Clinical Evaluation of a Lead Mobilization Test Using the Chelating Agent Dimercaptosuccinic Acid

Perrine Hoet, Jean-Pierre Buchet, Laurence Decerf, Benoît Lavallée, Vincent Haufroid, and Dominique Lison

Background: The lead mobilization test reflects the mobilizable and likely toxicologically active fraction of the lead body burden. We propose a safe and convenient protocol for this test, to assess concomitant copper and zinc excretion and to determine the size of the chelatable lead pool in nonoccupationally exposed adults.

Methods: The study population included 80 white adults: 40 controls [median blood lead concentration (PbB), 25 µg/L] and 40 lead-exposed individuals (315 µg/L). After collection of 4- and 24-h baseline urine specimens and a blood sample, dimercaptosuccinic acid (DMSA) was administered orally (1 g), and additional 4- and 24-h urine specimens were obtained. Determinants of the chelatable urinary lead (DMSA-PbU) were traced by linear regression analysis.

Results: Urinary DMSA and lead excretion peaked within 2–3 h after DMSA administration. The amounts of DMSA, lead, copper, and zinc recovered in the 4-h urinary collections were highly correlated with those in 24-h collections (r = 0.857, 0.859, 0.958, and 0.757, respectively). At PbB concentrations >300 µg/L, the relationship between DMSA-PbU and PbB showed a steep increase and a widespread dispersion of DMSA-PbU around the regression line. After DMSA, copper and zinc excretion rates were increased up to 91- and 33-fold, respectively. No side effects were reported after DMSA.

Conclusions: Determination of DMSA-PbU in a 4-h collection after DMSA is convenient, apparently safe, and inexpensive. An upper reference limit value of 22 µg/4 h is proposed for Belgian reference individuals. The diagnostic value of DMSA-PbU is likely to be contributive for PbB >300 µg/L.

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Lead, one of the oldest known metals, is also one of the most widespread toxicants, and lead poisoning remains a health threat (1–5). Lead adversely affects many organs and systems, particularly the kidneys, blood, and reproductive and nervous systems, with the greatest sensitivity to neurotoxicity occurring during the fetal period and childhood. In 2004, an International Agency for Research on Cancer (IARC) group reached the conclusion that inorganic lead compounds are probably carcinogenic to humans (group 2A) (6). A threshold below which lead has no adverse effects has not been determined.

The lead concentration in whole blood (PbB) is the most widely used biomarker of lead exposure and is considered the best indicator of recent exposure (7–10). PbB is limited, however, because (a) a single PbB determination cannot distinguish between chronic and acute exposure because of the long half-life of lead and its constant mobilization from endogenous pools; (b) the relationship between PbB and lead exposure is nonlinear, the lead body burden increasing continuously with further exposure after PbB plateaus; and (c) there are nonlinear relationships between PbB and the toxic effects of lead, and there is a wide variation in sensitivity among individuals.

Nonstandard abbreviations: PbB, lead concentration in whole blood; LMT, lead mobilization test; PbU, lead concentration in urine; BW, body weight; CaNa₂EDTA, calcium disodium EDTA; DMSA, dimercaptosuccinic acid; CuU, copper concentration in urine; ZnU, zinc concentration in urine; DMSA-U, dimercaptosuccinic acid concentration in urine; and PbP, lead concentration in plasma.
More than 90% of the total lead body burden is stored in bones, and x-ray fluorescence measurements of bone lead reflect total body burden. Lead in bone may cause toxicity even after exposure has ceased, but because much of it is biologically inactive and x-ray fluorescence is expensive and not widely available, this method is not promising for routine evaluation.

Lead mobilization tests (LMTs; challenge/provocative/provocation test) measure the urinary lead concentration (PbU) after a single administration of a chelating agent and provide an evaluation of the more readily mobilizable pool (7,10–13). Thus, LMTs might reflect the toxicologically active fraction of the total lead body burden and be of more clinical relevance than PbB or lead in bones in assessing imminent health risks (7,14–17). In individuals with previous lead exposure but low recent exposure, PbB may be only slightly increased whereas the mobilizable pool may be significantly increased (17–19). LMTs are useful aids for deciding whether a worker can resume lead-exposed work after a period of removal from exposure because of high lead absorption or, after past exposure, to determine whether a chelation therapy should be initiated or to determine whether clinical manifestations of uncertain etiology are attributable to lead, in particular when PbB is not or is only slightly increased (18,20–24).

