

**In vivo x-ray fluorescence of bone lead in the study of  
human lead metabolism in a cohort of the general  
population and smelter employees.**

By

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In vivo x-ray fluorescence of bone lead in the study of human lead metabolism in a cohort of the general population and smelter employees.

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## Abstract

After developing the clover-leaf geometry system, the system was used for the first time in a major survey in 2008 to measure the lead levels of 497 smelter employees in the province of New Brunswick in Canada, who participated in a lead study during the time interval of 14 January to 6 March 2008. Participants were measured for lead concentration in the left tibia and right calcaneus, each for 30 min. The minimum detection limit (MDL) of the clover leaf geometry detector system was improved by a factor 3.1 compared to a conventional system. Improvement of the detection system makes low-level environmental lead exposure studies relating to public health possible. This is important because low-level lead exposure has been well known to have many adverse health effects. Also, since the delivered effective dose is small (50 nSv), the technique can be used to measure the bone lead of sensitive populations such as the elderly and children. A total of 263 of individuals participated in a pilot study of bone and blood lead measurements supported by Health Canada's Chemical Management Plan. The study was conducted in collaboration with McMaster University and St. Joseph's Health Centre located in Toronto. Participants were measured for bone lead (calcaneus and tibia) concentration and whole blood and serum lead levels. Among participants 17 (6.5%) of them were 1 to 5 years old; 37 (14%) of them 6 to 10; 44 (17%) of them 11 to 19; 47 (18%) of them 20 to 35; 36 (14%) of them 36 to 50; 46 (17.5%) of them 51 to 64, and finally 19 (7%) of them belonged to age range of 65 to 85 years old.

In most of the previous work, the relationship between bone lead concentration and Cumulative Blood Lead Index (CBLI) was considered to be linear. In the study of a cohort of smelter employees we observed a non-linear relationship between CBLI and bone lead contents. The study showed that for the higher values of CBLI (earlier hired smelter employees), the transfer rate of lead from blood to bone becomes more efficient. Oppositely, we observed that for a higher levels of CBLI, the transfer rate of lead from bone to blood is less efficient and lead stays in bone longer. In this study, for the first time the transfer rate of lead from bone to blood was estimated as a function of predictors including age, employment time, and body lead contents for smelter employees.

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## **Chapter 1**

### **Introduction**

#### **1.1 Lead**

Lead (Pb) is an odourless, bluish-grey, lustrous metal that is malleable, ductile, and resistant to chemical corrosion. It exists as the element and as organic and inorganic compounds. "Organic lead" refers to lead compounds which contain carbon, whereas "inorganic lead" refers to compounds, including elemental lead, which do not contain carbon (ARB, 1997e). Lead has been extensively used in industry to manufacture products for tank linings, piping, equipment for handling corrosive gases and liquids used in petroleum refining, halogenations, sulfonation, extraction, condensation, metallurgy, and for pigments for paints. It is also used in ceramics, plastics, electronic devices, as a component of lead batteries, and in the production of ammunition, solder, cable covering, and sheet lead (HSDB, 1995). Lead is a poor electrical conductor but a good sound and vibration absorber (Sax and Lewis, 1989). In nature, lead occurs in bedrock, soils, tills, sediments, surface waters, ground waters, and sea water (Health Canada 1995). Volcanic activity and natural erosion of lead deposits are primary natural sources of lead release which absorbs at low levels in foods due to its uptake from soil into plants, from water and sediments into fish, and from plants and animals that consume them (Adriano 2001). Everyone is exposed to trace amounts of lead through air, soil, household dust, food, drinking water and various consumer products. Lead and lead compounds exist in the particle phase in the atmosphere with the average half-life and lifetime of about 3.5 to 10 days (Balkanski *et al.*, 1993), and after removal from the atmosphere and being deposited

on the ground and soil, inorganic lead may be re-entrained in the atmosphere (ARB, 1997).

