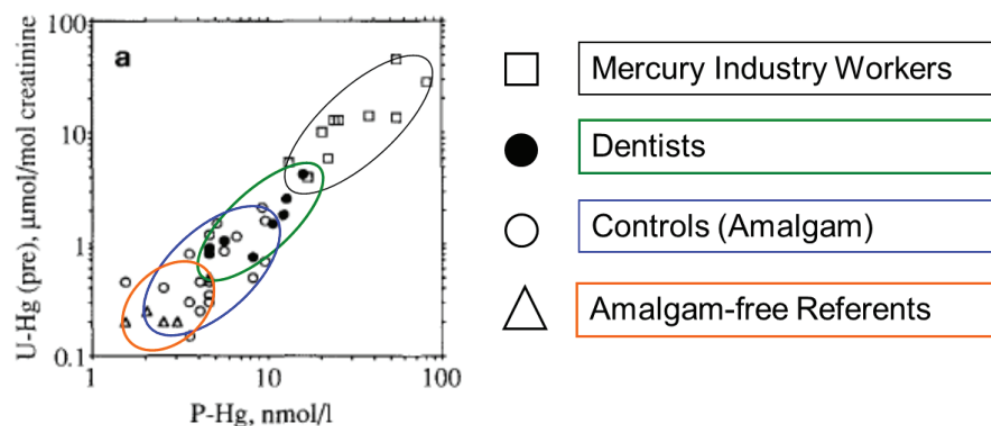
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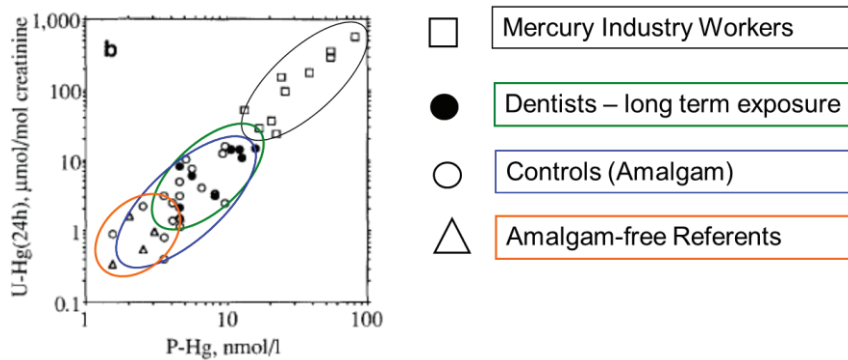
Received April 29, 1990 / Accepted March 21, 1991

Summary. In a mercury mobilization test, 0.3 g of the complexing agent sodium 2,3-dimercaptopropane-1-sulfonate (DMPS) was given orally to 10 workers with moderate occupational exposure to elemental mercury vapour, to 8 dentists with slight exposure, to 18 matched controls, and to 5 referents without amalgam fillings. In the workers, DMPS caused an increase in 24-h urinary mercury excretion by a factor of 10; in the dentists, 5.9; in the controls, 5.3; and in the amalgam-free referents, 3.8. Of the mercury excreted during 24 h, 59% appeared during the first 6 h. Close, albeit non-linear, associations were found between mobilized mercury and the pre-mobilization mercury levels in plasma and urine, but not with the duration of occupational exposure or the rough estimate of the integrated function of blood levels vs time. The present data indicate that mercury mobilized after a single DMPS dose in close connection with exposure is mainly an index of recent exposure and is not significantly affected by slow body pools or long-term exposure.

This DMPS study aimed to show long-term body burden in older dentists versus acute exposure in short-term factory workers. The test aimed to show long-term accumulation in dentists versus short-term acute exposure in industrial workers. The challenge test failed to show a different pattern than the pre-challenged testing of plasma and urine showed – i.e. the DMPS challenge just amplified previously-existing signals.



Above: Linear correlation between pre-challenged urine and pre-challenged plasma



Above: Linear Correlation between 300mg-PO DMPS-challenged urine and pre-challenged urine.

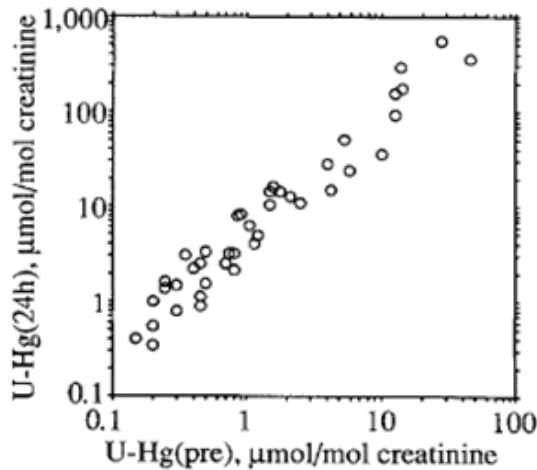


Fig. 2. The correlation between urinary mercury excretion during 24 h after [U-Hg(24h)] and before [U-Hg(pre)] ingestion of DMPS as calculated logarithmically. Correlation coefficient for the logarithmic values: $r = 0.97$

The mercury excretion provoked by DMPS intake was well associated with the pre-DMPS mercury levels in plasma and urine. As the latter are mainly indices of mercury exposure during previous weeks of months, the same should hold true for excretion induced by DMPS. Our hypothesis was that the body burden and, thus, the long-term exposure would be reflected by the DMPS-mobilization test. However, this hypothesis was not supported by our data.

Discussion: Clearly DMPS is very effective in mobilizing inorganic mercury, but the mobilization merely amplified a signal that existed in the ambient data. No “body burden” was revealed.