In children, the mobilization protocol is relatively well standardized, but in adults several protocols have been used, the one generally adopted being based on slow intravenous administration of 25 mg/kg of body weight (BW) or 1–2 g of calcium disodium EDTA (CaNa₂EDTA) diluted in 250–500 mL of 50 g/L dextrose or normal saline. The patient’s urine is then collected for the next 24 h (or possibly 6–8 h) (16,19,25,26). Drawbacks of this test include (a) side effects and the potential toxicity of CaNa₂EDTA even in the context of an LMT, (b) the long duration of urine collection and the difficulty of obtaining complete urine samples, and (c) the requirement of parenteral administration and hospitalization. Moreover, there is concern about possible redistribution of internal lead stores in target organs (27). In 1991, the Centers for Disease Control and Prevention (28) recommended that LMTs be used to determine whether chelation is indicated for children with a PbB of 250–440 μg/L, but because of the difficulty and costs of performing the CaNa₂EDTA LMT and the potential for increasing lead toxicity by use of CaNa₂EDTA alone, the American Academy of Pediatrics has considered this test obsolete since 1995 (29).

Dimercaptosuccinic acid (DMSA; Chemet®, Succimer®, Succicaptal®, a water-soluble derivative of dimercapto-1-propanol (British Anti Lewisite), was cleared by the US Food and Drug Administration in 1991 for the treatment of children with PbB >450 μg/L. DMSA appears to have several major advantages over CaNa₂EDTA (12,16,30–33): it can be administered orally, it is less acutely toxic and has a large therapeutic index, and it does not appear to redistribute lead to the brain or to induce significant elimination of essential trace elements. In addition to its role in the treatment of metal intoxication, DMSA offers considerable diagnostic potential for LMTs. Experience is limited, however (12,34,35); standard protocols are not in place and there is a need to define what constitutes significant chelation-induced lead diuresis after DMSA. The main purpose of the present study was to propose a protocol for an LMT that would be safe and easy to perform in ambulatory conditions. It was also important to verify the lack of increased excretion of essential trace elements, such as copper and zinc, and to determine the size of the chelatable pool of lead in nonoccupationally exposed adults.

Materials and Methods

STUDY GROUPS AND DESIGN OF THE STUDY

The total study included 80 white adults [mean (range) age, 42 (22–67) years; 31 women, 49 men]: 40 controls (17 females, 23 males), 37 individuals occupationally exposed to lead, 2 exposed via indoor shooting range practice, and 1 exposed via lead-contaminated water. At the outset, 44 controls were contacted. One woman was excluded because she was pregnant and 1 man because of severe allergic skin problem; 2 candidates refused to participate because of personal reasons. The lead workers comprised 17 individuals (12 females, 5 males) with long-term exposure to low lead concentrations from an enameling plant and 20 with various degrees of lead exposure (2 females, 18 males) recruited from the industrial toxicology outpatient clinic (exposure situations included maintenance of high-tension pylons, secondary smelting, and stained-glass window making).

Part A. Of the 40 controls, 5 individuals participated in a preliminary study aimed at investigating the urinary excretion pattern of DMSA, lead, copper, and zinc and determining the most appropriate urine collection period for the DMSA LMT. Participants provided a baseline 24-h urine collection divided into 2 consecutive sampling periods of 20 and 4 h. At the end of this urine collection, venous blood was taken from the cubital vein for the determination of PbB, and one 1-g dose of DMSA was administered orally (five 200-mg capsules of Succicaptal; SERB Laboratories). This sequence was followed by a 24-h urine collection fractionated at 2, 3, 4, 6, 9, 12, and 24 h. Two participants provided an additional 24-h urine collection (i.e., 24–48 h after DMSA administration).

Part B. For 8 other controls, after a baseline 24-h urine collection divided into 2 consecutive sampling periods of 20 and 4 h, a blood sample was taken and 1 g of DMSA was administered orally. An additional 24-h urine collection fractionated at 4 and 24 h was obtained.

Part C. On the basis of the results of the above studies, subsequent urine collections consisted of a 4-h baseline urine sample (before DMSA) and a 4-h urine collection...
after DMSA administration for 27 other controls and 27 lead-exposed individuals. For 13 additional lead workers, it was possible to obtain only a baseline spot urine sample. For all participants, PbB was measured before DMSA administration (for a description of the experimental design in the different groups, see Fig. 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol52/issue1/).