## **1.2 Health Effects of Lead**

Since the early 1970s, lead exposure in Canada has decreased substantially mainly because leaded gasoline and lead based paint were gradually phased-out and the use of lead solder is prohibited in food cans. Due to the health hazard of lead even in small amounts, it has been added as one of the first elements to the List of Toxic Substances (Schedule 1) of the original Canadian Environmental Protection Act (CEPA). Health Canada initiated a precise review of the current toxicological and toxicokinetic data on lead, in 2005, in response to increasing scientific evidence of effects on human health occurring at low levels of exposure. There is scientific evidence of potential adverse health effects including neurodevelopmental, neurodegenerative, cardiovascular, renal and reproductive effects at blood lead levels below 10 micrograms per decilitre ( $\mu\text{g}/\text{dL}$ ), also, there is sufficient evidence that blood lead levels below 5  $\mu\text{g}/\text{dL}$  are associated with adverse health effects. Lead not only can be harmful to the health of people of all ages, but also can cause infants' and children's intellectual deficits. The greatest effect of lead exposure on children is to neurodevelopment, specifically the reduction of Intelligence Quotient (IQ) score and attention-related behaviours. Research suggests that an incremental increase in blood lead levels of 1  $\mu\text{g}/\text{dL}$  is associated with approximately a 1 IQ point deficit (Lanphear *et al.* 2000, Schwartz 1994). The following provides a

summary of the health effects related to lead including *developmental neurotoxicity, neurodegenerative effects, cardiovascular effects and renal effects.*

### **1.2.1 Developmental Neurotoxicity:**

At lower doses, lead competes with calcium and mainly alters calcium homeostasis, causing disruption in neurotransmitter systems (Audesirk and Audesirk 1993; Banks *et al.* 1997; Leret *et al.* 2002; Mameli *et al.* 2001). The vulnerable areas to lead's neurochemical effects are prefrontal cortex, hippocampus, hypothalamus, nucleus accumbens, the brain stem and the cerebellum (Finkelstein *et al.* 1998; Kala and Jadhav 1995; Lasley *et al.* 2001). The neurotransmitters including noradrenaline, dopamine, serotonin and acetylcholine which are vital in the regulation of emotional behaviour are most affected by lead (DiPietro 2000; McIntosh *et al.* 1989; Sidhu and Nehry 2003). Lead disrupts the function of synapses and dendrites, which create cortical circuits for the transmission of signals from one area of the brain to another (Berger –Sweeney and Hohmann 1997; Rice and Barone 2000) by preventing signals from occurring normally between various brain regions, which can later manifest itself in global cognitive, behavioural or motor dysfunctions (Bellinger 1995; Rice and Barone 2000).

Bellinger *et al.* (1991) reported that the General Cognitive Index (GCI) decreased 3 points with the standard error (SE) of 1.4 for each natural logarithm unit increase in blood lead level at 24 months old. However, the authors did not find any association between prenatal exposures and GCI at 57 months old. Moreover, the authors found that

through 24 months of age, toddlers with umbilical cord blood lead levels between 10 and 25 ( $\mu\text{g/dL}$ ) showed persistent cognitive deficits on the Mental Development Index (MDI) of the Bayley Scales of Infant Development. Whereas, higher prenatal exposures were not associated with lower scores on the McCarthy Scales of Children's Abilities at 57 months old. The Fraser *et al.* (2006) study focused on 110 preschoolers of age 5 from Nunavik. The authors reported that lead directly affects both motor function and behaviour. The study suggested that postnatal blood lead concentrations correlated positively with impulsivity and activity (IA). Bouchard *et al.* (2009) investigated the association of current blood levels of major depression, panic, and generalized anxiety in 1987 adults aged 20 to 39. The authors demonstrated that increasing blood lead levels were associated with higher odds of major depression ( $P = 0.05$ ; 95% CI, 1.13-4.75) and panic disorder ( $P = 0.02$ ; 95% CI, 1.32- 18.48) but not generalized anxiety disorder ( $P = 0.78$ ).

