Master of Science Thesis

In vivo X-ray fluorescence (XRF) analysis of lead in finger bone; extended lead exposure range

The contribution to a collaborative study of lead in smelter workers

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Mätning av bly i fingerben med röntgenfluorescens (XRF) teknik i ett utvidgat intervall av blyexponering

Det är sedan länge välkänt att bly (Pb) och blyföreningar är giftiga. Exponering kan orsaka skador på njurar, blod-, nerv- och reproduktionssystem. Särskilt utsatta är personer som har eller har haft ett arbete med långvarig hantering av bly.

Normalt kontrolleras blyexponering i arbetsmiljön genom mätningar av blyhalt i luft, samt genom biologisk övervakning av arbetares blyhalt i blod (B-Pb). Mätvärden ställs i relation till bestämda gränsvärden för arbetsmiljön. Vid upphörd extern blyexponering sjunker halten bly i blod med en biologisk halveringstid i storleksordning månader, vilket då betyder att B-Pb reflekterar senaste tidens exponering. Vid blyexponering sker emellertid också en ackumulering av bly i skelettet. Här har bly en betydligt längre halveringstid, år eller tiotals år. Detta betyder dels att det hos långvarigt högexponerade finns en fortsatt endogen exponering genom att bly utsöndras från skelettet till blodet, och dels att benblyhalten kan ge en uppskattning av långtidsexponeringens storlek.

Förhandsinformation från ett tyskt företags center för hälsoservice visade på höga nivåer av B-Pb i en grupp smältverksarbetare. Samtidiga diskussioner inom EU om en förestående sänkning av gränsvärdet för tillåten B-Pb, tillgänglig teknik och intresse i likartade svenska studier gjorde att ett samarbete startade för att mer ingående bestämma blynivåer i utvalda tyska högexponerade arbetare. Detta arbete visar bidraget till den större studien av blynivåer hos smältverksarbetare. Tekniska och administrativa förberedelser gjordes för ett korrekt genomförande av insamling av data och prover på plats i Tyskland. Blyhalt i fingerben (Ben-Pb) mättes med den vid Medicinsk strålningsfysik/radiofysik i Malmö utvecklade *in vivo* röntgenfluorescens (XRF) tekniken. Kartläggning av blyhalt i blod, plasma och urin med plasma masspektrometri (ICP-MS) genomfördes av Yrkes- och Miljömedicinska avdelningen i Lund. Data från liknande tidigare studier inkluderades i analysen för jämförelse.

Gruppen av tyska aktiva respektive pensionerade arbetare hade medianvärden för ålder 44/59 år, och anställningstid 20/38 år samt Ben-Pb 71/150 µg/g. Maximalt uppmätt Ben-Pb var 222 µg/g. Ben-Pb ökade i genomsnitt med 4,2 µg/g per anställningsår, medan motsvarande siffra för en liknande svensk grupp arbetare var 1,6 µg/g. Hos en kombinerad grupp av tyska samt svenska arbetare, med en pensioneringstid på 2 år eller mer, förelåg bl a ett signifikant samband mellan B-Pb och Ben-Pb, men även andra signifikanta samband mellan parametrar observerades. *In vivo* XRF tekniken påvisas som ett värdefullt och hanterbart instrument i studien av bly. Erhållna resultat, såsom uppmätt Ben-Pb i den tyska gruppen, tyder på en långvarig exponering och vid hög nivå. Trots pensionering låg de tyska arbetarna ungefär dubbelt så högt i Ben-Pb som de aktiva arbetarna. I beaktandet av lägre gränsvärden leder det till ett nödvändigt genomförande av industriella säkerhetsåtgärder.

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In vivo X-ray fluorescence (XRF) analysis of lead in finger bone; extended lead exposure range

This work shows a contribution to a long-term study of lead in smelter workers where the objectives were to assess the historical lead (Pb) exposure and to study the relationships between lead concentrations in finger bone (Bone-Pb), whole blood (B-Pb), plasma (P-Pb), urine (U-Pb) and the duration of employment in workers at a secondary lead smelter. Moreover, results were compared with previous studies of populations with a wide range of lead exposure.

In 39 workers (29 active, 10 retired), recruited from those with the highest lead exposure at a German secondary lead smelter, levels of Bone-Pb was determined by the Malmö *in vivo* X-ray fluorescence (XRF) technique. The setup uses two ^{57}Co sources (γ-energy 122 and 136 keV) to generate photoelectric absorption in the K-shell of finger bone lead atoms (binding energy 88.005 keV). A high purity germanium (HPGe) detector detects subsequent characteristic lead X-rays and a large background from mainly incoherently scattered photons. The net number of registered characteristic pulses, in relation to phantom measurements and bone size, gives the Bone-Pb of the measured subject. The Department of Occupational and Environmental Medicine in Lund determined B-Pb, P-Pb and U-Pb by inductively coupled plasma mass spectrometry (ICP-MS). Results were compared with data from a similar previous study on workers with lower exposure, from a Swedish secondary lead smelter.

The median values in the active/retired German lead workers were: age 44/59 years, duration of employment 20/38 years and Bone-Pb 71/150 μg/g. Bone-Pb increased with duration of employment by 4.2 μg/g per year for German workers and by 1.6 μg/g per year for Swedish workers. The median Bone-Pb was three times higher in both active and retired German workers than in Swedish smelter workers with essentially the same age distribution and duration of employment.

In this work the *in vivo* XRF technique is being demonstrated as a valuable and manageable instrument when contributing to a larger collaborative study of lead. The high Bone-Pb values recorded for the German smelters implied a historical lead exposure of considerable magnitude. A long-term high lead exposure was also revealed in the B-Pb levels for both active and retired workers, which should be leading to the implementation of necessary industrial safety measures in order to respond to biological threshold limits.

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Foreword

The frame of this work has been the participation in a collaborative study of lead in a group of workers at a smelter factory. It was performed in cooperation between the Department of Medical Radiation Physics in Malmö, the Department of Occupational and Environmental Medicine in Lund and the Company Health Service Centre (BAD – Berufsgenossenschaftliche Arbeitsmedizinische Dienst – Gesundheitsvorsorge und Sicherheitstechnik, Braunschweig-Bienrode, Germany) connected to the factory where the study took place.

The core of the presented work describes specifically my main contribution to the study; participation in the preparation and the performance of the measurements of lead in finger bone with *in vivo* X-ray fluorescence (XRF) technique, and taking part in the following evaluation and analysis of the results.

The study was presented at the Swedish Medical Society's National Convention in Göteborg, 1998, at the 4th Topical Meeting on Industrial Radiation and Radioisotope Measurement Applications, Raleigh, North Carolina, USA 1999 and at the 5th International Symposium on In Vivo Body Composition Studies, Brookhaven National Laboratory, Upton, New York, USA 1999. It resulted in two publications in the international literature; "Lead accumulation in highly exposed smelter workers" (Olsson et al. 2000) and "Lead in finger bone, whole blood, plasma and urine in lead smelter workers: extended exposure range" (Schütz et al. 2005).

The Ethics committee of Lund University and the Radiation Protection Committee at Malmö University Hospital approved the study. All subjects gave their informed consent prior to their inclusion in the study.

Introduction

The heavy metal lead (Pb) is a toxic element and exposure to it may involve a serious health risk. In order to add knowledge about uptake and turnover of lead in the human body, it is of importance to perform measurements regarding the levels of lead. This is the main reason why lead is the centre of attention in this study. A brief overview of the research field is given.

