

# Predictors of dimercaptosuccinic acid chelatable lead and tibial lead in former organolead manufacturing workers

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## Abstract

**Objectives**—To identify predictors of tibial and dimercaptosuccinic acid (DMSA) chelatable lead in 543 organolead manufacturing workers with past exposure to organic and inorganic lead.

**Methods**—In this cross sectional study, tibial lead (by  $^{109}\text{Cd}$  K-shell x ray fluorescence), DMSA chelatable lead (4 hour urinary lead excretion after oral administration of 10 mg/kg), and several exposure measures were obtained on study participants, mean (SD) age 57.6 (7.6) years.

**Results**—Tibial lead concentrations ranged from  $-1.6$  to  $52.0$   $\mu\text{g}$  lead/g bone mineral, with a mean (SD) of  $14.4$  ( $9.3$ )  $\mu\text{g}/\text{g}$ . DMSA chelatable lead ranged from  $1.2$  to  $136$   $\mu\text{g}$ , with a mean (SD) of  $19.3$  ( $17.2$ )  $\mu\text{g}$ . In a multiple linear regression model of tibial lead, age ( $p<0.01$ ), duration of exposure ( $p<0.01$ ), current ( $p<0.01$ ) and past ( $p=0.05$ ) cigarette smoking, and diabetes ( $p=0.01$ ) were all independent positive predictors, whereas height ( $p=0.03$ ), and exercise inducing sweating ( $p=0.04$ ) were both negative predictors. The final regression model accounted for 31% of the variance in tibial lead concentrations; 27% was explained by age and duration of exposure alone. DMSA chelatable lead was directly associated with tibial lead ( $p=0.01$ ), cumulative exposure to inorganic lead ( $\mu\text{g}/\text{m}^3$ ,  $p=0.01$ ), current smoking ( $p<0.01$ ), and weight ( $p<0.01$ ), and negatively associated with diabetes ( $p=0.02$ ). The final model accounted for 11% of the variance in chelatable lead. When blood lead was added to this model of DMSA chelatable lead, tibial lead, cumulative exposure to inorganic lead, and diabetes were no longer significant; blood lead accounted for the largest proportion of variance ( $p<0.001$ ); and the total model  $r^2$  increased to 19%.

**Conclusions**—The low proportions of variance explained in models of both tibial and chelatable lead suggest that other factors are involved in the deposition of lead in bone and soft tissue. In epidemiological studies of the health effects of lead, evaluation of associations with both these measures may allow inferences to be made about whether health effects are likely to be recent, and thus potentially reversible, or chronic, and thus possibly irreversible. The data also provide direct evidence that

**in men the total amount of lead in the body that is bioavailable declines with age.** (Occup Environ Med 1999;56:22-29)

Keywords: bone lead; chelating agents; dimercaptosuccinic acid; lead; x ray fluorescence

In epidemiological studies of the health effects of lead, several biological measures are available to estimate lead in different kinetic pools and reflect different periods of exposure.<sup>1 2</sup> In such studies, it is important to try to separate recent from cumulative exposures, because recent exposures may be more likely to cause acute, and thus reversible effects, whereas chronic exposures may be more likely to cause chronic, and thus irreversible effects. The available biological measures—for example, blood lead, chelatable lead, bone lead, and such early biological effects as zinc protoporphyrin concentrations in blood or aminolevulinic acid concentrations in plasma or urine—may help to distinguish these different potential health effects of lead.

An extensive scientific literature describes investigations of predictors and kinetics of these different biological measures and compares these measures with each other. Blood lead concentrations are thought to represent a 120 day integrated measure of exposure to internal (bone and tissue lead) and external sources of lead, with a half life of about 30 days.<sup>2 3</sup> Epidemiological studies have relied heavily on evaluating associations with blood lead concentrations<sup>4</sup>; such associations are with a measure of integrated exposure over the past 4 months, and thus may be more likely to reflect recent, reversible effects. Chelatable lead can be estimated as 4–24 hour urinary lead excretion after intravenous administration of ethylene diamine tetra-acetic acid (EDTA) or oral administration of dimercaptosuccinic acid (DMSA). The DMSA has the advantages of oral administration, rapid urinary excretion, greater specificity for lead, and primary removal of lead from soft tissue rather than bone.<sup>5 6</sup> The lead chelatable by DMSA may reflect current bioavailable lead burden. Tibial lead is a measure of cumulative retained lifetime burden of lead.<sup>7 8</sup>

In the current study, predictors of tibial and DMSA chelatable lead are compared and contrasted in 543 former organolead manufacturing workers. Such knowledge is critical to making inferences about associations of these measures with health effects, and is an important step in the use of these measures in

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Table 1 Final recruitment of former organolead manufacturing workers contacted for screening and enrolment in the study during 1993–5

Final recruitment	n	%
All potentially eligible former workers:*	17760	100.0
High probability of no exposure to lead	10590	59.6
Possible exposure to lead but not selected for recruitment	3947	22.3
Possible exposure to lead and randomly selected for recruitment†	3223	18.1
Final subjects with possible exposure to lead selected for recruitment	3223	100.0
Recruited and enrolled:		
≥2 y	452	14.0
2 months–<2 y	251	7.8
Not eligible:		
Not exposed to tetraethyl lead	289	9.0
History of brain damage	41	1.3
Other health problems	14	0.4
Other‡	108	3.4
Refused (eligibility not established)	436	13.5
Unable to contact or enroll:		
Unable to locate	1145	35.5
Deceased	121	3.7
Out of state	366	11.4

\*Men, aged 40–70 in 1995, employed on or after 1 January 1950.

