Comparison of measures of lead exposure, dose, and chelatable lead burden after provocative chelation in organolead workers

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Abstract

Objectives—To describe 6 h urinary lead excretion (6 h PbU) after 1 g intravenous ethylene diamine tetraacetic acid (EDTA) in organolead manufacturing workers with mixed exposure to organic and inorganic lead; to determine the predictors of lead excretion (PbU); and to determine the extent to which internal lead stores and ongoing external exposure govern blood concentrations of lead (PbB).

Methods—A case series of 21 active workers were studied. Personal industrial hygiene data, grouped by 29 exposure zones, in combination with personal interviews about work location and times were used to derive several measures of recent and cumulative exposure to organic and inorganic lead. The average exposure concentrations assigned to the 29 zones ranged from 4 to 119 µg/m³ (0.02-0.57 µmol/l as lead) for organic lead and from 1 to 56 µg/m³ (0.004-0.27 µmol/l) for inorganic lead.

Results—After controlling for age, 6 h PbU was significantly and positively correlated with summary measures of PbB—for example, lifetime peak PbB, time weighted PbB—and zinc protoporphyrin concentrations—for example, lifetime peak zinc protoporphyrin, time weighted zinc protoporphyrin—but not with measures of estimated external exposure—for example, duration of exposure and cumulative exposure to inorganic or organic lead. Among workers with higher chelatable lead burdens (6 h PbU > 212·4 µg (1·03 µmol) divided at the median), there was no apparent relation between recent inorganic lead exposure and PbB at the time of chelation. Among workers with lower chelatable lead burdens (6 h PbU < 212·4 µg (1·03 µmol)), however, there was a significant relation between recent inorganic lead and PbBs.

Conclusion—These findings are consistent with the concept of physiological dampening. The high chelatable lead burden, a source of internal exposure, dampens the effect of external exposure on PbBs. The data suggest that in organolead workers with high chelatable lead burdens, PbBs may be more influenced by internal lead stores than by variations in airborne exposure to organic and inorganic lead.

Keywords: body burden; lead; provocative chelation; tetraethyl lead.

The organolead compounds most commonly encountered in the workplace are tetraethyl and tetramethyl lead, both components of additives for gasoline and aviation fuel. Use in the United States has greatly diminished since the introduction of catalytic converters into vehicles in 1975, but worldwide production and use is still considerable and represents an important occupational and environmental hazard.

After absorption, tetraethyl lead is rapidly dealkylated primarily in the liver by cytochrome P-450 mixed function oxidases to the toxic metabolite triethyl lead, or possibly directly to inorganic lead. Triethyl lead has been found to be relatively persistent in rodent tissues, with estimated half lives of 15 days in the liver and kidney, 7 days in the brain, and 3–5 days in blood. It is further metabolized to diethyl lead, an important urinary excretion product, then to inorganic lead, which can be excreted in the urine or stool, or stored in bone as the main portion of the body’s burden of lead. Precipitable lead (inorganic lead) is produced rapidly, but it disappears rapidly from tissues, probably due to uptake by bones. Studies in humans suggest that such bone lead represents 95% of the body’s burden of lead, with an estimated half life of elimination of 5–20 years.

Although ethylene diamine tetraacetic acid (EDTA) does not chelate organolead compounds in vitro or in vivo, enhancement of urinary lead excretion (PbU) after EDTA treatment of patients with acute organolead poisoning has been documented in several case reports. Arai reported that in a worker with tetraethyl lead poisoning 22 days after exposure, PbU output was 51% inorganic, 43% diethyl lead, and 6% triethyl lead. Administration of EDTA resulted in increased urinary excretion of inorganic lead but not organic lead.

No previous studies have compared measures of exposure, dose, and chelatable lead burden in workers exposed to organolead. Although several case series have been
reported concerning chelation in inorganic lead workers, exposure measures from epidemiological exposure assessment have not been used. In this study, the primary interest was to identify the determinants of blood lead concentrations (PbBs) in subjects exposed to lead. Specifically, we were interested in the extent to which internal lead stores and ongoing external exposure govern PbBs. A secondary aim was to describe lead excretion after administration of EDTA in organolead workers and determine the predictors of lead excretion in such workers.