Precautions were taken to avoid contamination of the urine, particularly among lead workers. The plastic containers used for urine samples were washed with nitric acid and tested to confirm that they did not release metals. The urine specimens were kept refrigerated until analyzed. Because the main objective of this study was to propose a practical and well-accepted protocol, the participants did not fast before DMSA administration. Participants were encouraged to drink water regularly and were warned about the probable mercaptan smell of their urine after DMSA intake. DMSA was well tolerated by most participants, but 2 complained of mild gastrointestinal symptoms. The serum activity of hepatic aminotransferases, measured in 15 individuals, did not show any increase 24 h after DMSA compared with before DMSA administration (data not shown).

Participation in the study was on a voluntary basis; all participants were informed about the objectives of the study and provided written informed consent. The study protocol was approved by the Board of Medical Ethics of the Université Catholique de Louvain.

Laboratory Analyses
All laboratory analyses were performed at the Industrial Toxicology and Occupational Medicine Unit (Université Catholique de Louvain). The laboratory has participated for more than 20 years in external quality assessment schemes organized by the German Society of Occupational and Environmental Medicine (Erlangen, Germany) and by the Centre de Toxicologie du Québec (Québec, Canada) and has shown a high degree of proficiency in metal analysis, particularly lead, copper, and zinc.

All measurements were done in duplicate. Lead and copper were analyzed by graphite-furnace atomic absorption spectrometry with Zeeman background correction, and zinc by flame atomic absorption spectrometry with deuterium correction. The limits of quantification (mean + 9 SD) were 2 μg/L for PbU and urinary copper (CuU), 20 μg/L for urinary zinc (ZnU), and 10 μg/L for PbB. Urinary creatinine was determined according to the Jaffe picrate method. Urine samples with creatinine concentrations <0.3 g/L or >3.0 g/L were excluded. Total DMSA (free DMSA plus oxidized and/or complexed DMSA) was measured by HPLC after reduction by dithiothreitol and reaction with monobromobimane to form a fluorescent derivative (36). Preliminary investigations performed in our laboratory showed that DMSA was stable in urine for at least 48 h when samples were kept refrigerated at 4 °C. Urine samples that could not be analyzed within 2 days were not tested for DMSA.

Data Analysis and Statistics
The urinary excretion rates measured before DMSA administration are designated baseline-PbU, baseline-CuU, and baseline-ZnU; those after DMSA administrations are designated DMSA-PbU, DMSA-CuU, and DMSA-ZnU.

Several measures of lead, copper, and zinc in urine were considered: the element concentration (μg/L); the amount excreted per gram of creatinine (μg/g creatinine); the absolute amount excreted in timed urine (μg/4 h or μg/24 h); the increment in element excretion after DMSA (postchelation excretion minus baseline excretion, in μg/4 h or μg/24 h); the ratio of chelated to baseline; the amount, increment, and ratio excreted per milligram of DMSA excreted [(μg/mg DMSA) per 4 or 24 h]; and the amount, increment, and ratio excreted per dose of administered DMSA corrected for BW.

Data showing skewed distributions were log-transformed. The Student t-test (2-tailed) for paired data was used to compare measures before and after DMSA administration. An unpaired t-test was used for comparison between controls and lead-exposed participants. Pearson correlation coefficients and simple linear regression were used to test the relationships between the different variables. P values <0.05 were considered significant.

Correlations were not improved by use of unadjusted or adjusted urinary concentrations. Hence, only the results of analyses with the absolute amounts (μg/4 h or μg/24 h before or after DMSA administration) are presented.

Results
Excretion Kinetics
We first examined the kinetics of the urinary excretion of DMSA, lead, zinc, and copper in 5 controls. Urinary DMSA and lead excretion peaked within 2–3 h after DMSA administration. For the 2 individuals who gave an additional 24-h urine collection, no more than 3% of the administered DMSA dose was detected in that sample (24–48 h after DMSA administration). Lead excretion returned progressively to baseline values within 12–24 h. Zinc and copper followed an excretion pattern similar to that of DMSA, but the peak excretion rate occurred somewhat earlier and returned to baseline values within 6–9 h (see Fig. 2 in the online Data Supplement).

