In vivo tibia lead measurements as an index of cumulative exposure in occupationally exposed subjects

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ABSTRACT In vivo tibia lead measurements of 20 non-occupationally exposed and 190 occupationally exposed people drawn from three factories were made using a non-invasive x-ray fluorescence technique in which characteristic x rays from lead are excited by gamma rays from a cadmium-109 source. The maximum skin dose to a small region of the shin was 0.45 mSv. The relation between tibia lead and blood lead was weak in workers from one factory (r = 0.11, p > 0.6) and among the non-occupationally exposed subjects (r = 0.07, p > 0.7); however, a stronger relation was observed in the other two factories (r = 0.45, p < 0.0001 and r = 0.53, p < 0.0001). Correlation coefficients between tibia lead and duration of employment were consistently higher at all three factories respectively (r = 0.86, p < 0.0001; r = 0.61, p < 0.0001; r = 0.80, p < 0.0001). A strong relation was observed between tibia lead and a simple, time integrated, blood lead index among workers from the two factories from which blood lead histories were available. The regression equation from two groups of workers (n = 88, 79) did not significantly differ despite different exposure conditions. The correlation coefficient for the combined data set (n = 167) was 0.84 (p < 0.0001). This shows clearly that tibia lead, measured in vivo by x-ray fluorescence, provides a good indicator of long term exposure to lead as assessed by a cumulative blood lead index.

As a consequence of the well established toxicity of lead, workers occupationally exposed to it in the United Kingdom and other industrialised countries are subjected to regular monitoring of blood lead concentrations. In continuous employment under relatively stable conditions the frequency of this monitoring in the United Kingdom depends on the level of exposure. For example, at blood lead concentrations of 40 μg/dl monitoring should be increased from 12 to six monthly intervals, whereas concentrations in excess of 60 μg/dl require three monthly monitoring. At concentrations exceeding 70 μg/dl subjects may be suspended from work involving lead exposure. The major pool of lead in blood has a half life of only two to three weeks, however, so that it is essentially only an index of recent exposure. Thus to establish the relation between health effects (nephrotoxicity, for example) and long term exposure to lead it is necessary to establish a means of monitoring cumulative exposure.

The principal organ of accumulation of lead is the bone, which contains over 95% of the body burden, and has a component with a long half life, of the order of 30 years. Consequently, measurement of skeletal lead concentrations could provide an index of cumulative exposure. There are, however, differences in metabolic activity between different bone types, cortical bone being generally less active than trabecular. This was one of the reasons for selecting the tibia as the initial measurement site, since it is one of the larger cortical bones. In addition, it is a superficial bone and measurement allows minimal disturbance to the subject.

We note here that an important feature of bone lead measurement is the need to normalise it to the mass of bone sampled. Where the bone composition
In vivo tibia lead measurements as an index of cumulative exposure in occupationally exposed subjects

is relatively stable, as with the tibia, it is feasible to normalise per mass of wet bone. The relation between wet bone mass and bone mineral in trabecular bone, however, is less well defined and changes with, among other things, age, particularly in women. Because our technique normalises to the gamma rays coherently scattered from both calcium and phosphorus, the most logical normalisation is therefore to the bone mineral mass. This is equivalent to quoting the lead content per mass of bone ash, a unit that is widely used for in vitro analysis. A possible alternative, particularly for those making biopsy measurements using atomic absorption spectrometry, is to normalise to the calcium content: however, the relation between the two procedures is readily established assuming bone mineral to consist of calcium hydroxyapatite (Ca$_{10}$(PO$_4$)$_6$(OH)$_2$). As our measurement programme is being extended to include trabecular bone we have therefore chosen to normalise to bone mineral mass throughout.