Lead hazards

There is an extensive amount of literature on the toxicology of lead. The human body is not known to require any lead at all. Instead, exposure to lead and lead compounds can cause serious health problems. People who handle lead in their workplace are especially exposed to its hazards. Long-term exposure may have a negative effect on several organs (Skerfving 1993). Complications can be seen in the nervous systems (Lille et al. 1994), the blood-forming organs, the kidneys (Ehrlich et al. 1998), the gastrointestinal tract, the cardiovascular system and the reproductive organs (Sallmen et al. 2000).

Lead kinetics

Lead enters the body mainly through inhalation and ingestion. The concentration of lead in blood (B-Pb) has been reported to have a biological half-life of about one month and therefore reflects recent lead exposure. Long-term and heavy lead exposure may result in a considerable accumulation of lead in the skeleton, containing more than 90% of the body burden and being the main lead depot in humans (Barry 1975). After exposure has ceased, the skeleton acts as an endogenous source of lead. The biological half-time of lead in various bone sites has been reported to range from two years to decades (Christoffersson et al. 1986; Schütz et al. 1987; Nilsson et al. 1991; Gerhardsson et al. 1993; Börjesson et al. 1997; Brito et al. 2001). Accordingly, bone lead (Bone-Pb) determination may be used as an estimate of long-term lead exposure. In subjects with an ongoing exposure and a high Bone-Pb value, the latter may contribute by as much as 50% to the current B-Pb (Ahlgren et al. 1980; Skerfving 1993; Smith et al. 1996). The internal exposure means a continuing health risk even though there is no present external lead exposure.

Aims

Information from the Company Health Service Centre at a secondary lead smelter in Germany showed that B-Pb concentrations periodically exceeded 620 µg/l in some of the workers (Appendix 1). A general concern for the workers' health, in connection with the, at the time, proposed decrease of the maximum permissible B-Pb value within Germany, initiated this study. The lead concentration in body fluids and bone, and their relationships, were of interest to compare with the results from other groups with differing exposure patterns and intensities in order to explore and increase the knowledge of the relationships between exposure indices in a supposedly extended concentration range. To enable these investigations, the data on German workers were to be compared with data obtained in previous similar studies of workers at a Swedish secondary lead smelter and lead exposed subjects from Ecuador and Russia.

The purpose of the presented work was to determine Bone-Pb *in vivo* as an estimation of the historical lead exposure, reflected in workers with various employment patterns. Besides assimilating the general lead study insight, this meant looking into and learning the available *in vivo* XRF theory and technique to perform the measurements as well as preparing for the study in terms of arranging with the correct documents and certificates to be able to use the equipment in Germany (Appendix 2) and taking part in the analysis of the results.

Background

Methods – *in vitro* – *in vivo*

The techniques of atomic absorption spectrometry (AAS) and inductively coupled mass spectrometry (ICP-MS) are used for *in vitro* measurements on samples in laboratories, for example to determine levels of lead concentration in whole blood (B-Pb), plasma (P-Pb) and urine (U-Pb). For details on these methods, see Börjesson et al. (1997) and Schütz et al. (1996).

In vivo measurement situations carry some slightly different but crucial conditions to handle compared to *in vitro* measurements, such as measurement depth and duration. The X-ray fluorescence (XRF) technique has been found to be adaptable to the search and quantification of certain elements, such as iodine, cadmium, mercury, gold and platinum. It has proven to be a valuable tool when measuring levels of lead in humans.

The other main non-invasive method used for measurements *in vivo*, neutron activation analysis (NAA), is useful only for elements with a suitable large cross-section for neutron capture, such as nitrogen, calcium, cadmium and aluminium. A detailed review on the applicability of XRF in medical sciences can be found by Börjesson and Mattsson (2004).

X-ray fluorescence (XRF)

Fluorescence is a kind of luminescence. That is when a substance is influenced, by anything but a thermal process, to emit electromagnetic radiation. Usually, the emission takes place when the substance goes from the introduced excited energy state back to the ground state. If it occurs immediately, within a time span of 10^{-8} seconds or so from the moment it is initiated, it is considered as fluorescence. In the case of continued luminescence, even though the influencing cause is removed, it may be called delayed fluorescence, or phosphorescence if it also corresponds to longer wavelength light. The idea of XRF is to get the incorporated element under investigation to fluoresce, where both the activating and the emitted light quantum, or photon, is in the X-ray energy spectrum.

According to the Bohr model of an atom the electrons surrounding the nucleus are confined to different orbits, constituting so called electron shells at discrete energy levels, labelled K, L, M, etc, and also further subdivided. The electrons in the K-shell are closest to the nucleus, and also the most tightly bound.

When an atom absorbs an incident photon which has an energy that exceeds an electron binding energy, it may result in the emission of a so called photoelectron from the current shell of the atom. This photoelectric absorption process leaves a vacancy in the electron shell. It will immediately be filled by another electron, usually coming from an outer shell and thus less tightly bound, with a subsequent rearrangement of shell electrons. As energy will be liberated when a vacancy is filled, it may be emitted as electromagnetic radiation (a photon) or be transferred to an electron with less binding energy (Auger electron) which is ejected from the atom. The probability of having photons emitted is represented by the fluorescence yield. It can be found tabulated among spectroscopic data.

The emitted photons will have an energy representing the difference between the discrete energy levels of the shells involved. These emitted X-ray energies are characteristic of the involved element, enabling identification. Another element will have a different set of possible characteristic X-rays. If the vacancy is created in the K-shell, the emitted energies associated are denoted K_α and K_β. When characteristic X-rays like these are captured by a detector and the energies identified, it will reveal the presence of the element. The number of detected photons, or counts, is proportional to the concentration of the element in the measurement volume.

There are some main benefits in favour for analysis of elements with a higher atomic number such as heavy metals like lead (Z = 82), making them appropriate candidates for investigation. The probability for a photoelectric process to occur is higher than for low atomic numbers, there is a higher fluorescent yield and the higher emitted energy is better at penetrating the surrounding tissue.

Most XRF techniques are aiming at the electrons in the K-shell, and may be called KXRF. When it comes to applying XRF on the lower energies following absorption in the L-shell, aside from being restrained to superficial tissues only, there are complicating but pertinent considerations concerning attenuation uncertainties in the bone and surrounding tissues. In the case of measuring lead, interference on the signal response might occur from lead in non-bone tissues, or, though less likely, the presence of arsenic with K_α X-rays (10.544 keV) on lead L_α X-rays (10.552 keV), which would be to close to distinguish (Todd 2002).

Source

Depending on which element to search for, there are always concerns such as what X-ray source and detection to use, what measurement site and geometry of the equipment. The source in XRF is either an X-ray tube or a radionuclide. The generated photons need to be able to reach through surrounding tissue, like skin and adipose, into the measurement volume.

It is crucial that the incident photon energy exceeds the binding energy of the K-shell electrons in the element under investigation, in order to render the photoelectric absorption process possible. When the energy is sufficient for the photo effect process, there is a sudden significant increase in the probability of it, called the K-edge (Figure 1).

Measurement site

In practice, the use of *in vivo* XRF is limited by the attenuation in tissue, of the incident and emitted photons, both regarding the loss of information carriers as well as the generated absorbed dose. Increasing the measurement time may improve the chance for the statistical treatment to discern the emitted characteristic X-rays registered on top of the background, but the subject also has to be able to be still during the measurement and the absorbed dose need to be kept as low as reasonably achievable.