†Workers were recruited from consecutive random samples of eligible subjects. Duration of employment in the lead area was first confirmed by interview at this stage. Recruitment ended when the target enrollment goal of 700 lead workers was reached.

‡Includes people ineligible by age, and women.

large scale epidemiological studies. To our knowledge, this study represents the largest bone lead and DMSA chelatable lead series published to date, and is the first measurement of either in workers exposed to organolead compounds.

## Methods

### STUDY DESIGN AND OVERVIEW

Data for the study were derived from a 4 year prospective evaluation of central and peripheral nervous system function in current and former employees of a chemical manufacturing factory in the eastern United States which produced tetraethyl (TEL) and tetramethyl (TML) lead. All subjects had past exposure to organic and inorganic lead and none were currently exposed to lead. Subjects were enrolled over a 3 year period and followed up from 2–4 years. During the 4 year data collection period, subjects completed two to four visits in which neurobehavioural function, peripheral nervous system function, blood specimens, tibial lead, chelatable lead, and blood pressure were measured or obtained. The current report is a cross sectional analysis of 543 tibial lead and 504 DMSA chelatable lead measurements obtained during the third year of the study.

### SELECTION OF STUDY SUBJECTS

The plant has manufactured a broad range of chemical products; the present study focused on workers with past employment in the organolead manufacturing area (termed the TEL factory). The TEL factory manufactured TEL from 1923 to 1991 and TML from 1960 to 1983.<sup>9</sup> Efforts were made to recruit two groups of subjects with past exposure to lead: workers with at least 2 years of employment in the TEL area, and workers with between 2 months and 2 years of exposure in the area (low exposure internal controls).

Workers with past lead exposure were identified after reviewing over 45 000 personnel records of current and former employees of the chemical manufacturing factory. Workers were eligible for recruitment if they were ever

employed in the factory on or after 1 January 1950, were male, and were between the ages of 40 and 70 years in 1995. From a review of personnel records, 17 760 men met these initial criteria.

The 17 760 workers were categorised into one of four groups based on the probability of having worked in the TEL area: (a) 2798 workers whose personnel records contained at least one of nine specific work area codes that indicated employment in the TEL manufacturing area; (b) a total of 158 of 222 lead workers who had participated in a 1990 cross sectional study in the plant<sup>9</sup>; (c) workers (n=4214) who held at least one of nine specific job titles (operator, electrician, mechanic, welder, millwright, pipefitter, painter, mason, and lead burner) deemed to signify possible exposure to organic or inorganic lead; and (d) workers who had no indication in their personnel records of having been employed in the TEL factory (n=10 590, table 1). Most of the workers with one of the specific job titles were involved in plant maintenance and repair, but had no specific designated workplace in the personnel record.

Of the 7170 potentially exposed male workers, 3223 were randomly selected for recruitment into the study in a series of consecutive random samples until the target enrollment goal of at least 700 lead workers was reached (table 1). Of the 3223 randomly selected subjects, 1632 (50.6%) could not be contacted or lived out of state (table 1); of the remaining 1591 subjects, 452 (28.4%) were not eligible to participate. Although eligibility status could not be established for the 436 subjects who refused participation, it was assumed that these were similar to those for whom eligibility could be established (specifically, 60.8% (703 / (703+452)) or 265 of the 436 were assumed to be eligible). Of the estimated 968 (703+265) eligible subjects who were contacted, 73% agreed to participate in the study.

### SUBJECT RECRUITMENT

Potentially eligible subjects were first sent a letter explaining the purpose of the study and study procedures. About a week later, subjects were contacted by telephone to obtain consent to administer a brief screening questionnaire. Subjects were excluded from participation if they reported a history of severe head or neck injury, stroke, brain tumour, or general poor health that would not allow compliance with study procedures. Subjects were excluded if they reported that they never spent time in the TEL factory.

Efforts were made to trace all subjects with non-working telephone numbers by calling directory assistance and using a database of United States telephone numbers, by reviewing company pension lists for updated address information, searching Department of Motor Vehicle records and the National Death Index, and by performing a credit search through Transunion (based on the worker's date of birth, social security number, and previous known addresses). For matching records, Transunion provided the person's most recent

address and a list of up to seven of his nearest former neighbours. Subjects with non-published telephone numbers were sent a letter describing the study and requesting that they contact the study office. The final status of all contacted study subjects is summarised in table 1.