x Ray fluorescence, which involves stimulation of characteristic x ray emission from the element of interest using a beam of photons, has been used by several groups to measure bone lead. The first to do so were Ahlgren and co-workers, who measured the lead $K_\alpha$ x ray emission (at 75-0 and 72-8 keV for $K_{\alpha 1}$ and $K_{\alpha 2}$, respectively) from lead in finger bones, and who determined bone mineral content using a measurement of the ratio of the coherently scattered gamma rays to those Compton scattered, a reliable technique that has been used independently to measure bone mineral. A similar approach was used by Price et al, both groups using a $^{57}$Co source, having gamma rays at 122 and 136 keV. Wielopolski et al, on the other hand, used different gamma ray sources in their measurements of the L x ray intensities in tibia: the energies of these x rays are 10-5 keV ($L_{\alpha}$) and 12-5 keV ($L_{\beta}$). As was noted by Wielopolski, the increased attenuation of these low energy photons compared with the K x rays means that, effectively, only the first 0-3 mm layer of the bone surface is sampled. Full details of all the experimental techniques will be found in the papers cited.

**x Ray fluorescence technique**

The x ray fluorescence technique used in the present paper has been discussed in detail by Somervaille et al, and the in vitro verification, by comparison with atomic absorption spectrometry, has been described in a related paper. A cadmium-109 source was used, having a gamma ray energy of 88-035 keV and the lead K x rays were monitored. Because the $^{109}$Cd gamma ray energy is only just above the lead K-edge (at 88-005 keV) the emitted K x ray intensity is proportional to the flux of uncollided source gamma rays. The technique takes advantage of this fact and normalises the lead signals to the intensity of coherently (elastically) back scattered gamma rays. For a typical tibia measurement, 98% of these coherent scatter signals arise from scattering in bone mineral, rather than from collagen or from overlying, or surrounding, tissue, because of the fifth or sixth power dependence of the coherent scattering cross section on atomic number, Z, at these large angles. This means that the lead signal is related directly to bone mineral content in a single measurement. It also means that the accuracy of the technique does not depend on either bone geometry or on the depth of overlying tissue, and that the latter affects only the measurement sensitivity.

For an overlying tissue depth of 3 mm the minimum detectable lead concentration in the tibia is $\sim$ 18 $\mu$g/g bone mineral ($\sim$ 10 $\mu$g/g wet bone using the bone composition data of Woodard). Because of the method of data analysis used (which is discussed below) we define this minimum detectable concentration to be when the lead peak is twice the uncertainty (total signal—background) in the limit that the lead peak approaches zero. Other groups often define it to be when the lead peak is three times the standard deviation on the underlying background; our definition is equivalent to a factor of 2.83 rather than 3.

In the present paper we report on the first measurements made using a $^{109}$Cd source with coherent scatter normalisation, which are in four groups of subjects—namely, a small group of normal subjects and three occupationally exposed populations.

**Measurement protocol**

The measurement geometry is shown in fig 1, and a view of the system in use on a subject is shown in fig 2. The $^{109}$Cd source, with an activity of between 3-7 and 7-4 GBq (100–200 mCi), is annular and coaxial with a planar hyperpure germanium (HpGe) detector. Through the centre of the source is a tungsten alloy collimator that defines the solid angle viewed by the detector; this geometry determines the bone volume sampled, attenuation and 1/r$^2$ effects then defining the relative detection efficiency from different parts of the volume.

The subjects were seated with the measurement axis at the mid-point of the tibia shaft. The source to skin surface distance was 3 cm, a sensor attached to a bell being used to monitor movement during the 20 minute measurement period. Although, because of the normalisation procedure used, such a precaution is not necessary from the accuracy point of view, it produces a more uniform measurement sensitivity. The radiation dose is small, being only 100 $\mu$Sv (10
mrem) to 1.5–2.0 kg of soft tissue and bone. The whole body dose equivalent of this irradiation is about 3 μSv (0.3 mrem) compared with the United Kingdom average annual dose from natural radiation background of 1.86 mSv (186 mrem). The corresponding maximum skin dose is 450 μSv (45 mrem). Such a low dose means that repeat measurements and, in particular, longitudinal studies may be readily undertaken without serious ethical implications.