Materials and methods
STUDY POPULATION
This case series consists of 21 employees of an organolead manufacturing facility in the eastern United States who sought medical care for possible health effects related to lead. All workers had mixed exposure to organic and inorganic lead and underwent provocative chelation with 1 g of intravenous EDTA. The population, one black and 19 white men and one white woman, had a mean (SD) age of 46.3 (6.0) y. Details of the plant, the exposures in the area, and the study population have been reported in a study of the neurobehavioral effects of inorganic and organic lead exposure in a larger group of 222 organolead workers from the plant.14 Organolead exposure was estimated to represent 65–70% of total lead exposure in the area. The 21 workers who underwent diagnostic chelation were part of a larger group of 58 workers who were clinically evaluated at their own request.

All workers who underwent chelation had symptoms or diagnostic evidence of health effects to the central or peripheral nervous system—for example, on neuropsychological tests or nerve conduction studies—without another medical explanation. Because PbB is not thought to be a valid measure of cumulative lead absorption, diagnostic chelation was performed to aid in the clinical evaluations. Data were abstracted from the medical records and linked with previously collected epidemiological data14 with approval of the Institutional Review Board.

COLLECTION OF DATA
Demographic, medical, and occupational information was obtained by questionnaire and interview, as previously described.14 Provocative chelation was performed with 1 g of EDTA given intravenously in 500 ml normal saline over 1 h, followed by a 6 h urine collection. For all workers, 24 h lead excretion was estimated from the 6 h urinary lead excretion (6 h PbU) value by the following equation: 24 h lead (μg) = 113·2 + 2·31 × 6 h PbU (μg).15

MEASURES OF EXPOSURE AND DOSE
Several measures of work-related external exposure to organic and inorganic lead and of internal dose were derived.14 Histories of individuals’ exposure were derived after linkage of personal industrial hygiene data with daily hours worked, obtained by interview, in 29 exposure zones in the lead area of the plant, for all jobs ever held in the lead area. The personal industrial hygiene data, available from the previous 12 years but with half the samples from 1989–90, were grouped by exposure zone and the arithmetic mean was used as the summary measure of the intensity of exposure in the zone. The assigned exposure intensities in the 29 zones ranged from 4–119 μg/m³ (0–0.02–0.57 μmol/m³ as lead) for organic lead and 1–56 μg/m³ (0.004–0.27 μmol/m³) for inorganic lead.14

The exposure histories consisted of mixed exposure to inorganic and organic lead, and were estimated for 100 day employment intervals in μg days/m³. The histories were used to create three main measures of external exposure: cumulative exposure to organic and inorganic lead during employment at the plant; recent (past 30 days) exposure to organic and inorganic lead; and lifetime weighted average intensity of exposure to organic and inorganic lead, dividing cumulative exposure by duration of exposure. Data were also derived from the histories of exposure and was the final exposure variable to be assessed. Questionnaire data showed that no workers had occupational exposure to lead before employment in the plant or significant current or past non-occupational exposure to lead.

The PbBs, PbUs, and zinc protoporphyrin (ZPP) concentrations were monitored by the employer and were abstracted from plant medical records. These were used to derive three measures each for PbU, PbB, and ZPP: lifetime peak values; the corresponding measures obtained closest in time to chelation (PbUc, PbBc, and ZPPc); and time weighted measures.

The PbBc and PbUc were assayed by a NIOSH certified commercial laboratory with graphite furnace atomic absorption spectrophotometry. Most historical PbBs (from medical records) were assayed by anode stripping voltammetry. Historical PbUs were generally assayed at the plant by a dithizone method (precipitate lead with phosphate collector; extract with chloroform; redissolve precipitate with acid, then alkalise; react with dithizone; assay with spectrophotometer). The ZPP assays were performed by haematofluorometry. The historical PbUs and PbUcs provided total urinary lead of all forms.