#### DATA COLLECTION

The study was approved by the committee for human research at the Johns Hopkins School of Hygiene and Public Health, and all subjects provided informed, written consent. In the first year of the study, subjects completed a general history questionnaire; an occupational history questionnaire; an occupational history interview for exposure assessment (see later); the University of Pennsylvania smell identification test (UPSIT); a 2.5 hour neurobehavioural testing session consisting of both examiner administered and computer administered tests; and provided a 10 ml blood specimen by venepuncture that was stored at  $-70^{\circ}\text{C}$  as plasma, buffy coat, red blood cells, and whole blood. Blood lead was measured from this sample obtained in the first year of the study.

Over 3 years, tibial lead was measured by  $^{109}\text{Cd}$  K  $x$  ray fluorescence (XRF) at the midtibial shaft<sup>7 8</sup> and chelatable lead burden was estimated as 4 hour urinary lead excretion after oral administration of 10 mg/kg DMSA.<sup>5 6</sup>

Tibial lead measurements were obtained in the third year of the study on 543 (77%) of the 703 lead workers being prospectively followed up. Of 452 workers with 2 or more years of employment in the TEL factory, 39 were not able to have a bone lead measurement due to death, relocation to an out of state address, or illness. Of 413 eligible workers, tibial lead measurement was obtained on 356 (86%). Of 251 workers with between 2 months and 2 years of employment, 233 were eligible for bone lead measurement, and tibial lead was obtained on 187 (80%). Of the 543 workers who had tibial lead measured, DMSA chelatable lead was completed on 504. Most of the workers who declined to have DMSA chelatable lead measured did so because of concerns about DMSA interfering with their own current medications.

#### EXPOSURE ASSESSMENT

The methods for assessing exposure to organic and inorganic lead built upon the techniques used in a previous cross sectional study.<sup>9</sup> During the initial interview, the former organolead workers were provided with a summary of their personnel record and a map diagram of the different buildings and areas in the TEL factory. During the interview, subjects were asked to describe the different areas in the plant in which they worked, grouped into 29 exposure zones, and the average percentage of time spent in these areas, for every job ever held in the area.

Personal industrial hygiene sampling data, consisting of about 1500 6–8 hour time weighted samples for each of organic and inorganic lead, were grouped by exposure zone, and the arithmetic means of samples used as estimates of organic and inorganic lead expo-

sure in the zones.<sup>9</sup> Although these samples were available during the period 1979–91, over 50% of the samples were obtained during 1989–91. As there were no data available to estimate temporal trends in exposure within the exposure zones, the arithmetic means of samples obtained from 1979 to 1991 were applied to all previous exposures in the area.

Self reported information on time spent in each zone by job were then multiplied by estimated exposures in the zones and summed for all zones and all jobs in the area to derive individual measures of cumulative exposure to organic and inorganic lead in  $\mu\text{g}/\text{m}^3$ . Cumulative exposure measures were validated by modelling each subject's last blood lead concentration with multiple linear regression.<sup>10</sup> The cumulative exposure measures were divided by duration of exposure to derive lifetime time weighted average intensity of exposure to organic or inorganic lead ( $\mu\text{g}/\text{m}^3$ ).

#### BIOLOGICAL MEASUREMENTS

To estimate chelatable lead burden, 4 hour urinary lead excretion after administration of 10 mg/kg DMSA was measured. Workers self administered the DMSA up to a maximum dose of 800 mg, based on a calculation of body fat content from measurement of skin folds at four body sites with a Lange skinfold caliper (Beta Technology) and their measured weight.<sup>11</sup> Blood and urine lead were measured by an OSHA certified commercial laboratory with NIOSH's standard addition methods<sup>12</sup> by graphite furnace atomic absorption spectrometry.

Tibial lead was assessed (in units of  $\mu\text{g}$  Pb/g of bone mineral) through a 30 minute measurement at the left midtibial shaft using  $^{109}\text{Cd}$  in a back scatter geometry to fluoresce K shell  $x$  rays from lead. These  $x$  rays are then measured to estimate the concentration of lead in bone.<sup>7 8</sup> The emitted K shell  $x$  rays are attenuated as they pass through bone and overlying tissues. The lead  $x$  rays are therefore normalised to the prominent  $\gamma$  ray peak in the spectrum which results from elastically scattered photons (elastic scatter peak); normalisation yields a measurement that is independent of  $\gamma$  ray source to subject distance, subject positioning, small subject movements, overlying tissue thickness, and bone size, shape, geometry, and density.<sup>8</sup>  $^{109}\text{Cd}$  based K shell XRF has been validated against atomic absorption spectrometry of lead in bone samples.<sup>13 14</sup> To ensure accurate and reproducible measurements, bone lead phantoms constructed of plaster of Paris with known concentrations of lead (based on atomic absorption spectrometry) ranging from 0 to 122  $\mu\text{g}$  Pb/g of plaster were regularly measured by the XRF system for calibration and quality control.