### **1.2.2 Neurodegenerative Effects**

Neurodegeneration is defined as the progressive loss of structure or function of neurons, including death of neurons causing neurodegenerative diseases such as Parkinson, Alzheimer, and Huntington (Stedman 2006). Based on recent studies, the mechanisms underlying the effect of lead on the central nervous system (CNS) in adult animals were examined to elucidate the role of lead in producing apoptotic cell death of neurons (Fox *et al.* 1997; Sharifi *et al.* 2002). Based on recent evidence, lead has been found to

accumulate in myelin, inhibits integral enzymes there, and may contribute to ultra-structural changes, or other changes in myelin (Dabrowska - Bouta *et al.* 2004), which in turn, supports the hypothesis of causation some of the neurodegenerative diseases (Bartzokis and Tishler 2000).

Struzynska *et al.* (1997) suggested that environmental exposure to lead influenced some morphological and biological properties of rat brain microvessels due to increasing of lead level in capillaries and synaptosomes. The authors reported that at low doses, lead induces blood-brain barrier (BBB) dysfunction. Also, lead accumulates in astroglia which are in charge of maintaining neuronal homeostasis in brain and controlling of the released glutamate. The results of studies indicate that lead toxicity in adult rat brain activates astrocytic processes connected with the controlling of glutamate homeostasis. Shih *et al.* (2006) found that long-term exposure to high levels of lead in the environment is associated with decrements in cognitive ability including language, processing speed, eye-hand coordination, executive functioning, verbal memory and learning, visual memory, and visuoconstruction in older Americans of ages 50 to 70. The authors suggested that for the mean (SD) blood lead level of 3.5 (2.2)  $\mu\text{g}/\text{dL}$  and tibia lead level of 18.7 (11.2)  $\mu\text{g}/\text{g}$ , higher tibia lead levels were consistently associated with worse cognitive function in all seven domains after adjusting for age, sex, and testing technician. However, blood lead was not associated with any cognitive domain.

### 1.2.3 Cardiovascular Disease

One of the main causes of mortality and a primary contributor to the burden of disease worldwide is cardiovascular disease (Lopez *et al.* 2006). Environmental toxicants, e.g. lead and other metals, may explain a part of the population variation in cardiovascular disease rates (Bhatnagar 2006; Weinhold 2004). The contribution of lead to cardiovascular disease is still incompletely understood. There is evidence that at blood lead levels below 10 µg/dL, lead exposure has been associated with cardiovascular mortality, stroke mortality, myocardial infarction mortality, cardiotoxicity, and peripheral arterial disease (Navas-Acien *et al.* 2004, 2007, 2008; Menke *et al.* 2006; Schober *et al.* 2006). Most of the past studies agreed with having a positive association between blood lead levels and blood pressure, where the estimated increase in systolic blood pressure associated with a 2-fold increase in blood lead levels (e.g. from 5 to 10 µg/dL) ranged across reviews from 0.6 to 1.25 mmHg (Sharp *et al.* 1987; Hertz-Picciotto and Croft 1993; Staessen *et al.* 1994, 1995; Navas-Acien *et al.* 2007). Based on the studies from 1988 onward (Kromhout 1988, Lustberg and Silbergeld 2002; Menke *et al.* 2006, Møller and Kristensen 1992), the authors were unanimous that lead exposure was positively associated with clinical cardiovascular end points for the general population. For the occupational studies, the association between the variables varied widely, with positive (Dingwall-Fordyce and Lane 1963; Lundstrom *et al.* 1997; Malcolm and Barnett 1982; Steenland *et al.* 1992), inverse and null associations (Robison 1974; Tollestrup *et al.* 1995; Sheffet *et al.* 1982).