ANALYSIS OF DATA
Data analysis was performed with the BMDP statistical software programs.16 Detailed descriptive statistics and summary measures were derived for all variables. Bivariate scatterplots and correlation coefficients were determined for pairs of study variables. Multiple linear regression was used to identify predictors of 6 h PbU and PbBc. The 6 h PbB regression examined the relative influence of inorganic vs organic lead exposure, recent vs cumulative exposure, and measures of external exposure vs internal dose, in the prediction of 6 h PbU. The PbBc regressions
examined the relative influence of inorganic Pb on organic lead exposure in the prediction of PbBc, and whether these most recent PbBs were more influenced by recent exposure or estimates of chelatable lead burden. Outliers were identified by examination of frequency distributions, scatterplots, and residual plots, and the influence of outliers on regression results was assessed. One outlier for recent inorganic lead exposure was eliminated from the analysis; the effect of this point on the regression is discussed in the results. Regressions were also evaluated for multicollinearity and departures from normality and were found to be free of these potential problems.

### Table 1 Summary of demographic, clinical, exposure, and dose measures

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
<th>Range</th>
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<tbody>
<tr>
<td>Age (y)</td>
<td>46 (6)</td>
<td>38-59</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>180 (7)</td>
<td>168-191</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>94 (15)</td>
<td>66-125</td>
</tr>
<tr>
<td>Duration of exposure (y)</td>
<td>14.3 (7.4)</td>
<td>3-26.5</td>
</tr>
<tr>
<td>Lifetime peak PbB: (µg/dl)</td>
<td>33.6 (13.9)</td>
<td>40-56.0</td>
</tr>
<tr>
<td>PbB just before chelation: (µg/dl)</td>
<td>1.02 (0.63)</td>
<td>0.19-2.71</td>
</tr>
<tr>
<td>Cumulative exposure during employment at plant: (µmol/y/m³)</td>
<td>20.6 (6.5)</td>
<td>8.0-30.0</td>
</tr>
<tr>
<td>Cumulative exposure, previous month: (µg/µmol)</td>
<td>3.96 (2.36)</td>
<td>0.18-8.73</td>
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</table>

### Results

The mean (SD) 6 h PbU after 1 g of intravenous EDTA was 215 (129) µg (1.04 (0.62) µmol), with a range from 31 to 495 µg (0.15-2.39 µmol). The estimated mean (SD) 24 h PbU was 609 (298) µg (2.94 (1.44) µmol), with a range of 184 to 1257 µg (0.89-6.07 µmol, table 1). The PbBcs ranged from 8 to 30 µg/dl (0.39-1.45 µmol/L), and study participants had a mean (SD) duration of exposure of 14.3 (7.4) years (table 1).

### PREDICTORS OF LEAD EXCRETION

Age and duration of exposure were negatively correlated with 6 h PbU, whereas the measures of PbB (lifetime peak, PbBc, and time weighted) and recent inorganic and organic lead exposure were positively correlated with 6 h PbU (correlation matrix in table 2). Time weighted ZPP and lifetime peak ZPP were also correlated with 6 h PbU (Pearson's r = 0.62 and 0.63, respectively). Several exposure and PbB measures were negatively correlated with age (table 2), suggesting that older workers had less exposed jobs.