Seven subjects had point tibial lead concentration estimates that were below zero. All point estimates were retained in the statistical analyses, including negative values, because this method minimises bias and does not require censoring of data.<sup>15</sup>

Table 2 Characteristics of 543 former organolead manufacturing workers who completed tibial lead measurements in 1996–7

Characteristic	Duration of exposure	
	≥ 2 y	2 months–<2 y
Study subjects, enrolled in previous years (n)	452	251
Eligible study subjects (n)*	413	233
Received tibial lead measurement (n (%))†	356 (86)	187 (80)
Age (y, mean (SD))	57.7 (7.7)	57.5 (7.2)
Exposure duration (y, mean (SD))	12.1 (10.2)	0.9 (0.5)
Duration since last exposure (y, mean (SD))	13.8 (9.9)	26.3 (10.1)
Education (%):		
Less than high school	7.8	7.0
High school	58.0	61.1
Some college	29.1	26.5
Completed college or graduate	5.0	5.4
Race (%):		
White	93.0	92.4
African-American	5.3	6.5
Other	1.7	1.1
Tobacco use (%):		
Never	29.5	23.8
Current	18.5	20.5
Past	52.0	55.7
Alcohol use (%):		
Never	3.7	2.7
Current	71.6	74.6
Past	24.7	22.7
Height (cm, mean (SD))	177.8 (6.9)	175.5 (6.5)
Weight (kg, mean (SD))	93.6 (16.4)	91.4 (16.2)
Tibial lead (µg/g, mean (SD))	15.6 (9.8)	12.1 (7.7)
Tibial lead measurement error (µg/g, mean (SD))	5.1 (1.0)	5.2 (1.0)
DMSA chelatable lead (µg, mean (SD))	17.1 (15.7)	20.4 (17.9)
Blood lead (µg/dl, mean (SD))	5.0 (2.8)	3.8 (1.9)

\*Excluding subjects who were dead, out of state, or too ill to participate.

†Of eligible subjects.

#### STATISTICAL ANALYSIS

The major goals of the analysis were to identify predictors of tibial lead and DMSA chelatable lead. Variables examined relative to tibial and chelatable lead were age, race, tobacco use, alcohol consumption, selected medical conditions that may affect bone or lead metabolism—for example, thyroid disease, diabetes—daily physical activities—for example, stairs climbed, miles walked—exercise, weight, height, chelatable lead, cumulative exposure, duration of exposure, and duration since last exposure. To examine the influence of lead exposure, lifetime time weighted

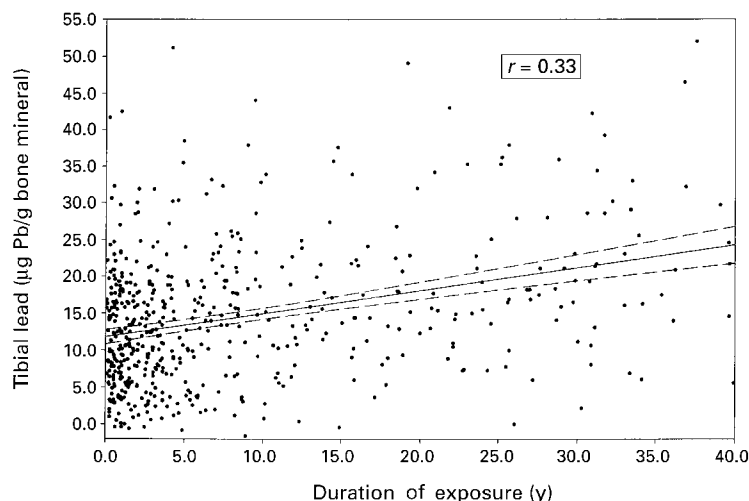


Figure 1 Crude (unadjusted for confounding variables) relation of duration of exposure (y) with tibial lead concentrations (µg Pb/g bone mineral) in 541 former organolead manufacturing workers with past exposure to organic and inorganic lead. Two subjects had missing information on duration of exposure. Dashed lines represent 95% CIs on either side of the linear regression line. The Pearson's  $r$  for the relation was 0.33 ( $p < 0.001$ ). Addition of a quadratic term for duration of exposure did not significantly improve the relation with tibial lead concentrations.

average intensity of exposure ( $\mu\text{g}/\text{m}^3$ ) for each of inorganic and organic lead, duration of exposure, and cumulative exposure (duration (y)  $\times$  intensity ( $\mu\text{g}/\text{m}^3$ )) for each of inorganic and organic lead were evaluated in the same linear regression models.

