

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

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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  $\mu\text{g}/24$  hr before chelation, and 7.8  $\mu\text{g}/24$  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>

Assessment of biological exposure is a key challenge in evaluating metal toxicity, for both clinicians and epidemiologists. Blood and urine measurements traditionally have been used, but these have several shortcomings, such as failure to reflect true body burden, failure to correlate with biological effects, high interperson variability following similar exposures, and relatively rapid clearance (1). X-ray fluorescence is being used increasingly to assess exposure to lead but not to other metals (2–5).

Because chelating agents bind metals and promote their urinary excretion, theoretically they can be used in challenge tests to assess metal levels. The rationale for diagnostic chelation challenge is straightforward: If a person has an elevated body burden of a metal, then administration of a chelating agent should cause a short-term increase in the urinary excretion of that metal. The most commonly used chelation challenge test has been EDTA administration following lead exposure (6,7), although British Anti-Lewisite and penicillamine have also been used (8). More recently, attention has focused on dimercaptosuccinic acid (DMSA), or succimer, a chelating agent approved by the U.S. Food and Drug Administration (U.S. FDA) in 1991 for the treatment of pediatric lead toxicity.

DMSA is used primarily in the treatment of metal toxicity, rather than in diagnosis. The most common therapeutic use has been in treating lead toxicity (9–11), but DMSA has also been used to treat a variety of other metal overexposures (12–14). Besides its treatment role, DMSA offers considerable diagnostic potential as a chelation challenge agent. First, it is convenient: DMSA is an oral agent, whereas EDTA must be administered parenterally. Second, DMSA has an excellent safety profile. Third, DMSA has been shown to mobilize a range of metals effectively in both animals and humans. Fourth, DMSA acts quickly. The blood concentration of DMSA peaks in 3 hr, and the half-life is 3.2 hr (15). DMSA-induced excretion of both lead (16) and mercury (17) peaks within 2 hr. In the clinical setting, chelation challenge would therefore require urinary collection only over several hours. For these reasons, DMSA chelation challenge could be a convenient, safe approach to assessing the biological burden of various metals. Indeed, DMSA chelation challenge has been used in several studies (16,18,19) and in clinical settings to assess lead burden.

Another metal that might be assessed in this way is mercury. DMSA mobilizes mercury effectively in both animals (20–25) and in humans (8,17,26–31). However, unlike lead, mercury undergoes relatively little

bioaccumulation. It is excreted with a half-life of 1–2 months (17,32–35). This suggests that the primary use of DMSA chelation challenge for mercury would occur in the first weeks after exposure. However, a long terminal elimination phase has been described (36), with mercury retention in nervous system, kidneys, and other soft tissues. Consequently, there could also be a role for DMSA chelation challenge some time after mercury exposure, especially if exposure had been prolonged and intense. Support for this notion comes from animal evidence (37) that DMSA draws mercury with special avidity from the kidneys—an important mercury storage site known to have a relatively slow turnover (38). Indeed, DMSA chelation challenge has been used clinically on a limited basis following mercury exposure (15,26,39). A related agent used in Europe, 2,3-dimercaptopropane-1-sulfonic acid (DMPS), has been used in a similar manner (40,41).

At present the interpretation of DMSA challenge tests for mercury is difficult because we lack reliable data on the normal range of mercury excretion in unexposed people following DMSA, the expected range of elevations following mercury exposure, the correlation between DMSA response and other measures of mercury exposure, and the clinical significance of elevations. Such data would be necessary to validate the DMSA chelation challenge response as a practical, informative biomarker of mercury exposure.

In this paper we report a study of DMSA chelation challenge testing among workers with long-term, high-level exposure to mercury in a chloralkali plant and among a comparison population of unexposed workers.