It has been found that *in vivo* measurements using KXRF is restricted to elements with an atomic number (Z) of about 45 or higher and for the lower Z in that interval only for the most superficial tissues of the body, like eye and skin. As for deeper situated organs, the higher characteristic X-ray energy that comes with increasing atomic number is needed. For example, when measuring cadmium (Z = 48) in kidneys, the characteristic X-rays are 98% absorbed by 50 mm of water, a typical kidney depth (Börjesson 1996). However, the measurement of lead in finger bone using KXRF is more advantageous due to higher photon energies in question and the short distances.

The skeleton consists mainly of two types of bone, about 80 % is cortical (compact) bone and about 20 % is trabecular (spongy). Lead turnover rate is considered to be much slower in the cortical parts, which makes them more suitable targets in the study of long term exposure.

Incorporated lead has a biological half-life in cortical bone of about a decade, as opposed to about 2 years in trabecular.

The *in vivo* XRF technique available at our department measures lead in the midpoint of the second phalanx of the left forefinger of the subject (described in detail by Ahlgren et al. 1980, Nilsson et al. 1991). The finger bone site is considered as nearly cortical and has been the measurement site in several studies of long term lead exposure since the technique developed in the mid 70s. Among other accessible cortical bone sites another subsequent technique using ^{109}Cd has been adapted to measure the lead level in the midpoint of the left shinbone (tibia) and the lower portion of the left heel bone (calcaneus) of the subject (Somervaille et al. 1989).

Detection equipment

The high energy resolution of solid state- or semiconductor detectors is a corner stone when using XRF for *in vivo* studies. There are more information carriers for each given pulse than any other detector type. A lower inherent statistical limit enables a better discerning registration of different energies. It also has good stability and timing characteristics, freedom from drift, thin entrance window and is a compact sized detector type simple to operate.

To be able to use them, most detectors are cooled with liquid nitrogen (-196°C) to prevent migration of thermally generated charge carriers when the necessary electric field is applied to the semiconductor material, i.e. a technique to greatly limit the electronic noise in the detector. Thermoelectrically cooled cadmium zinc telluride detectors may have a future potential for *in vivo* XRF.

Generally, a material of lower atomic number (Z) has characteristic X-rays of lower energy and a lower probability of attenuation in it. Silicon (Z = 14) detectors are very thin, at the most 1 – 3 mm, and usually used for more short range radiation like charged particle spectroscopy. In order to increase the detection efficiency for more penetrating radiation, lithium (Z = 3) may be added and thereby the active volume (or "intrinsic region") of the detection material can be made a little thicker (up to 5 – 10 mm). It is called lithium drifted silicon detector, Si(Li). Such a detector was proven to be a better choice than a germanium (Z = 32) detector when performing *in vivo* XRF studies of cadmium (Z = 48, $K_\alpha \approx 23$ keV), having characteristic X-rays of lower energies than lead (Z = 82, $K_{\alpha 1} = 75.0$, $K_{\alpha 2} = 72.8$ keV). The main reason for the improved minimum detectable level was less background registered due to the lower detection efficiency at photon energies above 30 keV (Nilsson et al. 1990).

Germanium can be refined to be ultra pure and detectors are manufactured with this material having an active volume with a thickness of more than 1 cm. These high purity germanium (HPGe) detectors are suitable for gamma-ray spectroscopy, or as in our case to capture and identify lead characteristic X-rays.

Following the detector there needs to be a fast analogue-to-digital converter (ADC) for the signals to be properly processed. There are limits for the source activity though.

Reproducibility and accuracy

Paired *in vivo* XRF measurements of lead in finger bone have been reported to give a reproducibility of 10 μg/g (SD of differences, 15 % of the mean, n = 16; Christoffersson et al. 1984) and 11 μg/g (SD of differences, 21 % of the mean, n = 6; Somervaille et al. 1989). The accuracy has been verified by following up two *in vivo* XRF measurements of 98 and 27 μg/g, by AAS analysis *post mortem*, which gave 91 and 36 μg/g, respectively (Christoffersson et al. 1984, Nilsson 1994).

Subjects

The German lead smelter has a long history of processing lead dating back to the 16th century (Appendix 3). Other metals, such as zinc, have also been processed.

The workforce consisted of approximately 130 subjects of whom about 100 were engaged in lead production. From the latter group, 29 male smelters were recruited to participate in the study. Ten retired smelters also volunteered. The main inclusion criteria were a long duration of employment and notations of high B-Pb values during previous years. The 39 lead smelters were or had been working in different lead processing departments. However, 15 of those 39 workers had periodically also worked at the zinc production plant.

Observations from the B-Pb records indicated that work in departments not involved in lead smelting, e.g. the zinc production plant and the transportation department, led to approximately 50 % lower B-Pb levels of that during work in lead departments. Based on this, three time-based cumulative exposure indices were used: (1) the duration of employment at the smelter, (2) the duration of employment in lead departments, and (3) a weighted duration of employment, where the years in lead departments were given the weight of 1.0 and the years in other departments were weighted 0.5.

A Swedish secondary lead smelter factory, where a similar lead study had been carried out previously, had a production in resemblance to that at the German smelter. The study results from this group of Swedish workers (71 active and 19 retired) were used for comparison. A detailed description of this material is found in Börjesson et al. (1997).

A summary on these participating workers are presented in Table 1. In addition to this there were also two other groups included in the complete study for comparison; a group of 42 male and female workers from a lead-accumulator plant in Russia and 34 lead exposed children from Ecuador. For information about that part of the study, see Schütz et al. (2005).

Methods and equipment

Lead measurements

The study present levels of lead concentrations in whole blood (B-Pb), plasma (P-Pb), urine (U-Pb) and finger bone (Bone-Pb). Briefly, the measurements of B-Pb, P-Pb and U-Pb were made on

samples of blood and urine that were collected from the smelter workers at the factory grounds in Germany. The samples were prepared and stored cold until required for analysis back in Sweden.

Lead levels were determined using ICP-MS by the Department of Occupational and Environmental Medicine in Lund (Appendix 4). Detailed information about this part of the study can be found in an article by Schütz et al. (2005). The same technique was used in the comparing studies on Russian workers (Bergdahl et al. 1998) and Ecuadorian children (Bergdahl et al. 1999). In the Swedish group, AAS was used to determine lead levels in the body fluids (Börjesson et al. 1997).

The lead level in finger bone, Bone-Pb, was measured using *in vivo* X-ray fluorescence (XRF) technique. This has been developed and used by the Malmö XRF group since the beginning of the 1970´s as a convenient tool for repeated *in vivo* measurements and surveys of Bone-Pb of exposed industrial workers (Ahlgren et al. 1976; Christoffersson et al. 1984; Nilsson et al. 1991; Börjesson et al. 1997; Börjesson and Mattsson 2004).

The system is based on two sources of ^{57}Co (Amersham* UK; 470 MBq in total), which generate photon energies of 122 keV (87%) and 136 keV (10%). They are positioned in holders made of high purity tin, which are mounted opposite to each other in an arrangement made of PMMA (polymethylmethacrylate). The person to be measured places his/her left forefinger in the holder (Figure 2). Between the sources about 1 cm^3 in the mid part of the second phalanx will be irradiated. A planar high purity germanium (HPGe) detector (Princeton Gammatech; Ø 16 mm, thickness 5.2 mm) is placed at right angle to the incident radiation.