Multiple linear regression (BMDP Statistical Software, Los Angeles, California) was used to model tibial lead and DMSA chelatable lead, controlling for such variables as age, duration of exposure, and tobacco and alcohol consumption. Variables that were considered potential confounders initially were first entered into the models, and additional study variables of interest were next added to these initial models. Only variables that were independent predictors of tibial lead or chelatable lead were retained in the final models. All linear regression models were evaluated for the influence of outliers, multicollinearity, departures from normality, and heteroscedasticity. To examine non-linear effects, linear and quadratic terms were evaluated for all continuous variables.

To directly compare and contrast bone and chelatable lead, the difference between tibial lead and chelatable lead was used as the dependent variable in a series of regressions. To standardise units between these two variables, tibial lead and chelatable lead were first Z transformed (Z transformed variables have a mean (SD) of 0 (1)), and the differences between tibial lead and chelatable lead were obtained.

#### Results

The subjects who received tibial lead measurements were primarily white (93%) and had mean (SD, range) age of 57.6 (7.6, 41.8–73.8) years, tibial lead concentrations of 14.4 (9.3, –1.6–52.0)  $\mu\text{g}$  Pb/g bone mineral, DMSA chelatable lead of 19.3 (17.2, 1.2–136)  $\mu\text{g}$ , and blood lead concentrations of 4.6 (2.6, 1–20)  $\mu\text{g}/\text{dl}$  (table 2). Blood lead concentrations were moderately correlated with both tibial lead (Pearson's  $r=0.43$ ,  $p < 0.001$ ) and DMSA chelatable lead concentrations (Pearson's  $r=0.40$ ,  $p < 0.001$ ).

Tibial lead concentrations increased with both increasing age (Pearson's  $r=0.45$ ,  $p < 0.001$ ) and duration of exposure (fig 1), but the correlation of tibial lead with age was higher than that for duration of exposure. Tibial lead concentrations did not decline with increasing duration since last exposure to lead in the workplace (Pearson's  $r=0.01$ ,  $p > 0.05$ ) and were only modestly correlated with DMSA chelatable lead (Pearson's  $r=0.17$ ,  $p < 0.01$ , fig 2). Notably, the respective correlations between tibial lead and cumulative or lifetime time weighted average exposure variables were lower than those with duration of exposure (tibial lead  $v$  cumulative exposure to inorganic lead, cumulative exposure to organic lead, lifetime time weighted average exposure to organic lead, and lifetime time weighted average exposure to inorganic lead were 0.31, 0.31, 0.07, and –0.03, respectively).

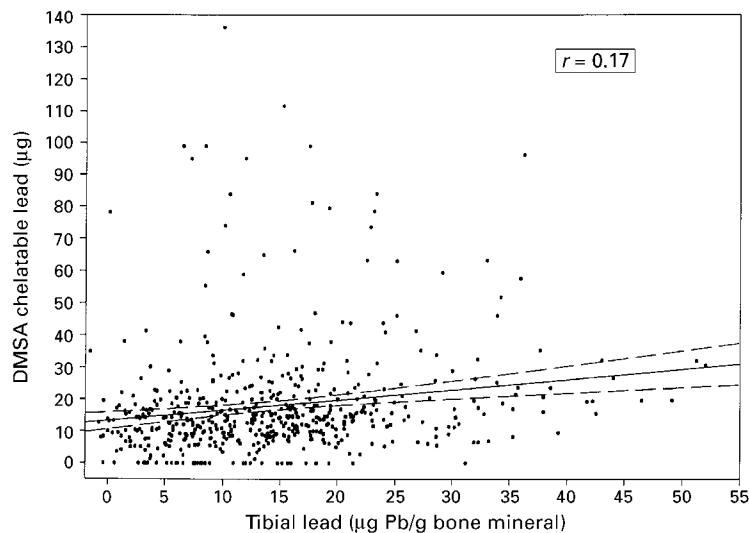


Figure 2 Crude (unadjusted for confounding variables) relation of DMSA chelatable lead (4 h lead excretion in  $\mu\text{g}$ ) with tibial lead concentrations ( $\mu\text{g Pb/g bone mineral}$ ) in 504 former organolead manufacturing workers with past exposure to organic and inorganic lead. Thirty nine subjects with tibial lead measurements did not have DMSA chelatable lead measured. Dashed lines represent 95% CIs on either side of the linear regression line. The Pearson's  $r$  for the relation was 0.17 ( $p < 0.01$ ).

#### PREDICTORS OF TIBIAL LEAD

Several independent predictors of tibial lead concentrations were identified. Tibial lead concentrations increased with both increasing age and duration of exposure (table 3). The relation with duration of exposure was not linear, but rather plateaued at higher durations of exposure (note the negative slope of the  $\beta$  coefficient for the quadratic term for duration of exposure in table 3). Current smokers, past smokers, and people diagnosed by a physician as being diabetic all had higher tibial lead concentrations, whereas subjects who reported exercise that induced sweating had lower tibial lead concentrations. Tibial lead concentrations decreased slightly with increasing height. Of note, tibial lead concentrations increased with increasing smoking pack-years in 381 current and past smokers (the  $\beta$  coefficient for pack-years among current and past smokers was  $0.036 \mu\text{g Pb/g bone mineral/pack-year}$ ,  $p = 0.04$ ).