### Methods

**Study subjects.** This study was conducted as part of a larger study of the health effects of

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mercury among former employees of a chloralkali plant in Brunswick, Georgia (42). The plant had operated from 1956 to 1994. We identified 221 former employees who had worked in the plant for at least a year, who were still alive at the time of the study in 1998, and who could be contacted. We also identified a large pool of unexposed persons who worked for three local employers: a local government, a quasi-governmental tourist authority, and a paper company. Individuals from this pool were selected according to a scheme that matched their age-race-sex distribution to that of the exposed subjects, and were invited to participate in the study. Participation consisted of completing a detailed questionnaire, physical examination, neurological and neurobehavioral testing, and blood and urine testing, in addition to the portions of the study specifically related to the chelation challenge. These elements of the study are reported in detail in the companion paper (42). Of note, the questionnaire and physical examination were designed to assess several sources of exposure to mercury. The questionnaire asked about other occupational sources of mercury exposure and about dietary sources, including fish. The physical examination included a count of the number of tooth surfaces with mercury amalgam fillings.

We performed an extensive exposure assessment as part of the larger study. Using personnel records, we recorded each former employee's job history within the plant. We also identified the air mercury exposure levels at each part of the plant, for each job title, for each year of the plant's operation. These estimates, generated from historical air sampling data, were validated by comparison with available urinary mercury sampling and with modeled air levels based on mercury throughput data and room air change parameters (43). We then created a job-exposure-year matrix and reconstructed an exposure profile for each former employee. We used three metrics of exposure: average exposure (in micrograms per cubic meter), cumulative exposure (in micrograms per cubic meter per year), and peak exposure (in micrograms per cubic meter). Mercury exposure had been high in the cell room and in other parts of the plant, with air levels averaging above  $100 \mu\text{g}/\text{m}^3$  for some employees (43), comparable to the exposures reported from contemporary chloralkali plants (44–46).

**Sample collection and analysis.** Each subject collected a baseline 24-hr urine sample. Approximately 2 weeks later the subjects returned for a second test session. At that time we administered two doses of DMSA, 10 mg/kg, at 8-hr intervals. Each subject commenced a second 24-hr urine collection

at the time of the first dose. Both urine collections, the baseline and the post-DMSA, used plastic containers provided by the Centers for Disease Control and Prevention (CDC; Atlanta, GA); all lots were tested to confirm that they were metal-free.

Both the baseline and the post-DMSA urine samples were kept refrigerated during the collection and were returned on the day the collection was completed (or, in rare cases, on the following day). We measured the volume of each 24-hr collection and then decanted approximately 50 mL into metal-free plastic specimen containers for transfer to the laboratory. The specimens were kept refrigerated throughout. If a 24-hr urine collection had a volume  $< 500$  mL or a total creatinine  $< 700$  mg it was considered incomplete and was excluded.

On each sample we measured the creatinine and the mercury levels. All measurements were performed at the laboratories of the CDC National Center for Environmental Health in Atlanta. We measured mercury in undigested urine by cold vapor atomic absorption analysis based on the method of Littlejohn et al. (47) using modified reagents as described by Greenwood et al. (48). Standard quality-assurance procedures, including replicate testing and the use of blanks and standards, were followed. Urinary mercury concentration was standardized to creatinine concentration and expressed in units of milligrams per gram creatinine.

**Data analysis.** We considered three metrics of urinary mercury response to DMSA: the absolute amount of mercury excreted in response to DMSA (in micrograms per 24 hr), the change in mercury excreted following DMSA (postchelation mercury excretion minus baseline mercury excretion, in micrograms per 24 hr, henceforth referred to as difference), and the ratio of the mercury output in the second collection to the mercury output in the first collection, henceforth referred to as the ratio.

We characterized the distribution of each variable among the exposed and the unexposed, and the entire study group. We then undertook four analyses to assess the association between mercury exposure and chelation response.