The measured photon spectrum from a subject having a tibia lead concentration of 123 μg/g bone mineral (69 μg/g wet bone) is shown in fig 3. To extract the peak areas from the underlying Compton background a non-linear least squares fitting procedure was used. We note that, in addition to the two Kα peaks that are apparent in fig 3, there are also smaller lead signals from the Kβ lines at 84.9, 87.23, 84.5 keV respectively. Each peak was therefore assumed to be Gaussian in shape and pairs of peaks (Kαx and Kαβ, Kβx and Kββ) and the elastic scatter peak) were fitted on a background approximated by a single or double exponential function.

To relate these measurements to absolute bone lead concentrations, tibia shaped, lead doped plaster of Paris phantoms were used. Because the lead content was related to bone mineral in the measurement field through the coherent scattering normalisation it was assumed to be Gaussian in shape and pairs of peaks (Kαx and Kαβ, Kβx and Kββ) and the elastic scatter peak) were fitted on a background approximated by a single or double exponential function.

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necessary to allow for differences in the coherent scattering intensity between the plaster of Paris matrix (CaSO₄. 2H₂O) and bone. The cortical bone composition assumed was that of Woodard. Using her data we may also determine the concentration in terms of μg lead per g wet bone, a figure that is often quoted: the ratio of (concentration in wet bone) to (concentration in bone mineral) was found to be 0.57. We note, however, that for trabecular bone, which is more metabolically active and for which the collagen content varies more widely, one would always seek to relate lead measurements to bone mineral content.

In addition to making the bone lead measurements, blood and urine samples were also obtained. These samples were analysed for a wide range of parameters including, in blood, lead, haemoglobin (Hb), and free erythrocyte protoporphyrin (FEP) whereas the urine samples were analysed for lead and for δ-aminolaevulinic acid (ALA). These analyses were performed at the Occupational Medicine and Hygiene Laboratory of the Health and Safety Executive at Cricklewood and monitored by routine quality control procedures. The lead related work history of each subject together with details of diet, smoking and drinking habits, hobbies involving lead, and of general health were also obtained, using a computerised questionnaire.

**STUDY POPULATION**

Four groups of subjects have been studied. The first, consisting of 12 men and eight women, were not occupationally exposed to lead and were volunteers, principally from the department of physics. They do not in any way constitute a proper normal population but do provide a base line for comparison. The other three groups were all volunteers occupationally exposed to inorganic lead or its compounds.

Fifteen men were monitored from factory A, a precious metal smelter. Their exposure to lead (both as fume and dust) arose through its use in a molten lead alloy for extracting precious metals from scrap material. Although a small population, as a pilot study it provided valuable information as well as experience in conducting bone lead measurements in the factory environment.

Factory B was a much larger plant engaged in lead acid battery manufacture. Here 88 people were monitored, including five women, and a wide range of occupations were represented with corresponding variation in exposure levels. Exposure was to metallic lead, lead oxide, and tribasic lead sulphate (PbO₃ PbSO₄) both as dust and, in some cases, fume. Once again, all the bone lead measurements took place inside the factory, in this case in the medical department.

In factory C 87 people were monitored, including six women. This plant was concerned with manufacturing lead crystal glass. The large majority of the volunteers here could be divided into two main exposure groups; those working with the molten glass, where exposure was to both fume and dust, and those engaged in glass cutting, where the exposure was mainly to dust. In this third survey all the measurements were carried out in a specially converted caravan, which greatly simplified the practical difficulties in setting up such a survey and caused considerably less disruption to the factory.

**RESULTS AND DISCUSSION**

The table shows a summary of the data including the more usual biochemical indices of lead exposure, such as blood and urine lead, FEP, ALA, and Hb. We note that, to compare the group means, the uncertainties quoted are standard errors of the mean of N results (σ/√(N−2)) and not the standard deviations about the mean (σ).