The detection system registers pulses from photons with a spectrum of energies. There is a large background of mainly incoherently scattered photons from interaction in the bone and soft tissues. Coherently scattered X-rays are seen at 122 and 136 keV if the amplifier is tuned for it. The presence of bone lead atoms will cause a fraction of the incident photons to undergo photoelectric absorption and the number of detected fluorescence photons is proportional to the concentration of the element.

The calibration procedure involves a correction for the individual finger bone size and measurements on finger phantoms containing known levels of lead (Ahlgren and Mattsson 1979). The measurement volume is estimated from two orthogonal radiographs of the finger bone, taken with mobile diagnostic radiography equipment (Appendix 5. Calculation of Bone-Pb concentration). The Bone-Pb is given as µg lead per gram wet bone weight.

The absorbed dose from an *in vivo* XRF measurement of lead in finger bone is about 1 mGy in the centre of the finger and about 3 mGy in the skin (Nilsson et al. 1991). These figures are based on measurements with small LiF dosimeters done by Ahlgren et al. (1980). As the irradiated volume is about 1 cm^3 (0.01 kg) the energy imparted is about 0.01 mJ, roughly giving a mean whole body (100 kg) dose equivalent of 0.1 µSv. In addition to this, the correction for finger bone size also contributes with an absorbed dose in the same order of magnitude as the lead measurement of the finger.

* Now part of GE Healthcare.

One of the ^{57}Co sources in the available equipment needed to be replaced prior to the measurements, owing to physical decay (half-life; $T_{½}$ = 272 days). This also brought in aspect the availability and affordability at the time. The activities of the in Germany used sources were 100 and 370 MBq. Due to the exchange of source the sampling time was adjusted to keep a similar detection limit as in the prior Swedish comparative study. Since the decay of the sources during the period of measurements (5 consecutive days) was only about 1 %, no specific correction was necessary.

In order to get the detection equipment ready for the measurements, ^{241}Am (26,3 and 59,5 keV) source was used when adjusting the energy calibration, i.e. as an extrapolation to also be able to register the lead peaks at 75,0 keV ($K_{\alpha 1}$) and 72,8 keV ($K_{\alpha 2}$). As a double check, measurements were made on a little piece of lead, packed tight and clean in double plastic bags and placed in the measurement position.

The measurements of Bone-Pb were performed in a house at the site of the smelter. In this environment there was an increased risk of lead contamination of the measurement equipment. Daily measurements, at least an hour long, on a clean, lead free, finger sized plastic rod showing no trace of lead verified that the XRF equipment set up was kept free from lead contamination.

Spectrum analysis

If lead is present at a detectable level the generated spectrum reveals characteristic lead K_{α} X-rays ($E_{K\alpha 1}$ = 75.0 keV and $E_{K\alpha 2}$ = 72.8 keV). A large characteristic peak to scattered background ratio is statistically desirable. Calculation of the number of counts in peak and background areas in all spectrums were performed with the help of a Microsoft Quick Basic computer program developed in Malmö. The background was calculated by fitting a third degree polynomial before subtraction in order to get the net number of counts in the peak area corresponding to K_{α} from Pb. This is better than a straight line approximation according to Börjesson (1996; paper IV).

Detection limit

There are different ways of defining and calculating a minimum detectable concentration (MDC). In agreement with previous studies (Mattsson et al. 1987, Börjesson 1996) it is in our study calculated for a specific measurement time according to the formula

$$MDC = 3 * C/N_{Peak} * (N_{Bkg})^{1/2}$$

where C is the lead concentration in the finger phantom bone substitution (200 µg lead per gram bone ash with 20% bone mineral contents), N_{Peak} is the number of counts in the peak which arise from detected fluorescent lead and N_{Bkg} is the number of counts in the background under the peak. These counts registered by the detector system are considered to be normally distributed.

The standard deviation (σ) of the background (N_{Bkg}) are calculated as the square root of the number of counts, $\sigma = (N_{Bkg})^{1/2}$. In other words, the MDC is equivalent to a net number of counts that equals three times the standard deviation of the background counts. It will be the lowest level that still has a statistically safe margin to be a reliable estimation.

Our set up had an MDC that was between 8 and 16 μg/g, depending on the diameter of the finger bone, for a measurement live time of 30 min.

Statistical treatment

Preliminary analyses showed that Bone-Pb and the other variables did not follow a normal distribution pattern. It was closer to a log-normal behaviour. Thus non-parametric statistics (Mann-Whitney U test, Spearman's rank correlation coefficients) were applied. Statistical significance was considered to be reached when $P < 0.05$.

To obtain more information from the data in the full study, using the flexibility and power of parametric methods, a linear regression analysis was also used to evaluate the association between ^{10}log P-Pb and B-Pb among active and retired lead workers. Model fits were checked by means of residual analyses (Altman 1991). These analyses were done with the help of SPSS (Statistical Package for the Social Sciences, version 10.0).

Results

Bone-Pb together with B-Pb, P-Pb, U-Pb in the German smelters are presented in Table 1. For comparison, data from an earlier study of a Swedish group of smelter workers are included. There were significant differences ($P < 0.05$) between active and retired German smelters. The active workers had higher B-Pb (median 1.5x), P-Pb (2.5x) and U-Pb (1.9x) than the retired workers had. On the other hand, the retired workers had higher Bone-Pb (median 2.1x), longer duration of employment (1.9x) and longer weighted duration of employment (1.4x). Among both retired and active workers there were subjects with Bone-Pb values approximately twice as high as we had ever recorded with the XRF technique in Swedish smelter workers.

Bone-Pb and duration of employment in the lead departments were significantly correlated ($r_s = 0.51$, $P = 0.001$) in the total group ($n = 39$) of German smelters. The correlation coefficient increased when the total duration of employment at the factory ($r_s = 0.65$, $P < 0.001$) or the weighted duration of employment ($r_s = 0.69$, $P < 0.001$) was used. A much stronger correlation ($r_s = 0.91$, $P < 0.001$; Figure 3) between the bone lead value and the weighted duration of employment was obtained when the analysis was confined to those workers that had almost exclusively (> 80 % of the time) worked in the lead departments (17/39 workers).

The slope of the linear regression line between Bone-Pb and weighted duration of employment was significantly steeper for German workers than for Swedish workers, 4.2 (95% CI: 2.7-5.7) versus 1.6 (95% CI: 1.3-1.9) μg/g per year (Figure 4). The intercept, however, was the same in both groups, 8 μg/g.

In active German workers there were positive correlations between Bone-Pb and the duration of employment in lead departments ($r_s = 0.63$, $P > 0.001$), the duration of employment ($r_s = 0.55$, $P = 0.002$), and the weighted duration of employment ($r_s = 0.65$, $P < 0.001$). The retired German

workers showed no significant correlations between Bone-Pb on the one hand and B-Pb, P-Pb, U-Pb or any of the employment indices on the other. However, there were strong relationships between P-Pb and B-Pb ($r_s = 0.90$, $P < 0.001$), U-Pb and B-Pb ($r_s = 0.78$, $P = 0.008$) and between U-Pb and P-Pb ($r_s = 0.85$, $P = 0.002$).