Lustberg and Silbergeld (2002) suggested individuals with blood lead levels of 20 to 29  $\mu\text{g}/\text{dL}$  ( $n=4292$ ) experienced significantly increased all cause, circulatory, and cardiovascular mortality from 1976 through 1992. Navas-Acien *et al.* (2004) analyzed data from 2125 participants who were 40 years of age and older in the 1999 to 2000 National Health and Nutrition Examination Survey (NHANES) and concluded that blood lead, at levels well below current safety standards, were associated with an increased prevalence of peripheral arterial disease in the general US population. In conclusion, Navas-Acien *et al.* (2007) reported, based on the all observational studies from database searches and citations regarding lead and cardiovascular end points, that there is sufficient evidence to infer a casual relationship of lead exposure with hypertension. Also, the authors found suggestive (not sufficient) evidence to conclude a casual relationship of lead exposure with clinical cardiovascular outcomes and with heart rate variability.

#### **1.2.4 Renal Effects**

At blood lead levels above 50  $\mu\text{g}/\text{dL}$ , lead may also cause harmful effects on the renal system [ATSDR 2007a]. Ekong *et al.* (2006) reported that lead exposure, at very much lower doses than those causing lead nephropathy, acts as a cofactor with more established renal risk factors to increase the risk for chronic kidney disease (CKD) and the rate of progression. The authors reported that adverse renal effects have been reported at mean blood lead levels of 5  $\mu\text{g}/\text{dl}$ .

### **1.2.5 Lead and Bone**

Lead circulates in the bloodstream and either accumulates in bone or is excreted from the body. Lead has a long residence time in bone and up to 90% and 70% of the absorbed lead accumulates in bone in adults and children respectively (Barry 1975). Lead in bone is constantly mobilized and released back into systemic circulation, but under certain conditions such as pregnancy, lactation, menopause, andropause, post-menopause, extended bed rest, hyperparathyroidism, and osteoporosis (Silbergeld *et al.* 1988; Franklin *et al.* 1997; Gulson *et al.* 1997, 1999, 2003) the rate of remobilization can increase. The organic matrix of bone is strengthened by deposits of calcium phosphate crystal. This crystal contributes in formation and organization of the bone (i.e. osteoblasts, osteocytes, and osteoclasts). Having the excess of other metals in bone (i.e. lead) can change the calcium to phosphorus ratio which, in turn, affects the composition and ion exchange mechanism that may contribute to osteoporosis disease (Barrere *et al.* 2006). Excess of lead may also bring delay in “bridging cartilage formation” which postpones the fracture healing of the bones (Carmouche *et al.* 2005).

### **1.3 Sources of Exposure and Concentrations in Environment**

The various sources of lead exposure are situations such as living in or working in older buildings that contain fading lead paint, soil, and contaminated ambient air; diet and health habits (e.g. smoking or pica), and consumer products (e.g. costume jewellery, toys, leaded crystal, art supplies). People are exposed to lead through ingestion (by food and



drinking water) or inhalation. Since infants and children have different behaviours from adults, including crawling, greater frequency of hand-to-mouth contact, they are exposed to lead additionally by oral intake of paint chips, house dust and soil contaminated with lead (ATSDR 2007, 2010; Bushnik *et al.* 2010; EFSA 2010). Concentration of lead in ambient air, soil, indoor air and house dust, food, and drinking water has been reported by Health Canada in their Final Human Health State of the Science Report in 2013 on lead as is presented in the following sections.

### **1.3.1 Ambient Air**

From 1970 to 1974 the average air lead concentration in Canada was reported to be about  $0.60 \mu\text{g}\cdot\text{m}^{-3}$ . Following the introduction of unleaded gasoline in Canada in 1975 and the prohibition of using leaded gasoline for vehicles in 1990, ambient air lead concentrations in Canada have declined significantly to about  $0.02 \mu\text{g}\cdot\text{m}^{-3}$  in 1990 and  $<0.0015 \mu\text{g}\cdot\text{m}^{-3}$  in 2008 (Environment Canada 2010b). The only main sources of lead in ambient air in the United States and Canada are small aircraft with piston engines as they are allowed to use leaded gasoline (U.S. EPA 2010a). Miranda *et al.* (2011) investigated the relationship between lead produced by aviation gasoline and blood lead levels in children 9 months to 7 years of age living in North Carolina. The authors suggested that children living within 500 m of an airport have higher blood lead levels than other children. Also, for children living 1000 m away from the airport the evidence of elevated blood lead level was observed.