First, we examined the correlation of exposure ranks and chelation challenge response ranks. We reasoned that if exposure were associated with chelation challenge response, the subjects exposed most heavily would have some of the highest chelation challenge response ranks and, similarly, that those with the most active response to chelation challenge would have some of the highest exposure ranks. We therefore identified the 15 most heavily exposed former workers, according to each

of the three exposure metrics we used (average, cumulative, and peak exposure), arrayed them according to their exposure ranks, and observed their chelation challenge response ranks. Conversely, we identified the 15 most active responders to chelation challenge, in terms of both difference and ratio, arrayed them according to their chelation challenge response ranks, and observed their exposure ranks.

Second, in a more formal assessment of this correlation, we calculated the Spearman rank-order correlation coefficients for the association between exposure and chelation challenge response, using ranks. We selected this nonparametric test because not all variables were normally distributed. We repeated this analysis for three metrics of exposure—cumulative, mean, and peak—and for two metrics of chelation challenge response—difference and ratio—producing six correlation coefficients.

Third, in an extension of this approach, we carried out multiple linear regression, with occupational mercury exposure and number of dental amalgam surfaces as independent variables, and chelation challenge response as the dependent variable. In this analysis, the occupational mercury exposure was set at zero for all unexposed subjects. To satisfy the linear regression assumption that the residuals follow a normal distribution, both metrics of chelation challenge response—difference and ratio—were transformed. Van der Waerden's transformation into the normalized rank was applied to the difference, and the ratio was transformed using the natural logarithm. This regression was run on the combined group of exposed and unexposed subjects, and on the exposed and unexposed subsets separately.

Finally, because a possible association might be apparent only in subjects with relatively recent exposure, we repeated all analyses, restricting them to those former employees whose employment had lasted into the five years before our testing.

## Results

Of the 221 eligible former employees of the chloralkali plant, 156 participated completely or partly in the study. There were nine subjects with diabetes, one with renal failure, and 27 who did not provide two usable 24-hr urine collections or who had missing data, leaving 119 exposed subjects. Of the 190 unexposed subjects invited to participate based on the age-race-sex distribution of the former employees, 138 participated. There were two unexposed subjects with diabetes, and 35 who did not provide two usable 24-hr urine collections or who had missing data, leaving 101 unexposed subjects. The results are based on data from these two groups.

The exposed workers who participated in the chelation study had an exposure profile virtually identical to that of the larger population of exposed workers, as reported elsewhere (42). The mean duration of exposure was 7.0 years, and the mean time since last exposure was 6.1 years. The mean workplace mercury exposure level was 33.8  $\mu\text{g}/\text{m}^3$ , the mean of the peak exposure levels was 71.9  $\mu\text{g}/\text{m}^3$ , and the mean cumulative exposure was 236.8  $\mu\text{g}/\text{m}^3$ -years.

Table 1 shows data on urinary mercury for the exposed and unexposed groups. Although the exposed workers tend to have greater mercury excretion than the unexposed workers, the differences do not reach statistical significance. Among the exposed workers, only one subject had a relatively high postchelation urinary mercury output; otherwise the distributions of the exposed and unexposed subjects were nearly identical.

Tables 2 and 3 show the results of the rank correlation analysis. These tables show data only for the exposed subjects, each of whom was ranked on several metrics of exposure and on the urinary mercury response to chelation. Table 2 shows the 15 highest-ranking subjects in terms of exposure (expressed in three ways: cumulative, mean, and peak exposure), with their respective ranks on chelation challenge response (expressed in two ways: as post/pre-chelation absolute difference and as the post:pre ratio). Table 3 shows the reverse: the 15 highest-ranking postchelation mercury excretors (expressed in two ways), with their respective exposure ranks (expressed in three ways). Because there are three exposure metrics and two metrics of chelation challenge response, each panel of the table shows six comparisons. In each case, visual inspection reveals that many of the highest-scoring subjects for one parameter had low scores on the other parameter.

The analysis whose results appear in Tables 2 and 3 was limited to exposed subjects, since only they were eligible to be ranked on both exposure and chelation challenge response. However, we also constructed an alternative version of Table 3 that included unexposed subjects (data not shown). Of the top-scoring subjects in terms of chelation challenge response, eight (using the difference score) or nine (using the ratio score) were unexposed. Thus, more than half of the most active responders to chelation challenge had no history of occupational mercury exposure.