Extending the lead exposure range by combining the German and Swedish former smelters but including only those who had been retired for 2 years or more (i.e. a group of 7 + 15 = 22 workers), we obtained a significant correlation between B-Pb and Bone-Pb ($r_s = 0.69$, $P < 0.001$) as well as between U-Pb and Bone-Pb ($r_s = 0.52$, $P = 0.016$). In this combined group there were also significant correlations between P-Pb and B-Pb ($r_s = 0.86$, $P < 0.001$), U-Pb and B-Pb ($r_s = 0.78$, $P < 0.001$) and U-Pb and P-Pb ($r_s = 0.79$, $P < 0.001$) but not between P-Pb and Bone-Pb ($r_s = 0.37$, $P = 0.09$).

Discussion

Since 1970, lead exposure at the German smelter had periodically and partly been under surveillance by stationary sampling of lead concentration in air. The level had frequently exceeded the value of 100 μg/m^3, an established limit for the workplace concentration (Maximale Arbeitsplatz-Konzentration, MAK). The exposure was presumably even higher during earlier decades. In accordance with European criteria, the existing medical control programme was complemented by more regular B-Pb determinations in the 1980s. Workers were removed from lead exposure when their B-Pb value exceeded 700 μg/l.

A gradual improvement in the working conditions decreased the number of values exceeding the B-Pb limit (700 μg/l) during the 1990s. During the 5-year period prior to our study, the annual mean B-Pb fluctuated between 390 μg/l and 500 μg/l in the total population of active lead workers at the German smelter (company data). The medical control programme reported prior to the study a median B-Pb level of 410 μg/l (range 80-680 μg/l) among the smelter population as a whole ($N \approx 100$), which was slightly more than the year before. The median B-Pb of 500 μg/l among the 29 active workers in our study indicated a somewhat higher lead exposure in the studied group compared with the remaining workers.

Medical records show that workers with identical work tasks could have differences in B-Pb values by a factor of 2-3, probably due to differences in personal work routine and individual biological factors affecting the uptake, distribution and elimination of lead from the body.

Pre-study information from the Company Health Service Centre at the German smelter showed that high concentrations of lead in blood, up to, or even exceeding, 700 μg/l had been observed now and then for many years. Thus, German workers with similar work tasks and approximately the same duration of exposure showed significantly higher lead concentrations in blood, plasma, urine and bone than the Swedish workers. Accordingly, pooling of data from German and Swedish workers made it possible to extend the Bone-Pb range from about 100 μg/g to 220 μg/g wet weight (Figure 3 and Figure 4).

In the low exposure end is the normal population where the concentration of lead in bone is only a few μg/g and below our detection limit, as reasonably indicated by our calculated intercept of about 8 μg/g. It can be noted that in the comparing Swedish group of workers several Bone-Pb levels were below the MDC (Table 1, Figure 4). From the statistical fluctuations in the spectrum analysis some even got a negative value. However, in order to keep a consistent approach to the values as a group received from the same analysis, it is important that all data is included. Excluding data points will lead to errors in the calculated median value.

Finger bone is more cortical than trabecular (Somervaille et al. 1989). Moreover, in the middle section of the left forefinger, where the XRF was measured, the fraction of trabecular bone is lower than at the ends. Hence, these measurements may be regarded as mainly representing cortical bone. Owing to the long biological half-live of lead in cortical bone, which constitutes approximately 80 % of the skeletal mass, such measurements will reflect the skeletal lead burden and will show a fairly good relationship to a calculated cumulative blood lead index, CBLI (Gerhardsson et al. 1993; Börjesson et al. 1997). Bone lead and CBLI indices can be useful for epidemiological dose-response studies in humans.

The data points representing the relation between Bone-Pb and duration of employment in Figure 4 are scattered around the same regression line for the subgroups of active and retired Swedish smelter workers. The retired German workers, however, seem to form a separate group, which mainly resides above the regression line of the active German workers. This suggests that the lead exposure during earlier decades was considerably higher at this smelter than it is today. The relatively short period of retirement among the retired German workers, median 2 years versus 6 years in retired Swedish workers, may also contribute to the difference observed, as Bone-Pb will only slightly decrease in cortical bone during such a short time.

In active lead workers, the ongoing lead uptake via the lungs and gastrointestinal tract is dominant. In retired workers, however, the endogenous exposure through the skeletal lead pool will have a strong impact on the blood lead levels, giving the significant correlation between their Bone-Pb and B-Pb.

For one German subject a hip-bone biopsy was performed 3 years prior to our study. Analysis by AAS showed a lead concentration of 147 μg/g wet weight. The estimated lead concentration in finger bone in this study was 169 μg/g, thus indicating a reasonable agreement (15 %) between methods and bone lead at different sampling sites. Again, it indicates massive lead exposure during earlier decades.

Owing to the difficulties in constructing individual CBLIs more simplistic exposure measures had to be used, e.g. the number of years employed. This index was influenced by work in the lead department as well as work at other plant sections, e.g. the zinc department, which had lower lead exposure. When comparing B-Pb values collected at approximately the same time from workers at the lead and zinc departments, respectively, it was found that the zinc workers, on average, had B-Pb values that were approximately 50% of those of the lead worker group. A weighted index, based on empirical observations, gave a better association with Bone-Pb than an index that was solely based on the number of working years at the lead plant.

The average B-Pb level in the general population of Germany (adult men, 18-69 years of age) has been estimated to be approximately 31 µg/l (geometric mean; Becker et al. 2002). This value is fairly similar to the 37 µg/l observed among the occupationally unexposed referents in the Swedish study (Börjesson et al. 1997). Accordingly, the similar intercepts found in Figure 4 for the Bone-Pb prior to exposure seem reasonable, due to similar pre-occupational B-Pb levels in German and Swedish workers.

In view of decreased German occupational B-Pb limit (400 µg/l) it is evident from Figure 5 that a significant fraction of the active German smelter workers had B-Pb levels clearly exceeding this action level. The situation is aggravated if the subjects have accumulated lead in the skeleton over many years, resulting in an increased endogenous exposure, which will further elevate the B-Pb level. Accordingly, such subjects will need a long time away from lead work to adjust their B-Pb concentration to the limit value (DFG 2003) before returning to work again.

Five years passed between the measurements on the Swedish smelters and the German workers. However, the set-up and equipment used in the two sets of measurement series were essentially the same, but, owing to physical decay (^{57}Co; $T_{½}$ = 272 days), the radionuclides were not identical and did not have the same total activity. On the other hand, the calibration procedure used identical finger phantoms at both occasions, and the methodology followed the same strategies for measurements in phantoms as well as in subjects. Sampling time was adjusted for source activity, i.e. live time was prolonged for a weaker source as to produce similar detection limits at both occasions. Dead time loss was kept low at both occasions. Contamination of the measurement apparatus was accounted for by frequent measurements on a plastic rod during the whole series of measurements in 1992 and 1997. There was no sign of contamination on any occasion.

A suggested future possible benefit for the *in vivo* XRF technique using ^{57}Co could be to normalize against the coherently scattered peak in the detected spectrum (O'Meara et al. 2001). However, the measured value will reveal different concentration as the lead concentration is given per bone mineral content and not wet cortical bone weight. But they can be compared. The bone mineral content in cortical bone has been found to be 58 % (Woodard and White 1986). Converting a measured concentration of lead per wet cortical bone weight to lead per bone mineral weight leads to a factor of 1.72. Though, as the finger bone has been reported to be nearly cortical, this factor may be a slight underestimate (Somervaille et al. 1989).