Both Barry in an analysis of postmortem data and Somervaille et al, analysing the data on normals reported here, have shown that bone lead increases with age in non-occupationally exposed subjects, ris-

### Summary of data from the four groups

<table>
<thead>
<tr>
<th>Group averaged value of:</th>
<th>Non-occupationally exposed</th>
<th>No</th>
<th>Factory A</th>
<th>No</th>
<th>Factory B</th>
<th>No</th>
<th>Factory C</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49.3 ± 3.7</td>
<td>20</td>
<td>41.3 ± 3.5</td>
<td>15</td>
<td>45.5 ± 1.0</td>
<td>88</td>
<td>27.7 ± 1.0</td>
<td>87</td>
</tr>
<tr>
<td>Working at factory (years)</td>
<td>78 ± 1.4</td>
<td>14</td>
<td>14.8 ± 0.8</td>
<td>88</td>
<td>10.6 ± 1.1</td>
<td>87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tibia lead:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μg/g wet bone)</td>
<td>9.4 ± 2.1</td>
<td>20</td>
<td>30.9 ± 5.9</td>
<td>15</td>
<td>18.2 ± 1.7</td>
<td>88</td>
<td>17.5 ± 1.9</td>
<td>87</td>
</tr>
<tr>
<td>(μg/g ashed bone)</td>
<td>16.7 ± 3.7</td>
<td>20</td>
<td>54.8 ± 10.6</td>
<td>15</td>
<td>32.3 ± 3.0</td>
<td>88</td>
<td>31.0 ± 3.4</td>
<td>87</td>
</tr>
<tr>
<td>Blood lead (μg/dl)</td>
<td>13.1 ± 1.6</td>
<td>17</td>
<td>51.4 ± 3.4</td>
<td>15</td>
<td>32.3 ± 1.2</td>
<td>88</td>
<td>48.1 ± 1.8</td>
<td>87</td>
</tr>
<tr>
<td>Urine lead (μg/dl)</td>
<td>11.4 ± 3.7</td>
<td>5</td>
<td>93.6 ± 16.5</td>
<td>14</td>
<td>51.4 ± 9.4</td>
<td>88</td>
<td>74.3 ± 6.4</td>
<td>86</td>
</tr>
<tr>
<td>FEP (μg/dl)</td>
<td>12.0 ± 2.5</td>
<td>10</td>
<td>97.2 ± 16.4</td>
<td>15</td>
<td>32.2 ± 4.2</td>
<td>78</td>
<td>96.9 ± 10.6</td>
<td>54</td>
</tr>
<tr>
<td>ALA (urine) (μmol/mmol creat)</td>
<td>2.4 ± 0.2</td>
<td>15</td>
<td>4.5 ± 0.7</td>
<td>15</td>
<td>2.5 ± 0.2</td>
<td>88</td>
<td>3.9 ± 0.5</td>
<td>81</td>
</tr>
<tr>
<td>Haemoglobin (g/dl):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>15.1 ± 0.2</td>
<td>12</td>
<td>14.9 ± 0.4</td>
<td>15</td>
<td>15.0 ± 0.1</td>
<td>82</td>
<td>15.4 ± 0.1</td>
<td>80</td>
</tr>
<tr>
<td>Women</td>
<td>14.5 ± 0.2</td>
<td>8</td>
<td>—</td>
<td>—</td>
<td>14.6 ± 0.2</td>
<td>5</td>
<td>14.1 ± 0.1</td>
<td>6</td>
</tr>
</tbody>
</table>

Note Errors quoted are standard errors of the mean = σ/√(N−2).
ing by about 0.4 µg/g (wet bone) a year in the United Kingdom. Although the average age of the normal group was significantly higher than for the three exposed groups, their tibia lead concentrations were the lowest of all the four groups. The normal group concentrations for blood and urine lead, and for FEP, are also the lowest, although there was no significant difference in the haemoglobin concentrations.