### Table 2 Correlation matrix of study variables

<table>
<thead>
<tr>
<th></th>
<th>Age (y)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Duration of exposure (y)</th>
<th>Lifetime peak PbB (µg/dl)</th>
<th>PbBc (µg/dl)</th>
<th>CEINO (µg/µmol)</th>
<th>CEORG (µg/µmol)</th>
<th>RECINO (µg/µmol)</th>
<th>RECORG (µg/µmol)</th>
<th>6h PbU (µg)</th>
<th>RECORG (µg/µmol)</th>
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<tr>
<td>Age (y)</td>
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<tr>
<td>Weight (kg)</td>
<td>0.10</td>
<td>1.00</td>
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<tr>
<td>Duration of exposure (y)</td>
<td>0.10</td>
<td>0.22</td>
<td>0.18</td>
<td>1.00</td>
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<tr>
<td>Lifetime peak PbB (µg/dl)</td>
<td>-0.16</td>
<td>-0.01</td>
<td>-0.18</td>
<td>-0.42†</td>
<td>1.00</td>
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<tr>
<td>PbBc (µg/dl)</td>
<td>-0.52†</td>
<td>-0.03</td>
<td>-0.15</td>
<td>-0.37</td>
<td>0.78*</td>
<td>1.00</td>
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<tr>
<td>CEINO (µg/µmol)</td>
<td>-0.40†</td>
<td>-0.19</td>
<td>0.04</td>
<td>0.54*</td>
<td>0.21</td>
<td>0.25</td>
<td>1.00</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>CEORG (µg/µmol)</td>
<td>-0.14</td>
<td>-0.25</td>
<td>0.16</td>
<td>0.76*</td>
<td>-0.17</td>
<td>-0.15</td>
<td>0.73*</td>
<td>1.00</td>
<td></td>
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<tr>
<td>RECINO (µg/µmol)</td>
<td>-0.57*</td>
<td>0.34</td>
<td>0.31</td>
<td>-0.29</td>
<td>0.63*</td>
<td>0.56*</td>
<td>0.35</td>
<td>-0.03</td>
<td>1.00</td>
<td></td>
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</tr>
<tr>
<td>RECORG (µg/µmol)</td>
<td>-0.65*</td>
<td>0.19</td>
<td>0.41†</td>
<td>-0.05</td>
<td>0.65*</td>
<td>0.45*</td>
<td>0.40†</td>
<td>0.32</td>
<td>0.66*</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6h PbU (µg)</td>
<td>-0.47†</td>
<td>0.06</td>
<td>-0.09</td>
<td>-0.26</td>
<td>0.65*</td>
<td>0.62*</td>
<td>0.07</td>
<td>-0.07</td>
<td>0.35</td>
<td>0.44*</td>
<td></td>
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</tr>
</tbody>
</table>

*p < 0.05; †p < 0.1; CEINO = cumulative exposure to inorganic lead during employment at plant; CEORG = cumulative exposure to organic lead during employment at plant; RECINO = cumulative exposure to inorganic lead in the 30 days before chelation; RECORG = cumulative exposure to organic lead in the 30 days before chelation.

### Table 3 Results of multiple linear regressions modelling 6hPbU (µg) after 1 g intravenous EDTA, comparing measures of exposure and dose

<table>
<thead>
<tr>
<th>Model</th>
<th>Variable</th>
<th>β (SE β)</th>
<th>P value</th>
<th>Total model r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recent exposure to organic lead and age</td>
<td>RECORG (µg/µmol)</td>
<td>9.33 (8.50)</td>
<td>0.29</td>
<td>0.27</td>
</tr>
<tr>
<td>Recent exposure to inorganic lead and age</td>
<td>Age (y)</td>
<td>-7.15 (5.10)</td>
<td>0.18</td>
<td>0.23</td>
</tr>
<tr>
<td>Recent exposure to inorganic lead and age</td>
<td>Age (y)</td>
<td>-8.70 (5.38)</td>
<td>0.12</td>
<td>0.27</td>
</tr>
<tr>
<td>Duration of exposure and age</td>
<td>Age (y)</td>
<td>-3.85 (3.54)</td>
<td>0.29</td>
<td>0.53</td>
</tr>
<tr>
<td>Lifetime peak PbBc and age</td>
<td>Age (y)</td>
<td>-9.69 (4.33)</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Lifetime peak PbBc and age</td>
<td>Age (y)</td>
<td>-3.53 (3.57)</td>
<td>0.08</td>
<td></td>
</tr>
</tbody>
</table>

*SE β = SE of β coefficient; †P value from t test of (β coefficient/SE β). Abbreviations as for table 2.
height, weight, cigarette smoking, renal function, and job title were not associated with 6 h PbU.

**PREDICTORS OF BLOOD LEAD AT THE TIME OF CHELATION**

In bivariate analyses, PbBc was positively correlated with recent organic and inorganic lead exposure (Table 2); and lifetime weighted average intensity of exposure to total (r = 0.48, P = 0.03) and inorganic (r = 0.64, P = 0.003) lead. The PbBc was negatively correlated with age and exposure duration. In multiple linear regression analyses, recent inorganic lead exposure and chelatable lead burden (6 h PbU) were both independent predictors of PbBc (Table 4).