The KXRF measurement setup, which uses ^{109}Cd as a source and measures lead in tibia, has been adopted for *in vivo* lead studies by several research groups. As the photon energy is 88.035 keV and just above the K-edge (E_K = 88.005 keV), this technique benefits accordingly from a better probability for photo effect. Effectively, only uncollided entering photons will be of importance for either initiating the characteristic lead K X-rays or the coherently scattered X-rays used for normalisation in this system. The first version had an annular source with a HPGe detector in the middle, both facing the same direction – towards the bone to measure. Developments lead to a small point source in front of a HPGe detector with larger area (Ø = 51 mm; 2040 mm^2), though the source was positioned in front of the detector, collimated to face the tibia, and therefore blocking the mid part of the detector. A significant improvement in overall precision was found when testing different digital shaping parameters (Fleming and Forbes 2001).

Further, a more recent and promising version has four HPGe with 16 mm diameter (200 mm^2) and 10 mm thickness placed around the ^{109}Cd source, each with individual pre-amplifiers and digital signal analysers. Measurements show that an improved minimum detectable level is achieved compared to the conventional version (Nie et al. 2006). Another similar and promising system uses four low energy germanium detectors (4 x 500 mm^2) and reports the highest precision yet among bone lead XRF systems (Fleming and Mills 2007).

Conclusion

The implementation of XRF on *in vivo* measurement of Bone-Pb has proven to work as a reliable and uncomplicated technique. It has contributed to the mapping of lead levels in a group of smelter workers in Germany where there had been high levels of lead exposure reported. Joined with the company doctor, certificates for performing this study were arranged. My main contribution of this work to the study, concerns the measurements of Bone-Pb. It meant learning, checking and using the equipment correctly before and during the study, and the evaluation afterwards with the following broadened analysis that comes when collaborating with other disciplines. Also planning and preparing for the transportation, with all papers needed to travel with a radioactive substance and using it in Germany.

The median Bone-Pb was approximately three-times higher in both active and retired German lead workers than in a previous study of Swedish smelter workers with essentially the same age distribution and duration of employment. The high Bone-Pb values observed in the selected group of German workers confirmed a historical lead exposure of considerable intensity. The long term high lead exposure also showed up in the B-Pb levels for both active and retired workers, leading to the implementation of necessary industrial safety measures in order to respond to biological threshold limits.

Acknowledgements

Thanks to the good cooperation of the German authorities it was possible to achieve all necessary permits to conduct the study. Without all the efforts of Jimmy Börjesson, Sören Mattsson, Lars Gerhardsson, Anker Jensen and the late Andrejs Schütz, this work would not have been done.

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Figures and tables

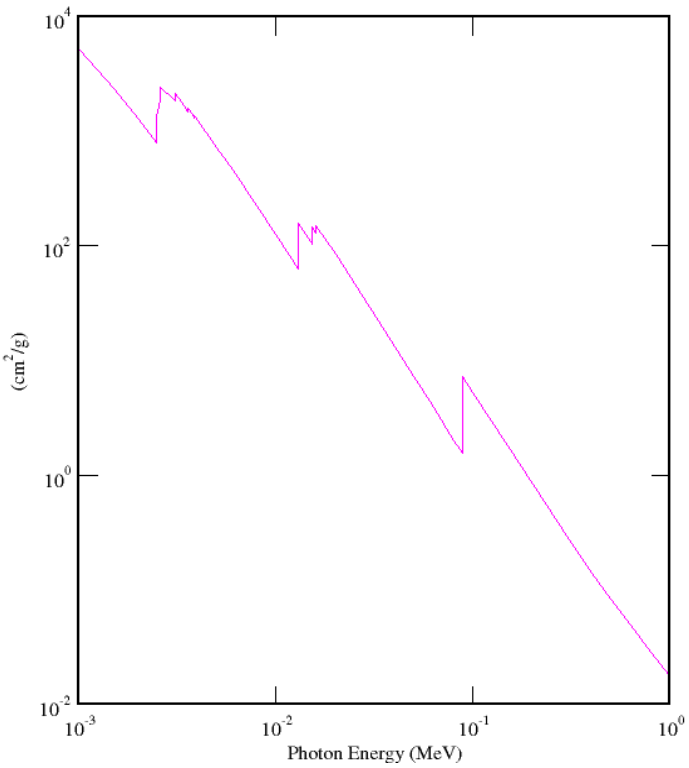

Figure 1. Cross-section (or probability) for photoelectric absorption in lead. The K-edge for lead is at 88.005 keV. The Graph is from XCOM Photon Cross Sections Database (NIST Standard Reference Database; http://physics.nist.gov/PhysRefData/Xcom/Text/XCOM.html).

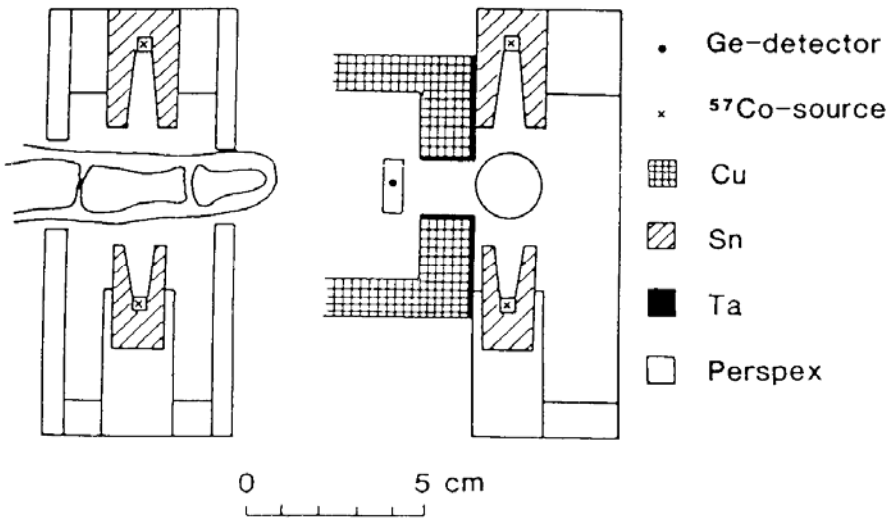

Figure 2. Set up for measuring lead concentration in finger bone (Bone-Pb). From Nilsson et al. 1991.

Table 1. Characteristics of German and Swedish smelter workers and lead concentrations in plasma, blood, urine and finger bone. All values are given as median with range in parentheses. The Swedish workers were all working in the lead department, thus, the duration of employment and the duration of work in lead departments were set equal for the Swedish workers. (Published in Schütz el at. 2005)

Variable	German		Swedish	
	Active	Retired	Active	Retired
Number of subjects	29	10	71	19
Age (years)[a]	44 (29–57)	59 (50–62)	41 (21–64)	69 (58–78)
Duration of employment (years)[a]	20 (6–39)	38 (10–42)	8 (2–42)	28 (6–44)
Duration of employment in lead departments (years)	11 (1–34)	13 (5–40)	8 (2–42)	28 (6–44)
Weighted duration of employment (years)[a]	18 (4–36)	25 (8–41)	–	–
Duration of retirement (years)	–	2 (0–3)	–	6 (1–14)
P-Pb (μg/l)[a]	2.7 (1.5–7.7)	1.1 (0.50–3.9)	1.4 (0.43–37)	0.77 (0.44–4.3)
B-Pb (μg/l)[a]	500 (390–750)	330 (140–620)	330 (80–930)	170 (90–330)
U-Pb (μmol/mol creatinine)[a]	25 (9.6–86)	13 (2.0–28)	11 (1.7–44)	6.6 (2.8–21)
Bone-Pb (μg/g)[a]	71 (21–183)	150 (42–222)	23 (−13–99)	55 (3–88)