### 1.3.2 Soil /Indoor Air and House Dust

Soils and sediments are primary sinks for lead compounds. Lead has a strong tendency to adsorb to soils and is generally retained in the upper soil layers permanently unless it is removed through remediation (ATSDR 2007). In Canada, the overall arithmetic mean of lead concentrations in glacial till has been reported to be 9.65 (mg/kg) with the range from 1 to 152 (mg/kg) based on 7398 samples collected throughout Canada for the particle size fraction < 63 µm (Rencz *et al.* 2006). In cities, around industrial sources, next to homes, and structures such as lighthouses lead levels in soil tend to be higher (CMHC 2009), where lead-contaminated soil can be tracked into residences and contribute to the lead content of indoor settled dust. Lanphear *et al.* (1998) reported that lead-contaminated house dust is the major source of lead intake for children who have moderate to high blood lead levels (between 10 to 25 µg/dL).

Furthermore, indoor environments can be a significant source of exposure to lead as Canadians spend up to 90% of their time indoors (e.g. at home, at school, or in the workplace) (Leech *et al.* 1996). In the United States, the most significant source of lead in indoor environment is the lead based paint in homes before 1978 (U.S. EPA 2010c) since lead from lead-based paint can be dispersed when the paint degrades and can then contaminate household dust, which, in turn, can become re-suspended in air.

### 1.3.3 Food and Drinking Water

The primary sources of lead absorption to foods are through uptake from soil into plants and deposition onto plant surfaces; for instance, leafy vegetables grown in lead-bearing soil will contain lead in their leaves and have lead-containing particles on their surface (ATSDR 2007), and other animals may be exposed to lead through the foods they eat. Also, fish can absorb lead from water and sediments (Health Canada 2011a). Lead may be introduced to foods during transport to market, processing, and kitchen preparation including cooking with lead-contaminated water, or storing food in lead-containing vessels (U.S. EPA 1986a; Health Canada 1992; ATSDR 2010). Moreover, hunting wild animals with lead bullets is another potential source of dietary lead exposure (Tsuji *et al.* 2008; Health Canada 2011a). Health Canada has carried out a Total Diet Study Since 1969 up to 2007. Studies illustrate that the dietary intake of lead (in units of  $\mu\text{g}/\text{kg}$  body weight per day) by average Canadians of all ages and sexes has decreased since 1981 from 0.8 to about 0.1 in 2007.

Drinking water can be another source of lead exposure since lead can be introduced into drinking water as a result of dissolution of old lead-based solders used to join copper pipes within homes and buildings, and plumbing fittings, and other components containing lead. The age of the plumbing system, length of time the water sits in the pipes, and the seasonal variations in temperature between the summer and winter are some of the factors that influence the amount of lead of drinking water (Health

Canada 2009a). With the warmer temperatures of the summer months, lead concentration increases (Britton and Richards 1981; Karalekas *et al.* 1983; Colling *et al.* 1987, 1992; Douglas *et al.* 2004), where the highest lead concentrations in Ottawa, Ontario drinking water has been reported to be seen from May to November (Douglas *et al.* 2004). Another factor that may increase the amount of dissolved lead in water is due to the use of decontaminator chloramine. A change of disinfectant from free chlorine to chloramine may cause an elevation in drinking water lead concentrations which resulted in elevated blood lead levels for children ( $\leq 1.3$  years) consuming that water (Edwards *et al.* 2009).

#### **1.4 Toxicokinetics of Lead Metabolism**

The term toxicokinetics refers to the study and modeling of the movement of toxic substances and their residence time in different compartments of the body. Several biokinetic models for lead have now been published as has been listed as follows.