The final model, with six variables (age, duration of exposure, smoking, diabetes, height, and exercise that induced sweating), accounted for 31% of the variance in tibial lead concentrations. Of this, 27% was explained by age and duration of exposure (linear and quad-

ratic terms) alone; the other four variables accounted for about 1% of the variance each.

Other medical conditions—for example, thyroid disease, arthritis—different methods of modelling exposure—for example, cumulative exposure, lifetime time weighted average exposure, for both inorganic and organic lead—duration since last exposure, education, race, alcohol consumption, duration since stopping smoking or drinking, and different measures for exercise—for example, weight lifting, running, swimming—were all evaluated for their influence on tibial lead concentrations, and none were found to be independent predictors of tibial lead concentrations.

#### PREDICTORS OF CHELATABLE LEAD CONCENTRATIONS

Tibial lead, cumulative exposure to inorganic lead, and weight were independent, positive predictors of DMSA chelatable lead (table 4). Current smokers had higher chelatable lead, consistent with their higher tibial lead concentrations. Of interest, although people with diabetes diagnosed by a physician had higher tibial lead concentrations ( $8.9 \mu\text{g/g}$  higher than in people without diabetes), they had lower chelatable lead ( $6.7 \mu\text{g}$  lower, table 4, model 1). When blood lead was added to this model, tibial lead, cumulative exposure to inorganic lead, and diabetes no longer reached significance; blood lead accounted for the largest proportion of variance in DMSA chelatable lead; and the total model  $r^2$  increased to 19% (table 4, model 2). As with the tibial lead models, the other factors previously mentioned were not significant predictors of chelatable lead when added to the model.

Tibial lead and cumulative exposure to inorganic lead both independently predicted DMSA chelatable lead. The  $\beta$  coefficients for either of these variables declined by about 25% when the other was added to the linear regression model.

#### PREDICTORS OF TIBIAL LEAD: CHELATABLE LEAD DIFFERENCE

In multiple linear regression, the difference between tibial and chelatable lead (expressed in standardised units) was used to assess predictors of the relative amount of lead in tibia and soft tissue. This difference increased with increasing age ( $\beta = 0.054$ ,  $p < 0.01$ ) and duration of exposure ( $\beta = 0.018$ ,  $p < 0.01$ ), and was higher

Table 3 Linear regression modelling\* results identifying predictors of concentrations of tibial lead in 541 former organolead manufacturing workers, 1996–7

Independent variables	Units of $\beta$ coefficient	$\beta$ coefficient	SE $\beta$	p Value†	Model $r^2$
Age	$\mu\text{g Pb/g bone mineral/y}$	0.477	0.048	<0.01	31%
Exposure duration	$\mu\text{g Pb/g bone mineral/y}$	0.370	0.062	<0.01	
Exposure duration squared‡	$\mu\text{g Pb/g bone mineral/y}^2$	-0.007	0.004	0.05	
Current smoking	$\mu\text{g Pb/g bone mineral}$	2.940	1.003	<0.01	
Past smoking	$\mu\text{g Pb/g bone mineral}$	1.545	0.796	0.05	
Diabetes	$\mu\text{g Pb/g bone mineral}$	3.327	1.302	0.01	
Height	$\mu\text{g Pb/g bone mineral/cm}$	-0.108	0.051	0.03	
Exercise inducing sweating	$\mu\text{g Pb/g bone mineral}$	-1.480	0.709	0.04	

\*Final model presented after evaluating the influence of age, education, tobacco and alcohol use, race, exercise, medical history, and several methods of evaluating exposure to inorganic and organic lead, including cumulative exposure, lifetime weighted average intensity of exposure, and duration of exposure (see methods).

†From  $\beta/\text{SE } \beta$ ; p values may not correspond exactly because of rounding, to three significant digits, of the  $\beta$  coefficients and the SEs.

‡To account for the non-linear association between duration of exposure and concentrations of tibial lead.