An interesting association was found among chelatable lead burden, recent inorganic lead exposure, and PbBc. For control for duration of exposure among workers with high chelatable lead burdens (6 h PbU > 212.4 µg (1.03 µmol), divided at the median), there was no apparent relation between recent inorganic lead exposure and PbBc. However, among workers with low chelatable lead burdens (6 h PbU < 212.4 µg (1.03 µmol)) there was a dose-related increase in PbBc with increasing recent inorganic lead exposure (Table 4). The 11 workers with high chelatable lead burdens (6 h PbU = 212.4 µg (1.03 µmol)) had an estimated mean (SD) 24 h PbU of 842 (184) µg (4.07 (0.89) µmol) (range 604 to 1257 µg (2.92-6.07 µmol)).

One outlier for recent inorganic lead exposure was identified. When this point was eliminated from the linear regression model of PbBc, there were no changes in the β coefficients in the model, but the SE of the interaction term increased and hence the significance declined from P = 0.04 to P = 0.11.

**Discussion**

Despite modest rises in PbBs, over half of 21 organolead workers evaluated for possible health effects related to lead had high chelatable lead burdens. At the time of chelation, PbBs ranged from 17 to 30 µg/dl (0.82-1.45 µmol/l) in the 11 workers with 24 h PbUs estimated to be >600 µg (2.90 µmol), a generally accepted positivity criterion. Even with low PbBs, several measures of PbBc were strongly associated with chelatable lead burden in this group of workers.

The data suggest that among workers with high chelatable lead burdens, recent inorganic lead exposure does not exert a strong influence on PbBs. In contrast, among workers with low chelatable lead burdens, recent inorganic lead exposure is an important predictor of PbBc, and the data show a strong relation between exposure and effect. Caution must be expressed, however, because the significance, but not the magnitude, of this association was altered by the elimination of a single outlier for recent inorganic lead exposure.

This finding would be expected for toxins with long half lives of elimination (t½ > 40 h), and is consistent with the concept of physiologic damping. The high chelatable lead burden, a source of internal exposure, dampens the transmission of external exposure variations to PbBs. The relative contributions of internal lead stores and recent lead exposure to PbBs found in this study contrasts with findings by other investigators that PbB is more influenced by daily lead absorption than by lead released from bone deposits. Measures of external lead exposure (cumulative or recent inorganic or organic lead exposure) were not significant predictors of chelatable lead stores. Measures of internal dose (PbB) were expected to be better predictors of chelatable lead stores than external exposure measures because of their ability to account for all routes of exposure, sources of exposure—for example, non-vocational inorganic lead exposure—and use of personal protective equipment. Also, PbB is likely to be in equilibrium with these chelatable lead stores.

Although negatively correlated (r = −0.26), the association between 6 h PbU and duration of exposure was not significant. For toxins with long elimination half lives, duration of exposure is thought to be a good surrogate for cumulative dose, and hence retained body burden. Data on associations between duration of exposure and provocative chelation results are scant, but some suggest that duration of exposure is a predictor of chelation yield in workers exposed to inorganic lead. It is possible that chelatable lead stores are not an accurate reflection of body burden, which perhaps accounts for this lack of association.

Lead kinetic studies have shown that the
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half life of lead varies among its storage compartments. The elimination of lead from trabecular bone, for example, is more rapid than from cortical bone, and the rate of elimination of lead stores decreases with time. Postmortem analysis has suggested that with advancing age an increasing proportion of bone lead content is stored in cortical bone rather than trabecular bone. This would suggest that chelation yields may decrease over time, and hence with advancing age and increasing duration of exposure. We cannot be certain that the finding that older workers had lower PbU is because of decreased availability of lead stores to EDTA, or because older workers in this study had lower recent exposures to inorganic and organic lead.

Conclusions

Provocative chelation showed high chelatable lead burdens in 11 of 21 organolead workers with an average of 14 years of exposure in the industry. The PbU after intravenous EDTA was not strongly associated with any measure of external exposure to inorganic and organic lead after control for age. Lifetime peak PbB, to a lesser degree other blood lead measures, and age accounted for over 50% of the variability in chelation results in multiple linear regression analyses. The PbBs were found to be independently influenced by both recent inorganic lead exposure and internal lead stores, but workers with high chelatable lead burdens did not show increases in PbBs with increasing recent exposure to inorganic lead.

9 Ari F. Determination of triethyllead, diethyllead, and inorganic lead in urine by atomic absorption spectrometry. Ind Health 1986;24:139-50.