Rabinowitz *et al.* (1976) fitted three compartment models to their  $^{204}\text{Pb}$  tracer data. They studied the steady state kinetics of lead metabolism in five healthy 53 year old men, weighing on average 70 kg, smoking eight cigarettes per day. Participants' daily intake was supplemented with 79-204  $\mu\text{g}$   $^{204}\text{Pb}$  for 1-124 days (average 367  $\mu\text{g}/\text{d}$ ). Three main compartments labeled with  $^{204}\text{Pb}$  and  $^{207}\text{Pb}$  tracers included, blood that contained 1.7-2.0 mg of lead with a mean life of 35 days; Soft tissue that contained 0.3-0.9 mg of lead, and had an approximate mean life of 40 days, and finally the last compartment was skeleton which enclosed the vast quantity of bone lead with a very slow mean life. Figure

I summarized the schematic of the steady state kinetics of lead metabolism suggested by the authors.

In addition to three compartments, mentioned above, Batschelet *et al.* (1979) included the lungs and the digestive tract to the model. The authors suggested that the digestive tract is not only fed by dietary lead but also by some lead contained in saliva, gastric secretion, and bile. The recommended model has been shown in figure 2 using Rabinowitz *et al.* (1976) experiment. The authors reported biological half lives of 15.5, 34.7 and  $22.6 \times 10^3$  days in blood, soft tissues and bone respectively.

Marcus (1985) suggested a nine-compartment model for the Rabinowitz data as has been shown in figure 3. Due to the large lead content and long residence time of lead in bone, the linear compartmental model was suggested by the author.

A compartmental model (figure 4) of a 70 kg man for lead intake, distribution, and transport was suggested based on previous models and experimental results for lead in human body by Bert *et al.* (1989). To predict lead levels in blood, bone, and other compartments as a function of time resulting inputs from inhalation and ingestion, a set of first order, linearly ordinary differential equations with constant coefficients was used (Bert *et al.* 1989). Their model demonstrated an excellent agreement with the measurements of blood lead for a controlled study by Rabinowitz *et al.* (1976).

O'Flaherty (1991) considered a physiologically based toxicokinetic model in the growing rat from birth to growth, where the model was capable of integrating exposure over time by considering growth, development, and aging. In this model, age was considered as a main factor that controls lead distribution to bone and soft tissue, lead

absorption from the gastrointestinal tract, lead elimination, and transfers of lead between plasma and bone.

Leggett (1993) recommended a model which describes the time-dependent distribution and excretion of lead that has been injected or absorbed into blood. Figure 5 illustrates the transport of lead between compartments, where the rate of lead transfer is assumed to follow first order kinetics provided that the concentration of lead in red blood cells stays below a nonlinear threshold concentration. If the concentration of lead in red blood cells exceeds the threshold, the transfer rate from diffusible plasma to red blood cells will decrease, and at the same time, the deposition fractions in other compartments are increased due to decreased competition from the red blood cells. However, first order transport between all other compartments is assumed to be maintained at all levels of exposure.