Examination of the data from the three exposed groups shows that their exposure histories and biochemical signatures have several differences. Figure 4 shows the distribution of lead exposure (identified by both blood and tibia lead concentrations) as well as the age distribution for all four groups. Interestingly, the blood lead distributions from factories A and C are somewhat similar, as are their mean values (51.4 and 48.1 µg/dl respectively), but the tibia lead concentrations seen in factory C were, on average, lower than in factory A. This may seem somewhat anomalous, since the average number of years worked in both factories are similar (7-8 and 10-0 years respectively). Workers from factory A, however, reported that working conditions had improved over the past years whereas conditions in factory C seemed to have remained more constant. In addition the age distribution of the population studied from factory C differed somewhat from that of factory A, the means being 27.7 and 41.3 years respectively, and 40% of those surveyed from factory A said that they had been previously employed in lead or lead related industries compared with only 15% in factory C.

Since the concentration of lead in the blood samples taken at the time of each survey reflects the exposure conditions at the time, whereas the tibia lead concentrations reflect more cumulative exposure, we would, perhaps, expect the relation between blood and tibia lead to be weak. This is certainly true of the non-occupationally exposed group (correlation coefficient r = 0.07, p > 0.7) and also of factory A (r = 0.11, p > 0.6). A stronger relation is apparent in factory B (r = 0.45, p < 0.0001) and in factory C (r = 0.53, p < 0.0001), however, which may reflect working practices that have varied less with time in both these firms. In addition, age is an important confounding variable in terms of tibia lead, and indeed significant relations exist between age and tibia lead in all three exposed groups from factories A, B, and C (r = 0.71, p = 0.003; r = 0.49, p < 0.0001; r = 0.74,
In vivo tibia lead measurements as an index of cumulative exposure in occupationally exposed subjects

p < 0.0001 respectively) as well as for the non-occupationally exposed group (r = 0.80, p < 0.002). But age and duration of employment are also dependent on each other, and similar relations exist between tibia lead concentrations and the time spent working at each factory. Regression coefficients for factories A, B, and C are 0.86 (p < 0.0001), 0.61 (p < 0.0001), and 0.80 (p < 0.0001) respectively. Clearly tibia lead concentrations are strongly influenced by the duration of exposure and not simply its magnitude.

Cumulative exposure indices

As has already been noted, blood lead concentrations are indicative of recent exposure, and therefore a time integrated blood lead index, viz \( \int_0^T B(t) \, dt \) (where \( B(t) \) is the blood lead concentration at time \( t \), and \( T \) is the total exposure time) should be a measure of total exposure. Indeed, if the residence time for lead in the tibia is long compared with the exposure time \( T \) then this time integrated blood lead index and tibia lead should be strongly correlated.

This sort of relation has been investigated by Christoffersson et al who made in vivo lead measurements not on the tibia shaft but on finger bones. Unlike Christoffersson, we have not attempted to separate out the background contribution to bone lead from the environment, nor do we measure the same bone site. Therefore our own results and those of Christoffersson et al are not directly comparable although, as we shall see, we reach similar conclusions.

To investigate the relation between tibia lead and a time integrated blood lead index for the data described here, time integrated blood lead concentrations were calculated from the medical records of factories B and C. We are indebted to the medical staff of both these factories for their help in this.

Clearly, continuous blood lead data is not available in the form required for the cumulative blood lead integral given earlier. There are gaps in the data, particularly in earlier years, and the measurement intervals are often non-uniform, varying from a few weeks to annually. The integral has therefore been calculated numerically using the trapezoidal rule, because of the non-uniform time intervals, viz

\[
\int_0^T B(t) \, dt = \sum_{i=1}^{n} \left( B_i + B_{i+1} \right) \Delta t
\]

where \( B_i \) and \( B_{i+1} \) are the \( i \)th and \( (i + 1) \)th blood lead measurements respectively and \( \Delta t \) is the time interval between measurements.

For periods greater than a year for which no data exists the overall average concentrations have been assumed. It is appreciated that where gaps exist, particularly in the early working years of the longer serving subjects, this may significantly underestimate exposure, but we did not feel justified in extrapolating the data to try and get a better estimate. The effect of this assumption would be to increase the variance in the relation between stored lead and the blood lead index.