[a]Statistical significance ($P < 0.05$) between active and retired German smelter workers. A bone lead concentration below zero results from fitting a measured pulse height distribution with its inherent statistical fluctuations

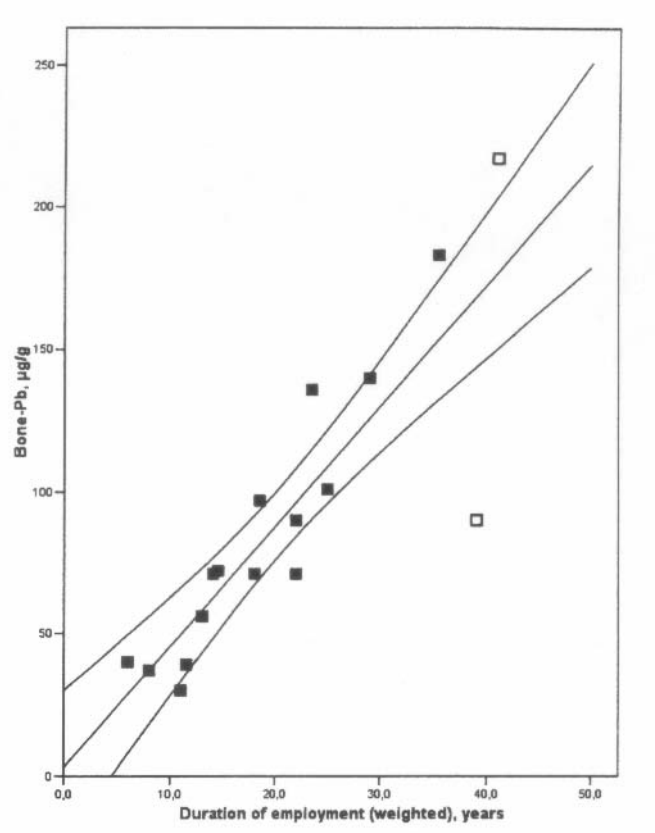

Figure 3. Relationship between lead concentration in finger bone and weighted duration of employment in German workers (n = 17 of 39 workers; *solid squares* German active, *open squares* German retired) with a duration of employment that was dominant (> 80 % of the employment duration occupied with direct lead handling). The regression line and the 95 % confidence interval for the mean are presented. (Published in Schütz el at. 2005)

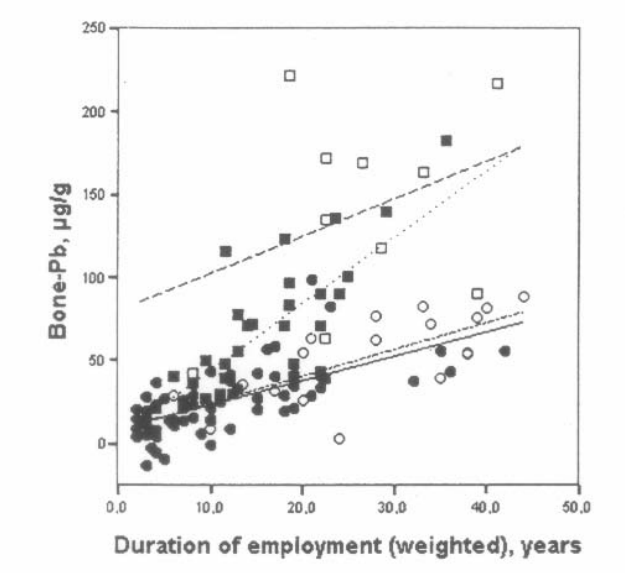

Figure 4. Relationship between lead concentration in finger bone and duration of employment in German and Swedish smelter workers (*solid squares* German active, *open squares* German retired, *solid circles* Swedish active, *open circles* Swedish retired). The respective regression line for each subgroup is shown (· · · German active, - - - German retired, — Swedish active, - · - · - Swedish retired). A bone lead concentration below zero results from fitting measured pulse height distribution with its inherent statistical fluctuations. (Published in Schütz el at. 2005)

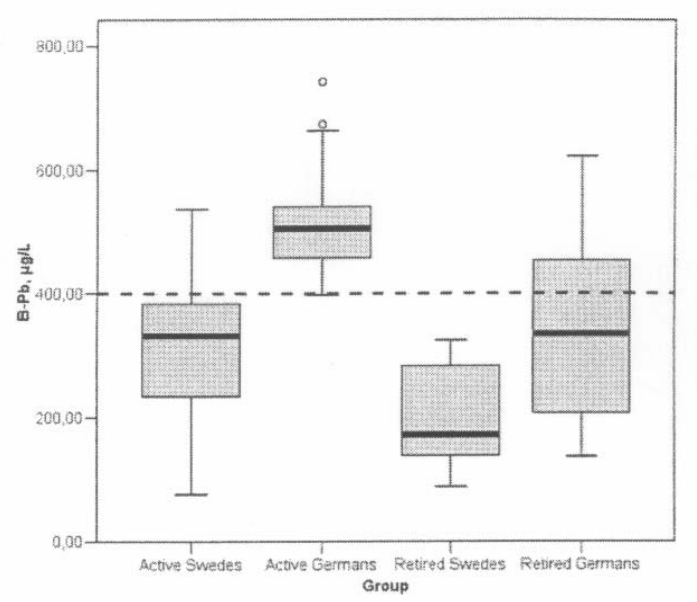

Figure 5. Box plot of blood lead concentrations in active and retired German and Swedish smelter workers, showing the median, the 25 % and 75 % percentiles, and the lowest and highest values that are not outliers. Two outliers among the active German smelters are indicated by *open circles*. The *broken line* at 400 µg/l illustrates the new occupational threshold limit value (TLV) for B-Pb within Germany. (Published in Schütz el at. 2005)

Appendix 1. Lead exposure surveillance

In order to keep the level of lead exposure low in a work environment it is of importance that the workers observe good hygiene. The lead content in air can be measured, though during the last decades, so called biological monitoring of blood lead concentration (B-Pb) has been the preferred indicator of risk assessment for lead exposed workers. The B-Pb level has been put in relation to official biological threshold limits, a level where immediate action should be taken to eliminate exposure, e.g. by transferring the subject into a lead free environment.

For many years the maximum permissible B-Pb level has been 700 µg/l within the European Community. With other used units seen in literature it equals 70 [µg/dl], or using the molecular weight of lead it equals 700 [µg/l] / 207,19 [g/mol] = 3,4 [µmol/l]. However, at the time of this study, lower limits for workers were discussed in Germany. Eventually the maximum level was decided to be 400 µg/l for workers, with a limit of 300 µg/l for younger women (<45 years of age) (Deutschen Forschungsgemeinschaft; DFG 2003). In Sweden the threshold limit value (TLV) for B-Pb in male workers has been 520 µg/l.

Appendix 2. Measurement preparations

The Department of Medical Radiation Physics at the Malmö University Hospital and the Department of Occupational and Environmental Medicine at the Lund University Hospital was in contact with a Company Health Service Centre (BAD – Berufsgenossenschaftliche Arbeitsmedizinische Dienst – Gesundheitsvorsorge und Sicherheitstechnik, Braunschweig-Bienrode) in Germany in order to participate in this collaborative study of lead in a group of smelter workers.