We also compared the 24- and 4-h collections before and after DMSA administrations in 8 additional participants. DMSA excretion was quite variable among individuals (see Table 1 in the online Data Supplement). The fraction of DMSA (as total DMSA) recovered in urine was 4%–15% of the administered dose in the first 4-h collection and 10%–27% in the 24-h collection. Approximately 63% (SD, 17%; range, 32%–97%) of the total DMSA excreted in the urine over 24 h was excreted within the first 4 h. The amount of DMSA recovered in the 4-h collection was...
highly correlated with the amount excreted during the 24-h collection \((r = 0.857; P < 0.0001)\). The 4- and 24-h cumulative excretions of lead, copper, and zinc after DMSA administration were also highly correlated \([r = 0.859 \text{ for lead}; r = 0.958 \text{ for copper}; r = 0.757 \text{ for zinc}\) \((P < 0.001 \text{ for all})\).

On the basis of these findings, we decided to use 4-h urine collection periods before and after DMSA administration for further investigations.

**MAIN STUDY**

The characteristics and lead concentrations and excretions rates for the total study population are given in Table 1. The urinary volume outputs before and after DMSA were not statistically different, although there was a trend to a higher output after DMSA administration. The mean DMSA excretion rate was highly variable and slightly but not significantly higher in men \((76.6 \mu g/4 \text{ h}; \text{range}, 7.2–154.3 \mu g/4 \text{ h})\) than in women \((60.83 \mu g/4 \text{ h}; \text{range}, 37.35–124.75 \mu g/4 \text{ h})\) and was significantly lower in the lead-exposed individuals than in the controls. There was no correlation between DMSA excretion and age or BW in the control group, the lead-exposed group, or the total population.

**LEAD CHELATION**

**Controls.** Baseline-PbU and PbB were not correlated in controls. Urinary excretion of lead significantly increased after DMSA administration \((P < 0.0001)\). The 97.5th percentile of DMSA-PbU was 21.8 \mu g/4 \text{ h}. The ratio of DMSA-PbU to baseline-PbU was extremely variable among individuals.

To identify the determinants of DMSA-PbU \((\mu g/4 \text{ h})\), we examined the correlations of this variable with urinary DMSA \((\mu g/4 \text{ h})\), baseline-PbU \((\mu g/4 \text{ h})\), and PbB \((\mu g/L; \text{Table 2})\). We found no correlation between baseline-PbU and DMSA-PbU, but did find a moderate correlation when data were log-transformed, in which case baseline-PbU accounted for 12.6% of DMSA-PbU. Log(DMSA-PbU) and log(DMSA-U) were weakly correlated.

**Lead-exposed individuals.** The mean \((SD)\) PbB was 146 (94) \mu g/L \((\text{range}, 46–400 \mu g/L)\) in the 17 workers from the enameling plant, and 518 (18) \mu g/L \((12–86 \mu g/L)\) in the other 23 lead-exposed individuals. Overall, the mean \((SD)\) excretion rate of baseline-PbU was 8.67 (10.9) \mu g/4 \text{ h} \((\text{range}, 1–41.4 \mu g/4 \text{ h})\). In contrast to the lack of correlation in controls, we found a good correlation between baseline-PbU and PbB in the lead-exposed workers \((r = 0.85; P < 0.0001)\).

We observed a significant correlation between DMSA-PbU and baseline-PbU in the lead-exposed workers \((r = 0.576; P < 0.05)\); however, the values ranged widely around the regression line. A similar correlation was