## LEAD METABOLISM MODEL

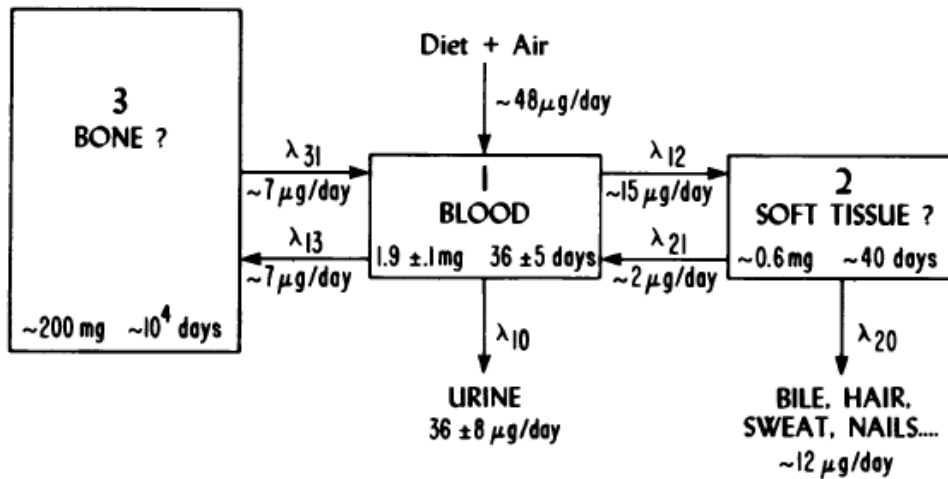


Figure 1: A three-compartmental model of human lead metabolism derived from tracer and balance data from five healthy men, where the lead content and mean life of each pool and the rates of lead movement between pools ( $\lambda$ ) are shown. Numerical values represent the mean values  $\pm$  standard deviation for all subjects for whom data were available. Based on the kinetic analysis of the data, 1.9 mg of lead receive from the gastrointestinal tract. Compartment two includes soft tissues. Lead exchanges between compartments one and three and compartments one and two. Compartment two contains approximately 0.6 mg of lead and gives rise to hair, nails, and at least some alimentary tract secretions. Most of the lead in the body stores in skeleton. The biological half life of lead in blood, soft tissues and skeleton are  $36 \pm 5$  days, 30 to 55 days, and 27 years respectively. Loss of lead from the body via pool two ( $\lambda_{20}$ ) is from hair, nails, sweat and alimentary tract losses, such as salivary, biliary, gastric, and pancreatic secretions, and loss of lead via pool one ( $\lambda_{10}$ ) is from urine (Rabinowitz *et al.* 1976).

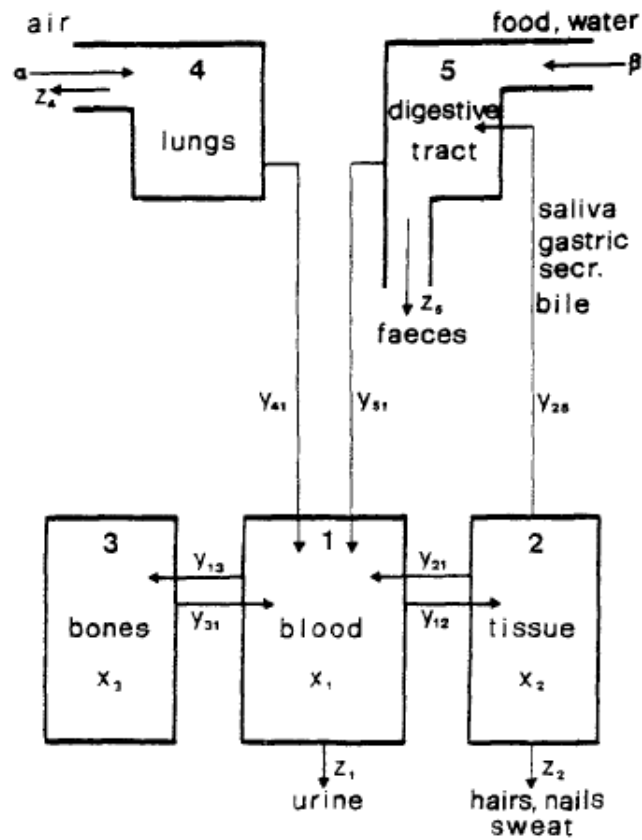


Figure 2: A three compartment model of lead kinetics in human body. The daily inputs from air and food (and water) are presented by  $a$  and  $\beta$  respectively. The daily outputs are presented by  $z_1$  (urine),  $z_2$  (hair, nails, sweat),  $z_4$  (air, mucus),  $z_5$  (faeces). The amount of lead stored in the compartments are  $x_1$  (blood),  $x_2$  (tissue),  $x_3$  (bones). The daily exchange portions of lead ( $\mu\text{g}/\text{d}$ ) are denoted by  $y_{ik}$ , where  $i$  and  $k$  means the compartments of origin and destination respectively (Batschelet *et al.* 1979).