Table 4 shows the Spearman rank-order correlation coefficients for the associations between exposure and chelation challenge response. Two of the results—for the difference scores correlated with average and peak exposure—reached marginal statistical

**Table 1.** Mercury excretion before and after DMSA chelation.

Values	Exposed (n = 119)	Unexposed (n = 101)	p-Value for difference
Baseline values			
Urinary Hg concentration, uncorrected ( $\mu\text{g Hg}/\text{L}$ )			
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 ( $\mu\text{g Hg}/\text{g creatinine}$ )			
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 ( $\mu\text{g}/24$ 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 ( $\mu\text{g}/24$ 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, $\mu\text{g}/24$ 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 <sup>a</sup>			
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	

<sup>a</sup>Excludes one unexposed subject whose baseline Hg excretion was 0.

**Table 2.** Association of exposure ranks and chelation challenge response ranks among the most heavily exposed subjects.

Exposure rank	Cumulative exposure		Mean exposure		Peak exposure	
	Difference	Ratio	Difference	Ratio	Difference	Ratio
1	12	11	1	6	80	86
2	15	8	56	49	4	3
3	80	86	71	64	15	8
4	36	15	109	114	74	81
5	74	81	88	59	22	43
6	19	33	23	29	19	33
7	96	91	14	19	12	11
8	16	37	69	85	96	91
9	70	74	35	34	38	57
10	111	108	39	70	73	83
11	5	16	103	100	69	85
12	54	7	31	39	20	4
13	4	3	19	33	14	19
14	81	89	38	57	39	70
15	75	56	93	96	28	25

n = 119; ranks can range between 1 and 119.

**Table 3.** Association of exposure ranks and chelation challenge response ranks among subjects with the highest chelation challenge ranks.

Chelation challenge rank	Difference			Ratio		
	Cumulative	Mean	Peak	Cumulative	Mean	Peak
1	99	1.0	26.0	59	88.0	71.5
2	59	88.0	71.5	48	101.0	103.0
3	32	87.0	78.5	13	27.0	4.5
4	13	27.0	4.5	66	30.0	11.5
5	11	55.0	18.5	69	38.0	53.5
6	94	66.0	103.0	99	1.0	26.0
7	100	89.5	94.0	12	20.5	18.5
8	48	101.0	103.0	2	49.0	4.5
9	69	38.0	53.5	100	89.5	94.0
10	73	108.0	97.0	31	36.0	46.5
11	27	93.0	93.0	1	24.0	4.5
12	1	24.0	4.5	19	58.0	51.0
13	74	48.0	86.0	104	40.0	63.5
14	29	7.0	11.5	94	66.0	103.0
15	2	49.0	4.5	4	47.0	46.5

n = 119; ranks can range between 1 and 119.

significance ( $p = 0.05$  and  $0.04$ , respectively). However, multiple comparisons were made, and a Bonferroni correction procedure would reduce the statistical significance of these two results.

The multiple linear regression analysis, including both exposure scores and dental amalgam surfaces as independent variables, showed no significant associations between any measure of exposure and chelation challenge response (Table 5). There was a significant association between the number of dental amalgam surfaces and the chelation challenge when the two groups, exposed and unexposed, were analyzed together. However, this association went in the unexpected direction: More amalgam surfaces were associated with a lower post/pre ratio. When exposed and unexposed subjects were analyzed separately, the number of amalgam surfaces was not significantly associated with the chelation challenge response (data not shown).

Finally, when we repeated the analyses including only the 88 former workers who had been employed within the 5 years before the study, we found no significant associations between any measure of occupational mercury exposure and any measure of chelation challenge response. In particular, the two borderline statistically significant findings shown in Table 2 became nonsignificant ( $p = 0.61$  and  $0.42$ , respectively). In addition, the significant (if unexpected) associations shown in Table 5 between dental amalgam surfaces and chelation challenge response became nonsignificant.