| Table 1. Characteristics of the population and lead marker values. |
|--------------------------|------------------|------------------|
| Controls \((n = 40; 23 \text{ males, 17 females})\) | Mean (SD) | Range | Median |
| Age, years | 41 (12) | 22–67 | 41 |
| Body weight, kg | 70 (10) | 51–87 | 70 |
| PbB, \mu g/L | 28 (9.6) | 12–46 | 25 |
| PbU \((n = 36)\), \mu g/4 \text{ h} | | | |
| Baseline-PbU | 0.63 (0.5) | 0.02–2.43 | 0.43 |
| DMSA-PbU | 8.6 (4.5) | 1.73–23.1 | 7.7 |
| Ratio baseline-PbU/DMSA-PbU | 24 | 3.6–127 | |
| DMSA-U \((n = 33)\), mg/4 \text{ h} | 78.7 (32.7) | 26.7–154.3 | 76.7 |
| DMSA-PbU/DMSA-U \((n = 33)\), \mu g/mg | 0.123 (0.07) | 0.022–0.32 | 0.125 |
| Lead-exposed individuals \((n = 40; 30 \text{ males, 10 females})\) | | | |
| Age, years | 42 (7) | 23–57 | 44 |
| Body weight, kg | 74 (12) | 56–120 | 75 |
| Duration of exposure, years | 9.3 (8.7) | 0.3–36 | 14.2 |
| PbB, \mu g/L | 367 (250) | 46–86 | 315 |
| PbU \((n = 25)\), \mu g/4 \text{ h} | | | |
| Baseline-PbU | 8.7 (10.9) | 1.08–41.4 | 3.2 |
| DMSA-PbU | 377.6 (538) | 14.7–2598 | 117 |
| Ratio baseline-PbU/DMSA-PbU | 26.5 | 3.7–86.8 | |
| DMSA-U \((n = 22)\), mg/4 \text{ h} | 53.5 (21) | 7.2–103.9 | 49 |
| DMSA-PbU/DMSA-U \((n = 22)\), \mu g/mg | 1.43 (1.17) | 0.34–4.95 | 0.9 |

\(a\) Arithmetic mean.

\(b\) DMSA-PbU vs baseline-PbU, \(P < 0.0001\).

\(c\) Exposed vs controls: \(^\text{a} P < 0.0001; \(^\text{b} P < 0.005.\)
observed between log(DMSA-PbU) and baseline-PbU (Table 2 and Fig. 1A).

We found a significant correlation between DMSA-PbU and PbB. The spread over the regression line was even more important than in controls (Fig. 1B). Only 41%, 27%, and 15% of DMSA-PbU, from blood lead concentrations of 150, 250, and 500 μg/L, respectively, was accounted for by PbB. Log transformation slightly improved the determination coefficient ($r^2$ from 0.479 to 0.564). The slope of the regression line was, however, much steeper than in controls (Table 2). When we considered controls and exposed-individuals together, we observed a steep increase in DMSA-PbU above a PbB concentration of $\approx$300 μg/L (Fig. 1C).

We observed a moderate correlation between log(DMSA-PbU) and log(DMSA-U) ($r = 0.54; P < 0.05$).

As observed in controls, we found no correlation between DMSA-PbU and BW, between DMSA-PbU and DMSA-U corrected for BW, or between DMSA-PbU and the amount of DMSA administered corrected for BW. Similarly, we found no correlation between delta-PbU or DMSA-PbU/baseline-PbU and the amount of DMSA excreted.

ZINC AND COPPER MOBILIZATION
The main outcome measures are summarized in Table 3. Copper and zinc excretion rates (baseline and chelated) did not differ in controls and lead-exposed individuals (not shown) and were thus treated together. DMSA caused significant increases in the excretion rates of zinc and copper (Table 3). The mean ratio of chelated concentrations to baseline concentrations of copper and zinc were 6.76 and 1.37, respectively, but there were large interindividual variations for both (0.73–91 for copper and 0.16–32.9 for zinc). The 97.5th percentiles of the baseline concentrations were 4.5 μg/4 h for copper and 198 μg/4 h for zinc; the 97.5th percentiles of the chelated concentrations were 28.5 μg/4 h for copper and 258 μg/4 h for zinc.

We found no correlation between baseline-CuU and DMSA-CuU, but did find a significant correlation between baseline-ZnU and DMSA-ZnU ($r = 0.602$; see Table 2 in the online Data Supplement). DMSA-CuU and DMSA excretion were weakly correlated in the total study population ($r = 0.320$); whereas these variables were not correlated in the controls, the correlation was excellent ($r = 0.809; P < 0.0001$) in lead workers (data not shown).

We found no correlation between the ratio of the chelated to the baseline concentrations of the elements and the amount of DMSA excreted or the dose of administered DMSA corrected for BW. We also found no correlation among the excretion rates of the different elements (lead, copper, and zinc) in either the total study population or when controls and lead-exposed individuals were considered separately.