In calculating this blood lead index several other major assumptions were also made. The first was that the blood lead contribution from the exchangeable bone fraction compartment was assumed to be negligible. Secondly, the variation in the transfer constant from blood lead to bone lead with both age and with differing blood lead concentrations is ignored. Thirdly, as has been already mentioned, the half life of lead in the cortical bone of the tibia shaft is assumed to be long and its effect therefore negligible.

Finally, in terms of background exposure, no allowance has been made for bone lead burden accumulated before employment at each factory. As was stated earlier, a percentage of people will have had previous work contact with lead but in both factories B and C this was a small fraction of the populations (16% and 15% respectively). In many cases, however, in particular in factory C, the major part of the pre-employment period will have been between birth and age 16, when bone growth and calcium turnover is at a maximum. We find it difficult to estimate the effect of this unknown exposure.

![Tibia lead vs time integrated blood lead index for factory B](http://oem.bmj.com/)

Fig 5 Tibia lead vs time integrated blood lead index for factory B.
Despite these uncertainties, the relation between tibia lead concentrations and the integrated blood lead index is extremely strong (figs 5 and 6), the correlation coefficients being 0.82 for factory B (p < 0.0001) and 0.86 for factory C (p < 0.0001). The equations given by a least squares fit to both factory data sets are shown on the figures. Although both firms use inorganic lead, the two manufacturing processes differ and both groups contain people working in differing exposure environments. As may be seen, however, the two factory lines are similar. A combination of the two data sets (n = 167) gives a correlation coefficient r = 0.84, (p < 0.0001) with a least squares fit to the data of: tibia Pb (µg/g wet bone) = 0.0296 (±0.0015) x (integrated blood lead index) - 2.285 (±1.02).

We see, therefore, that tibia lead concentrations are an accurate index of long term lead exposure and, as such, are a powerful tool in the investigation of any long term health effects. Because of this, a detailed biochemical screening, using both the blood and urine samples taken at the time of each survey, has been undertaken to investigate the relation between cumulative exposure and health effects, and this will be the subject of a separate paper.

Clearly, a detailed investigation of the relation between blood lead and skeletal lead burden requires both a knowledge of lead concentrations at other bone sites together with metabolic modelling of lead uptake, storage, and excretion. To provide the data required we are now making measurements in both tibia and calcaneus, the latter being both large and mainly trabecular.

In addition to providing information on cumulative lead exposure, bone lead concentrations could help produce insight in other ways. For example, both because statutory blood lead monitoring in the United Kingdom is a comparatively recent requirement and because there are some groups occupationally exposed who are difficult to monitor—for instance, demolition and scrapyard workers—the use of bone lead concentrations to estimate an individual’s average blood lead concentration during employment and to give some indication of cumulative previous exposure could be valuable additional information to systemic Pb data in interpreting any medical symptoms in exposed workers. Clearly, however, such a procedure would be subject to the uncertainties arising from the spread in the data concerned.

Knowledge of bone lead concentrations for both occupationally and non-occupationally exposed subjects could also be important in disease states—for example, the current discussion concerning lead nephropathy or treatments where mobilisation of bone lead is possible, such as during cancer chemotherapy using the cis-platinum drugs. The technique used in this work can provide accurate bone lead measurements at most bone sites without the need for either elaborate phantom measurements or knowledge of the tissue overlay and bone geometry. As such we believe that it may play a valuable part in the study of lead toxicology related to industrial exposure, as well as in the more general field of lead metabolic studies.

The work reported here is supported by the United Kingdom Health and Safety Executive, who provided the personal support for two of us (LJS and DRC). This support is gratefully acknowledged, as is the help of Dr D C L Burges, of the Employment Medical Advisory Service of the Health and Safety Executive, in arranging one of the factory surveys. The collaboration and help of the workers, management, and medical staff in the factories concerned is also gratefully recorded.

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