The study was initiated at the end of 1995 and in June 1997 there was an intensive week spent in Germany, collecting information and samples and performing the XRF measurements. The preparations prior to this were partly technical, concerning the method and equipment, and partly administrative, in arranging with the necessary certificates for the study and the transportation to the location in Germany.

In order to be allowed to conduct the study, there was a lot of contact with German authorities. A thorough description of the intentions and technique was sent and approved beforehand. The XRF *in vivo* method was new in Germany and the regulations had to be interpreted especially according to this case. Radiation levels around the XRF equipment set up, with the two ^{57}Co sources, had to be documented and verified. Absorbed doses in air and to the measurement subject had to be declared.

Before the measurements began on the location, a technical control of the equipment was made by the German technical surveillance association TÜV (Technical Überwachungsverker Hannover/Sachsen-Anhalt). Arrangements were made with necessary permissions from the German authorities (Staatliches Gewerbeaufsichtsamt Braunschweig). This included a separate permission to use the X-ray machine on location and a permission to use the XRF apparatus on

humans in Germany. Mandatory leakage test certificates, less than 6 months old, were arranged for the two ^{57}Co sources (as specified in the most recently issued SSI FS 2006:2). No leakage was found from the older source (100 MBq), and the most recently bought source had cleared the leakage limit as specified by the deliverer (Amersham* UK): 0.18 kBq (0.005 μCi) for a 370 MBq source.

The director of the local BAD had to be named as overall responsible for the study, and the skills on radiation protection of the participating German licenced doctor had to be approved by the authorities (Ärztekammer Niedersachsen). Other institutions that were contacted were the BAD Gesundheitsvorsorge und Sicherheitstechnik Gmbh – Gesellschaft mit beschränkter Haftung (meaning Inc. or Ltd.) – Bonn, Niedersächsisches Landesamt für Ökologie and Philips Medizin Systeme Hamburg.

In case of emergency, additional necessary back up electronic devices and computer were generously supplied by the Radiation Physics Department in Göteborg. All equipment performance was tested prior to departure.

In order to make the transportation of equipment in Europe we needed to bring certain certificates and documents for the radioactive sources and the X-ray machine. These were possession certificates from the Swedish Radiation Protection Authority (Statens strålskyddsinstitut, SSI) and documents declaring the ^{57}Co sources as dangerous goods according to standard ADR-S directions. ADR stands for European Agreement Concerning the International Carriage of Dangerous Goods by Road.

In Sweden the regulations are issued by the Swedish Rescue Services Agency. According to ADR, radioactive material is Class 7. In this class, our ^{57}Co sources were registered among the least dangerous. As they were properly packed in a lead can and fixed in a wooden box the dose rate at the surface of the package was well below the limit of 5 μSv/h. The box was properly marked with labels according to ADR.

Since we also were travelling by ferry, we needed documents following the ADR standard at sea, called IMDG (International Maritime Dangerous Goods Code). In Sweden these regulations are issued by the Swedish Maritime Administration. The classification is similar to ADR. Normally, the documents follow a certain template, however, the shipping companies we were using specified somewhat different requirements. We had both Swedish and English versions of the ADR documents. The procedure was also to announce the transportation for the shipping company well ahead in time.

When transporting sealed radioactive sources between member states within the European Union, the responsible authority of the arriving country needed to issue an approval. SSI signed the document for the return trip (EU standard document 1493/93, according to SSI FS 2006:1, replacing SSI FS 1996:1 valid at the time of the study). Our contact in Germany arranged with the document for our journey going there (from the German Federal Office for Radiation Protection – Bundesamt für Strahlenschutz).

* Now part of GE Healthcare.

Appendix 3. Lead smelter and subjects

At the German lead smelter, where this study took place, mining and smelting of lead ore had begun in the 16th century. Since 1970 lead ore has no longer been processed. The production switched over to recycling of lead from lead batteries. Other metals, particularly zinc, have also been processed since 1930. The lead production at the time of the study (summer 1997) was approximately 25,000 tons/year.

The Swedish secondary lead smelter factory, where the similar previous lead study was done, also recycled lead, mainly from lead batteries, and the annual lead production was approximately 40,000 tons during the 1990s. The study results from this group of Swedish workers were used for comparison. It comprised 71 active and 19 retired workers. Between 1968 and the end of the 1990s, the mean B-Pb in active workers had decreased from around 620 µg/l to 270 µg/l. A detailed description of this material is found in Börjesson et al. (1997).

Appendix 4. Sampling and analysis of body fluids; blood, plasma and urine

In short, venous blood was obtained from the workers on site in Germany. Whole-blood samples were stored under refrigeration until required for analysis. Blood samples were centrifuged for separation and plasma samples received. Morning urine was collected in the homes by the workers, and samples taken and prepared before packed and stored frozen (-20°C) together with the plasma samples, until required for analysis when returned to Sweden. B-Pb, P-Pb and U-Pb were analysed at the Department of Occupational and Environmental Medicine, University Hospital, Lund, Sweden, whereas creatinine in urine was determined at the Department of Clinical Chemistry, University Hospital, Lund, Sweden.

Lead was determined using ICP-MS (PlasmaQuad2+, Fisons Instruments, Winford, UK) after certain dilutions. All samples were prepared in duplicate. More diluted blood and serum were tested with respect to precision and detection limits. Screening of all standard solutions used for calibration was carried out before multi-element stock solutions were made, which showed only negligible contaminations with other elements.

The analysis of lead in blood and urine by the ICP-MS technique showed a good agreement with the concentrations in the reference samples, both in the present study and in two comparing previous studies by Bergdahl et al. (1998, 1999). This was also the case for the comparing group of Swedish workers where AAS was used (Börjesson et al. 1997). Further detailed description is found in Schütz et al. (1996, 2005).

Appendix 5. Calculation of Bone-Pb concentration

A linear function can be described by $y = k \cdot x + m$. The slope of the line is given by $k = (y_2 - y_1)/(x_2 - x_1)$. The intercept can be given by $m = y_2 - k \cdot x_2$.

There are two finger phantoms with the same bone lead concentration ($C_{phantom}$ = 200 μg/g) but different bone thickness (d = 8 and 12.2 mm). If the number of registered net pulses (N) from characteristic K X-rays are proportional to the finger bone volume, this relation can for our setup also be described as a linear function, $N = k \cdot d^2 + m$, where d is the average diameter of the finger bone. The slope for this situation is given by the phantom measurements and $k = (N_{12.2} - 0.9 \cdot N_8) / (12.2^2 - 8^2)$. Note that there is a factor 0.9 also needed, according to empirical observations. The intercept can be given by $m = N_{12.2} - k \cdot 12.2^2$. That gives a complete description of the relation between the number of net pulses and the finger bone thickness, but valid only for $C_{phantom}$.

With the bone thickness dependence eliminated and a proportionate number of net pulses for different concentrations of bone lead, it is possible to calculate the bone lead concentration for the measured subject as

$$C_{subject} = C_{phantom} * N_{subject} / N_{phantom} = C_{phantom} * N_{subject} / (k \cdot d_{subject}^2 + m)$$

where k and m are calculated as mentioned above, with values given by the calibration measurements of the two finger phantoms.