## Discussion

Our study hypothesis was that DMSA chelation challenge might indicate the body

**Table 4.** Correlation coefficients for the association between exposure and chelation challenge response.

	Difference		Ratio	
	CC	$p$ -Value	CC	$p$ -Value
Cumulative	0.128	0.17	0.100	0.28
Mean	0.179	0.05	0.135	0.14
Peak	0.190	0.04	0.127	0.17

CC, correlation coefficient.

**Table 5.** Occupational Hg exposure and dental amalgam surfaces as predictors of chelation challenge response: results of linear regression.

	Hg exposure		Dental amalgam surfaces	
	Coefficient	$p$ -Value	Coefficient	$p$ -Value
Difference in Hg excretion after and before chelation				
Cumulative	0.0002	0.67	-0.0098	0.36
Mean	0.0022	0.45	-0.0095	0.37
Maximum	0.0010	0.51	-0.0096	0.37
Ratio of Hg excretion after to before chelation				
Cumulative	0.0001	0.83	-0.0182	0.03
Mean	0.0003	0.88	-0.0184	0.03
Maximum	0.0001	0.94	-0.0184	0.03

burden of mercury in a population with chronic occupational mercury exposure, tested several years after the end of exposure. The results do not support this hypothesis, and suggest that DMSA chelation challenge is not useful in quantifying past mercury exposure.

Our results do provide useful normative data on urinary mercury levels, both pre- and postchelation. Subjects in this study excreted an average of approximately  $4 \mu\text{g}$  of mercury in 24 hr before the administration of DMSA, a quantity that roughly doubled following two doses of DMSA (Table 1). These results did not vary with past occupational exposure status. Given our observed standard deviations (SDs), and assuming a normal range that extends to 2 SDs above the mean, the normal upper limit of 24-hr urinary mercury excretion would be approximately  $12 \mu\text{g}$  without chelation treatment, and  $20 \mu\text{g}$  after two doses of DMSA. We believe these are the first population data published on the mercury response to DMSA chelation challenge.

It is possible that our negative findings were due to misclassification of past exposures. However, we believe that such error is unlikely to explain our results; our exposure assessment was based on a large body of direct measurements, verified by internal validation procedures, and consistent with other studies of mercury levels in chloralkali plants (43). Moreover, exposure misclassification would not account for the fact that our exposed and unexposed subjects had similar profiles of urinary mercury excretion.

It is also possible that our urinary collection procedure—specifically, collecting urine for 24 hr rather than a shorter interval—accounted for the negative findings. Other studies have collected urine for shorter intervals, in the range of 8 hr, based on the rapid action of DMSA in effecting mercury excretion [e.g., Aposhian et al. (49)]. A longer collection may have diluted our results by diluting the mercury in our specimens, causing the results for the highly exposed and the unexposed to converge. However, given the uniformly low levels of urinary mercury

among both exposed and unexposed individuals, we believe it is unlikely that a shorter collection period would have altered our findings substantially.

We believe that the most likely cause of the inability of DMSA chelation challenge to quantify past mercury exposures was the elapsed time between the exposures and the testing. As discussed above, most mercury is cleared within 1–2 months, apparently to levels too low to be assayed by DMSA challenge. Other approaches to retrospective exposure assessment will be required in future studies of mercury epidemiology.

We also found that the response to DMSA chelation challenge did not increase with the number of mercury amalgam filling surfaces. Prior evidence suggests that dental amalgam fillings do cause systemic mercury absorption (50–52). In addition, at least two studies have reported an association between dental amalgam fillings and mercury excretion following chelation with either DMPS (49) or DMSA (29). The negative finding in our study may indicate that dental amalgam fillings do not create enough systemic mercury absorption to be detected by DMSA chelation with the protocol we used. It is also possible that our assessment of dental amalgam (counting surfaces rather than measuring surface area) or our challenge protocol (the DMSA dose we used and/or the timing of our collection) limited our ability to detect a true association.

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.

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