### Table 2. Correlations between DMSA-PbU (μg/4 h) and baseline-PbU (μg/4 h), PbB (μg/L), and DMSA-U (mg/4 h).a

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>r</th>
<th>P</th>
<th>$r^2$</th>
<th>Slope</th>
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<tr>
<td>DMSA-PbU vs baseline-PbU</td>
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<tr>
<td>Controls</td>
<td>36</td>
<td>0.356</td>
<td>&lt;0.05</td>
<td>0.126</td>
<td>0.21</td>
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<td>Lead-exposed</td>
<td>27</td>
<td>0.551</td>
<td>&lt;0.05</td>
<td>0.303</td>
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<td>DMSA-PbU vs PbB</td>
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<tr>
<td>Controls</td>
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<td>0.607</td>
<td>&lt;0.001</td>
<td>0.368</td>
<td>0.875</td>
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<td>0.751</td>
<td>&lt;0.001</td>
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<tr>
<td>Controls</td>
<td>33</td>
<td>0.327</td>
<td>0.05</td>
<td>0.107</td>
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<td>Lead-exposed</td>
<td>22</td>
<td>0.541</td>
<td>&lt;0.05</td>
<td>0.293</td>
<td>2.058</td>
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a All data were log-transformed before analysis.
Table 3. Zinc and copper urinary excretion rates (n = 58).

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>Range</th>
<th>Median</th>
<th>p</th>
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<tbody>
<tr>
<td>Copper, µg/4 h</td>
<td>2.2 (1.26)</td>
<td>0.15–8.1</td>
<td>1.98</td>
<td>&lt;0.0001</td>
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<td>Baseline-CuU</td>
<td>14.96 (8.2)</td>
<td>1.7–46.6</td>
<td>13.63</td>
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<tr>
<td>DMSA-CuU</td>
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<tr>
<td>Zinc, µg/4 h</td>
<td>82.8 (61.2)</td>
<td>3.8–337</td>
<td>74.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Baseline-ZnU</td>
<td>114.2 (57.2)</td>
<td>16.1–279.3</td>
<td>106.3</td>
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<tr>
<td>DMSA-ZnU</td>
<td></td>
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</table>

* Arithmetic mean.
* Value after DMSA administration vs baseline value (paired test).

Discussion

The main purpose of this study was to propose a protocol for an LMT that is easy to perform on an outpatient basis, is safe, and would be well accepted. The kinetic study in controls showed that DMSA acts quickly, inducing a peak PbU concentration within 2 h, and that the 4- and 24-h cumulative lead excretion rates are highly correlated. These results are similar to those of Lee et al. (12), who reported that PbU peaked ~2 h after DMSA administration and that cumulative lead excretion amounts at 2 and 4 h were highly correlated with the 8-h total (r = 0.76 and 0.95, respectively) in lead workers. Gerhardsson et al. (35) also observed that in both active and retired lead workers, DMSA-PbU in the 6- and 24-h collections were well correlated (r = 0.95; P <0.001). A serious limitation of a chelation test requiring a 24-h urine collection would be the lack of patient compliance. The kinetic pattern of DMSA excretion allows a shorter urine collection period, which is not only more convenient but also reduces the risk of incomplete sampling and the risk of contamination, thus increasing the reliability of the results.

DMSA-U at 4 h was significantly higher in controls than in lead-exposed individuals. This might be consistent with data suggesting that the pharmacokinetics of DMSA is altered in lead-poisoned individuals. A significant positive association between PbB and DMSA half-life has been observed in children with moderate lead exposure (37), and renal clearance of total DMSA is greater in healthy adults than in adults with lead poisoning (38).

In cases of lead poisoning, the amount of DMSA administered for therapeutic purposes is generally calculated according to BW (10–30 mg/kg of BW) and is often given in divided doses. A dose of 30 mg/kg was significantly more effective in increasing PbU than were doses of 20 and 10 mg/kg, with no difference between 10 and 20 mg/kg (31). In investigations including LMT with a single dose of 5 or 10 mg/kg DMSA in Korean lead workers and US former organolead workers, body mass index (weight) was not a significant predictor of DMSA-PbU except when PbB was used to model DMSA-PbU in multiple linear regression modeling (12, 39, 40). In the present study, again for the sake of an easy-to-use protocol, a single oral dose, not adjusted to BW, was given, and correlations were comparable to those with DMSA administration adjusted to BW. In our study population, the mean dose administered was ~14 mg/kg of BW.