Table 4 Linear regression modelling\* results identifying predictors of DMSA chelatable lead in 503 former organolead manufacturing workers, 1996–97

Independent variables	Units of $\beta$ coefficient	$\beta$ coefficient	SE $\beta$	p Value†	Model $r^2$
Model 1: without blood lead:					
Tibial lead	$\mu\text{g}/(\mu\text{g lead/g bone mineral})$	0.228	0.084	0.01	11%‡
Cumulative exposure to inorganic lead	$\mu\text{g}/\mu\text{g.y}/\text{m}^3$	0.013	0.005	0.01	
Current smoking	$\mu\text{g}$	8.872	1.852	<0.01	
Diabetes	$\mu\text{g}$	-6.748	2.794	0.02	
Weight	$\mu\text{g}/\text{kg}$	0.160	0.050	<0.01	
Weight squared§	$\mu\text{g}/\text{kg}^2$	-0.004	0.002	0.02	
Model 2: with blood lead:					
Tibial lead	$\mu\text{g}/(\mu\text{g lead/g bone mineral})$	0.007	0.087	0.94	19%
Cumulative exposure to inorganic lead	$\mu\text{g}/\mu\text{g.y}/\text{m}^3$	0.003	0.005	0.64	
Current smoking	$\mu\text{g}$	6.221	1.839	<0.01	
Diabetes	$\mu\text{g}$	-4.141	2.746	0.13	
Weight	$\mu\text{g}/\text{kg}$	0.149	0.049	<0.01	
Weight squared‡	$\mu\text{g}/\text{kg}^2$	-0.004	0.005	0.03	
Blood lead	$\mu\text{g}/\mu\text{g}/\text{dl}$	2.345	0.327	<0.01	

\*Final model presented after evaluating influence of age, education, tobacco and alcohol use, race, exercise, medical history, and several methods of evaluating exposure to inorganic and organic lead, including cumulative exposure, lifetime weighted average intensity of exposure, and duration of exposure (see methods).

†From  $\beta$ /SE  $\beta$ ; p values may not correspond exactly because of rounding, to three significant digits, of the  $\beta$  coefficients and the SEs.

‡The total model  $r^2$  increased to 15% when the duration of urine collection was added to the model, and this variable had an associated p Value of 0.04. This variable did not change the significance or  $\beta$  coefficient of any variables in the model, but was not included because 23 workers were missing this information.

§To account for the non-linear association between weight and of DMSA chelatable lead.

in ex-smokers ( $\beta=0.33$ ,  $p<0.01$ ) and diabetic people ( $\beta=0.70$ ,  $p<0.01$ ). This difference decreased with increasing weight ( $\beta=-0.079$ ,  $p=0.02$ ). Blood lead was not a predictor of the difference between tibial lead and chelatable lead, nor were any other variables ( $p\leq 0.05$ ). The associations with age and duration of exposure raised the specific question whether this difference increased with increasing time since last exposure, but time since last exposure was not a meaningful predictor.

### Discussion

In this large cross sectional study of 543 former organolead manufacturing workers, several independent predictors of tibial lead and DMSA chelatable lead were identified. Tibial lead concentrations increased with increasing age and duration of exposure, but age was not associated with chelatable lead. Cumulative exposure to inorganic lead (accounting for both exposure intensity and duration) was a better predictor of chelatable lead than was duration of exposure. Current smoking was associated with tibial and chelatable lead, but past smoking was only associated with tibial lead. Diabetic people had higher tibial lead but lower chelatable lead. Finally, weight was a positive predictor of chelatable lead, whereas height was a negative predictor of tibial lead concentrations. Notably, the final regression models accounted for only 31% of the variance in tibial lead and 11% (without blood lead) or 19% (with blood lead) of the variance in chelatable lead. Although some of the unexplained variance may be accounted for by measurement error in predictor variables and in tibial lead and chelatable lead measures, it is also possible that metabolic mediators of lead distribution, deposition, and excretion that differ among individual people may also account for some of this unexplained variation among study subjects.

In this study of former organolead manufacturing workers, mean blood lead, DMSA chelatable lead, and tibial lead were all low.

These are comparable with those that might be obtained from the general population of similar ages in the same geographical area. The former lead workers had an overall mean (range) of 18 (1–48.7) years since last exposure to lead, and 25% of workers had more than 16 years since last exposure to lead. No previous studies have evaluated tibial lead or DMSA chelatable lead in organolead workers. Although duration since last exposure certainly accounts for some subjects with low biomarker concentrations, the current data would also suggest that organolead exposure may contribute less to lead body burden than would a similar exposure to inorganic lead.

In subjects with environmental exposure, and not occupational exposure, to lead, age has been consistently identified as the strongest predictor of bone lead concentrations. It is thought to be a surrogate for lifetime cumulative lead exposure (relations of age with bone lead are discussed by Kosnett *et al.*,<sup>16</sup>  $r=0.83$ ; Morgan *et al.*,<sup>17</sup>  $r=0.62$ ; Drasch *et al.*,<sup>18</sup>  $r=0.62$ ). The current results are consistent with these studies; however, in this occupationally exposed group, duration of occupational exposure to lead was also an independent predictor of tibial lead concentrations. These findings are consistent with the hypothesis that age is a surrogate for non-occupational sources of exposure and that duration of exposure is a meaningful surrogate for occupational exposure to lead.