Urinary excretion of mercury after occupational exposure to mercury vapour and influence of the chelating agent meso-2,3-dimercaptosuccinic acid (DMSA)

Table 1 Exposure to mercury in different study groups before DMSA experiment

	Control (n = 16) Mean (SEM)	Removed from exposure (n = 11; battery plant) Mean (SEM)	Currently exposed* (n = 16; chloralkali plant) Mean (SEM)
Years of exposure to Hg vapour	—	3.5 (0.5) (1.0–6.5)†	7.0 (1.1) (2.3–15)
Years of removal from exposure	—	4.5 (0.6) (2.4–9.4)	—
Hg air (µg/m ³)	—	—	110† (18) (9–308)
HgB (µg/l)	1.6 (0.3) (1.0–6.5)	2.8 § (0.3) (1.2–4.3)	25.6 (3.5) (8.3–51.4)
HgU (µg/g creatinine)	2.1 (0.2) (1.4–3.7)	6.9§ (1.1) (3.0–13.3)	119 (10.1) (49–200)

Both blood and non-provoked urine show the differences in the populations, even three years after removal from the source. Pre- and Post-challenged urines were very well correlated.

Table 2 Concentrations of mercury in 24 hour urine samples before and after a single oral administration of 2 g DMSA in groups of workers differently exposed to mercury vapour

HgU (µg Hg/24 h)	Control group (n = 16)	Alkaline battery plant (removed from exposure) (n = 11)	Chloralkali plant (currently exposed)*		
			Before reduction of exposure (n = 16)	After reduction of exposure	
				Before holiday (n = 16)	After holiday (n = 16)
Before DMSA:					
Mean	4.1	10.4	184	78	66
SEM	0.3	1.5	15	8	6
Range	2.1–5.3	4.3–19.1	93–293	24–136	24–134
After DMSA:†					
Mean	8.3	31.1	793	257	174
SEM	0.4	5.2	66	23	20
Range	5.3–10.8	13.4–66.1	416–1269	106–459	49–324

SEM = Standard error of the mean.

*The same subjects were examined at three different occasions (for details, see Subjects and methods).

†Significant increases of the urinary mercury levels after DMSA administration in all the groups (paired *t* test; *p* < 0.001).

the renal markers in the different groups. The relation between the amount of mercury in the 24 hour urine specimens before (x axis) and after (y axis) administration of DMSA was examined. Table 3 shows that both parameters were highly associated in all the groups. In the chloralkali workers currently

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Diagnostic Value of a Chelating Agent in Patients with Symptoms Allegedly Caused by Amalgam Fillings

J Dent Res 79(3): 868-874, 2000

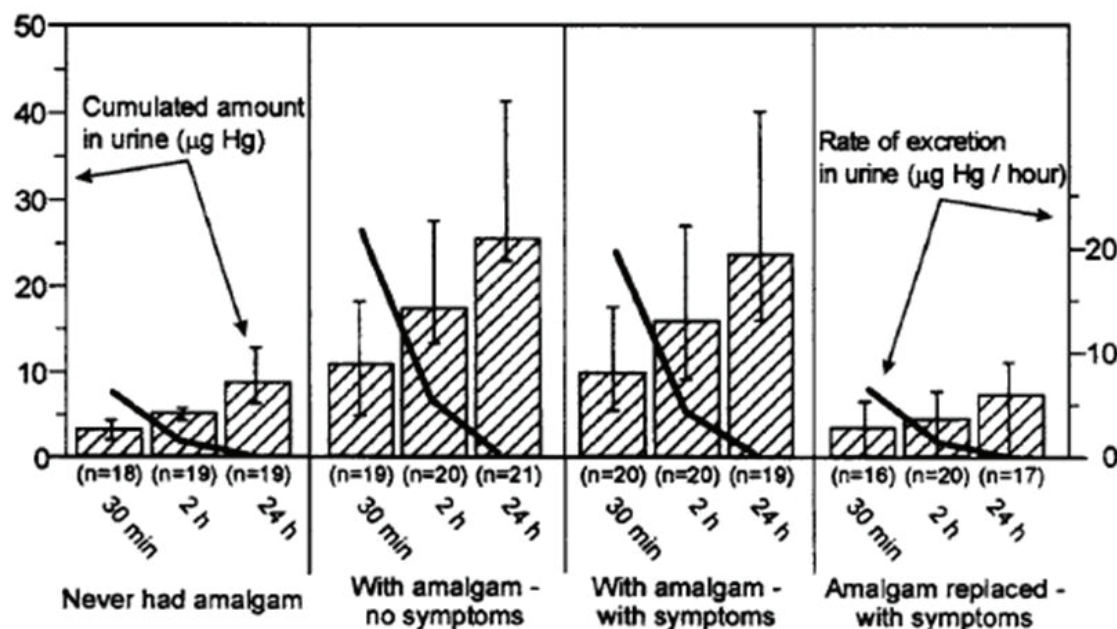


Figure 2. Cumulative amount (bars) and rate of excretion (curves) of Hg (µg) in urine after DMPS injection. Median values and quartiles (vertical lines).

Stenman and Grans, 1997). In Dauderer's intravenous test, the excretion of mercury in urine (first urination without time schedule) after injection of DMPS (4 mg/kg body weight) is measured. According to this test, a mercury level above 50 µg/L indicates the necessity of amalgam removal (Dauderer, 1989). There are several factors to consider regarding the DMPS test; however, reproducible and comparable results can be obtained only through standardized procedures (Aposhian *et al.*, 1995).

Discussion: DMPS challenge certainly shows recent loading, but fails to show difference between people who never had amalgam and people who formerly had amalgam and people who never had amalgam, thus failing to show historical exposure. A closer analysis of rate of excretion during chelation shows the difference between amalgam-free and amalgam removed, but this would not be obvious upon clinical observation.