The nonfasting condition of our study participants may have had an impact on the excretion kinetics of DMSA. Pharmacokinetic studies on 11-h fasted healthy volunteers have shown that ~15%–20% (range, 6.9% to >25%) of an orally administered dose (10 mg/kg of BW) is eliminated in the urine within 14–15 h (32, 37). Our results were similar; ~17% (range, 10%–27%) of the absorbed dose was found in urine by 24 h, 75%–99% of this amount being excreted within 12 h. It is therefore unlikely that fasting is an important confounder for the interpretation of DMSA LMT results.

DMSA was well tolerated by the study participants. It is considered to be safer than CaNa$_2$EDTA, and the side effects reported in the literature are rare and mild. Unlike CaNa$_2$EDTA, DMSA does not appear to form a toxic emetic complex with iron (30, 32, 41, 42). Among the major advantages of DMSA over CaNa$_2$EDTA is its relative specificity. It is generally not considered to induce substantial elimination of essential trace elements such as copper, zinc, iron, and manganese. Disagreement exists as to whether DMSA influences the urinary excretion of copper and zinc (43–46). We observed that DMSA increased copper and zinc excretions by 6.8-fold (up to 91-fold) and 1.4-fold (up to 33-fold), respectively. Although we found no correlation between DMSA-CuU and DMSA-U in controls, the correlation was excellent in lead-exposed individuals. The excretion rate of DMSA-CuU was comparable in both groups. The reason for this difference is not immediately apparent. We observed no correlation between DMSA-ZnU and DMSA-U. On the one hand, our observations confirm that DMSA might be useful for the treatment of Wilson disease (43); on the other hand, although this effect on essential trace elements does not pose a health hazard within the context of an LMT, the situation could be somewhat different within the context of a chelating treatment. Animal investigations have confirmed the embryotoxicity and fetotoxicity of DMSA, and it has been suggested that this toxicity might be mediated through changes in copper and zinc metabolism (47–50). Although these experiments were performed with much higher doses than those generally recommended, DMSA should be administered to pregnant women with great care.

The efficacy of DMSA in chelating lead has been demonstrated in lead-intoxicated children and adults (30–32, 41, 42). We found that DMSA rapidly and significantly increases PbU, even at very low exposures; in both controls and lead-exposed individuals, the mean ratio of DMSA-PbU to baseline-PbU was ~25.

Although determination of the bioavailable pool of lead is considered more relevant in terms of health risk assessment than PbB, no consensus exists regarding the usefulness of LMT. DMSA-PbU is considered an indicator of bioavailable lead burden (12, 13) and, as such, might better predict imminent toxicity (14, 15). DMSA-PbU has
been found to be more strongly related to urinary aminolevulinic acid (12) and a better predictor of lead-related symptoms, particularly of total symptom scores and neuromuscular symptoms, than other lead biomarkers, including PbB (15). Although PbB accounts for a large proportion of the variance in DMSA-PbU, suggesting that it may not be necessary to measure both PbB and DMSA-PbU in epidemiologic studies, at the individual level, DMSA-PbU may be a better predictor of one or more health outcomes and therefore be the most useful measurement (40).

Schwartz’s group has reported correlations between DMSA-PbU and PbB ranging from none to high (12, 13, 40, 51). In a first study, they found no association between PbB \( [n = 34; \text{mean (SD) PbB}, 513 (142) \mu g/L] \) and the 8-h DMSA-PbU value after a DMSA dose of 10 mg/kg of BW (12); however, in 1997, the same group reported that PbB \( [n = 57; \text{PbB} 254 (102) \mu g/L] \) was correlated \((r = 0.40)\) with the 4-h DMSA-PbU value after a DMSA dose of 5 mg/kg of BW and hypothesized that DMSA primarily accesses PbB stores at low doses (5 mg/kg) and PbB and soft tissues stores at higher doses (10 mg/kg) (13). However, in a later publication, they reported a good association between PbB (mean, 320–440 \( \mu g/L \); maximum, 860 \( \mu g/L \)) and 4-h DMSA-PbU(ln) after intake of 10 mg of DMSA/kg of BW (40). In former organolead manufacturing workers with low PbB (mean, 460 \( \mu g/L \); range, 10–200 \( \mu g/L \)), the 4-h DMSA-PbU was reported to be moderately correlated with PbB \((r = 0.40; P < 0.001)\) (51).