Duration of exposure was found to be the best measure of occupational exposure to lead as a predictor of tibial lead concentrations. Linear regression models evaluated duration of exposure (y), cumulative exposure (duration  $\times$  intensity in  $\text{y}\cdot\mu\text{g}/\text{m}^3$ ), and average exposure intensity ( $\mu\text{g}/\text{m}^3$ , cumulative exposure / duration of exposure) for each of organic and inorganic lead. Only duration of exposure was found to be an independent predictor of tibial lead concentrations. This finding is consistent with the concept that for exposures to toxicants with long half lives, body burden will be most

influenced by duration of exposure rather than variations in exposure intensity.<sup>19</sup>

The current study had considerably more power than earlier studies,<sup>5,6</sup> and identified several independent predictors of DMSA chelatable lead. Identification of current smoking and weight as predictors of chelatable lead is consistent with earlier studies. Current smokers had higher chelatable lead even after controlling for tibial lead concentrations, which were higher in current and past smokers. Higher chelatable lead in current smokers may be due to induction of enzymatic pathways. It is thought that DMSA must undergo enzymatic linkage to cysteine before it chelates lead,<sup>20</sup> and we speculate that this enzymatic linkage may be induced by tobacco use. Weight may be a surrogate measure for soft tissue mass, the lead stores from which DMSA chelates lead. Associations between DMSA chelatable lead and tibial lead, cumulative exposure to inorganic lead, and diabetes have not, to our knowledge, been previously reported.

Tibial lead concentrations are thought to be a measure of lifetime burden of retained lead, reflecting both cumulative exposure to lead and the influence of excretory pathways that eliminate lead from bone with an estimated half life of 10–15 years.<sup>21,22</sup> By contrast, DMSA chelatable lead are thought to reflect current bioavailable lead burden<sup>5</sup>; evidence suggests that DMSA removes lead from blood and soft tissue and minimally, if at all, from bone.<sup>5,6,23</sup> As these soft tissue sites, in former workers, are replenished from bone lead stores, it was expected that tibial lead concentrations would be an independent predictor of chelatable lead, as was found. The finding that age and duration of exposure predict tibial lead concentrations, but not chelatable lead, supports the notion that tibial lead reflects cumulative exposure. By contrast, DMSA chelatable lead is an estimate of current lead stores that can be accessed by DMSA, which can be termed current bioavailable lead stores. The finding that tibial lead and cumulative exposure to inorganic lead were no longer associated with chelatable lead after addition of blood lead to the model supports the notion that tibial lead and cumulative exposure to inorganic lead account for the blood and soft tissue stores from which DMSA chelates lead.

The finding that cumulative exposure to inorganic lead predicts DMSA chelatable lead suggests that this measure may account for lead deposition in and release from other bone sites that are not fully accounted for by the tibial lead measurement. It is known that lead deposition in bone is not homogeneous and release of lead from bone is likely to differ by bone type—for example, cortical *v* trabecular<sup>7</sup>; a single tibial lead measurement does not estimate the deposition of lead in other bone sites that can replenish the soft tissue sites from which DMSA removes lead.

We used multiple linear regression to model the differences, in standardised units, between tibial and chelatable lead to evaluate how the amount of total body lead that is chelatable differs by such factors as age, duration of expo-

sure, medical history, and smoking. The differences between tibial and chelatable lead increased with increasing age and duration of exposure, despite the findings that (a) tibial lead increased with increasing age and duration of exposure, and (b) chelatable lead increased with increasing tibial lead. The results of this model provide direct evidence that in men the total amount of lead in the body that is bioavailable decreases with age.

Although numerous studies have examined the associations between age, current blood lead concentrations, and duration of exposure with bone lead concentrations,<sup>24,27</sup> few studies in occupationally exposed subjects have examined the influence of other predictor variables. Hu *et al* reported that bone lead concentrations declined with increasing time since last exposure to lead,<sup>28</sup> by contrast with the finding reported here. In 127 carpenters with low to moderate bone lead and blood lead concentrations, Watanabe *et al* reported that age, duration since last exposure, and welding were independent predictors of tibial lead concentrations, whereas age, paint stripping, regular exercise (negative  $\beta$  coefficient), and carpet laying were independent predictors of patellar lead concentrations.<sup>29</sup>

In conclusion, the data show that the predictors of tibial lead and DMSA chelatable lead are different. The two measures are poorly correlated, suggesting that they would rank subjects differently along an exposure gradient. These and earlier data support the notion that tibial lead is a measure of cumulative lead absorption, whereas DMSA chelatable lead may be a measure of current bioavailable lead. This finding is very helpful and important in the evaluation of the health effects of lead. Evaluation of both measures, one as an estimate of recent exposure and the other as an estimate of cumulative exposure, should allow inferences to be made about whether observed health effects are likely to be acute, and thus reversible, or chronic, and thus probably irreversible. Such inferences could be used in the identification of optimal environmental and occupational monitoring approaches and risk management or intervention strategies, and the design of subsequent toxicological and epidemiological research.

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## Predictors of dimercaptosuccinic acid chelatable lead and tibial lead in former organolead manufacturing workers.

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