The curvilinear relationship we observed between PbB and DMSA-PbU is in accordance with the findings of Gerhardsson et al. (35) and reflects the nonlinear relationship between lead exposure and PbB. In adult volunteers exposed to lead for 18 weeks, PbB increased for \( \sim \) 12 weeks, and then plateaued between 270 and 370 \( \mu g/L \) (52), and in 96 pediatric populations from various parts of the world, the relationship between air lead and PbB showed PbB concentrations that plateaued at \( \sim \) 400 \( \mu g/L \) (53). Moreover, even if lead in blood is partitioned predominantly in erythrocytes (>98%), there is strong evidence of a curvilinear relationship between plasma lead (PbP), which is considered a labile, exchangeable fraction of lead in blood, and PbB (54–58). A possible explanation for the increasing fraction of PbP with increasing PbB is a gradual saturation of the binding sites in erythrocytes with highest affinity for lead, e.g., delta-aminolevulinic dehydratase (57, 59, 60). The increase of PbP might also be caused by altered cell morphology at high concentrations that leads to reduced availability or stability of lead-binding sites in the erythrocytes (61, 62). It is conceivable that although the relative increase in PbB would be negligible at a PbB concentration of \( \sim \) 300 \( \mu g/L \), the fraction of lead in blood (PbP) available to be distributed to the organs might increase considerably and would be reflected in the steep increase in the mobilized pool that we observed as PbB after DMSA administration.

In view of the difficulty of accurate measurement of very low concentrations of lead in biological materials and the concern regarding sample contamination from external sources or from hemolysis, PbP is not routinely determined.

LMT interpretation is difficult. To determine whether there is an increased mobilizable lead pool, the size of the chelatable pool in controls must first be measured. A CaNa2EDTA LMT considered to be positive covers chelated-PbU amounts of 350–1000 \( \mu g/24 \text{ h} \), with \( \sim 600 \mu g / 24 \text{ h} \) being the most generally used limit (16, 21, 25, 63–68). The importance of a “positive” result, however, is not always clear; possible interpretations include an increased bioavailable lead pool compared with controls, a limit that can be used to distinguish occupationally exposed individuals from persons with no such exposure, a safe limit, an indication for chelation treatment, or identification of individuals who will respond to chelation treatment.

These values are based on very old data obtained with CaNa2EDTA administered according to different protocols, and they no longer apply in view of the decrease in PbB in the general population since lead was removed from gasoline. Moreover, evidence from animals [see, for examples, Refs. (33, 69)] and humans (12) suggests that the lead pool mobilized by DMSA is not the same as the one mobilized by CaNa2EDTA. In the investigations conducted by Schwartz’s group, the DMSA LMT was applied to a limited number of controls. Their first report mentioned a DMSA LMT performed in 5 controls \([\text{mean (SD)} \text{ PbB}, 57 (13) \mu g/L]\) who all excreted <100 \( \mu g \text{ Pb/8 h} \) (mean 61 \( \mu g \text{ Pb/8 h} \); range, 30–97 \( \mu g \text{ Pb/8 h} \)) after receiving a DMSA dose of 10 mg/kg of BW (12). In a further study, they reported a mean 4-h DMSA-PbU of 23.7 \( \mu g \) (10.5–43.5 \( \mu g \)) in 13 controls (mean PbB, 59 \( \mu g/L \); range, 40–72 \( \mu g/L \)) (15). The PbB in our controls was somewhat lower (mean, 28 \( \mu g/L \); range, 12–46 \( \mu g/L \)). Considering the entire study group (controls and lead-exposed individuals), those with PbB <50 \( \mu g/L \) had a DMSA-PbU <23.2 \( \mu g/4 \text{ h} \), and all individuals with PbB <100 \( \mu g/L \) had a DMSA-PbU <42 \( \mu g/4 \text{ h} \). Reference values are generally based on the 97.5th percentile value, which was 21.8 \( \mu g/4 \text{ h} \) in controls.

In conclusion, we provide evidence that a short, practical, well-accepted, apparently safe, and inexpensive LMT involving a 4-h urine collection after a single oral dose of 1 g of DMSA can be used to detect a lead pool that is not reflected by PbB. Because a biomarker is of limited use without reference values, we propose 22 \( \mu g/4 \text{ h} \) as a tentative reference value for the adult Belgian general population. Currently, it is not known whether an individual would be at risk of developing toxic effects at lead concentrations above this proposed value. The biological significance of this reference value should be investigated in further studies.
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References