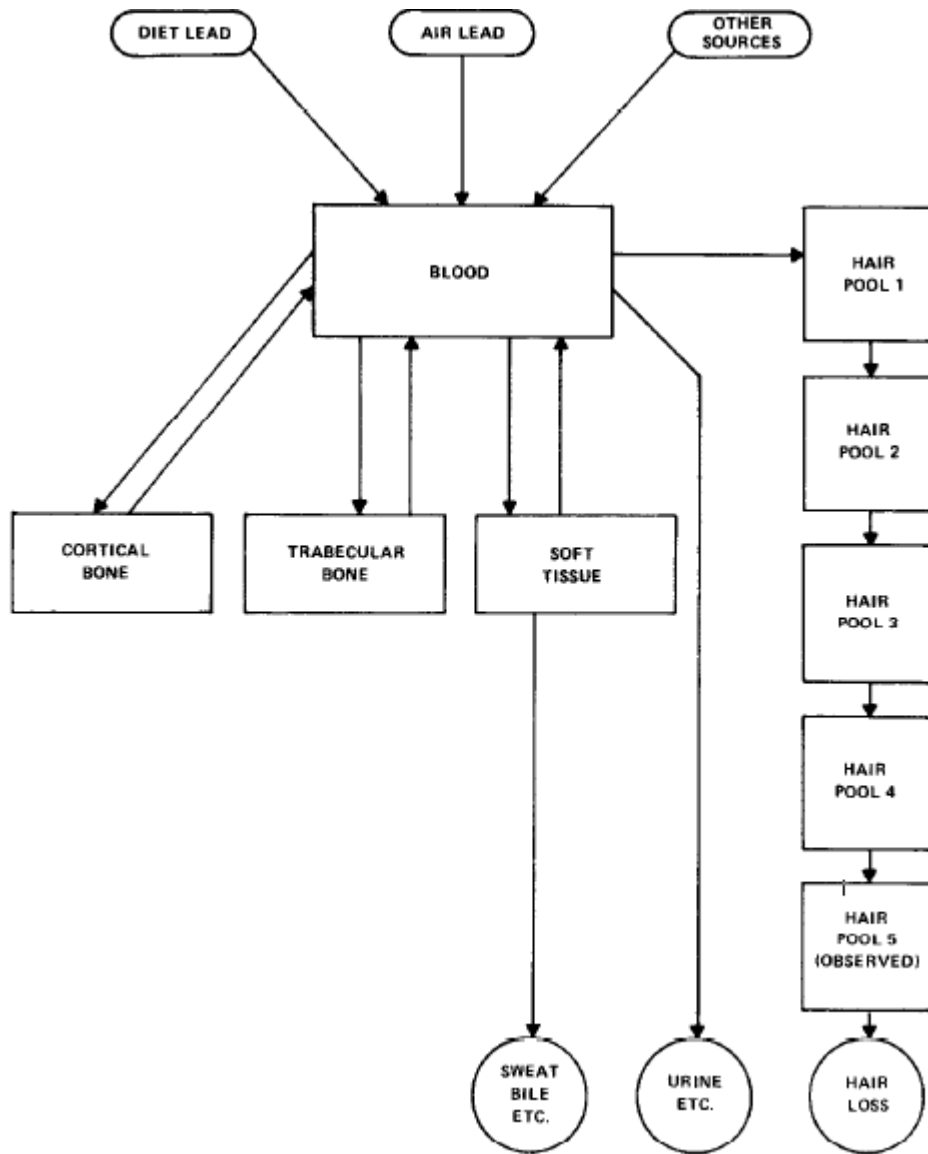


Figure 3: Compartmental model for lead in man (Marcus 1985)

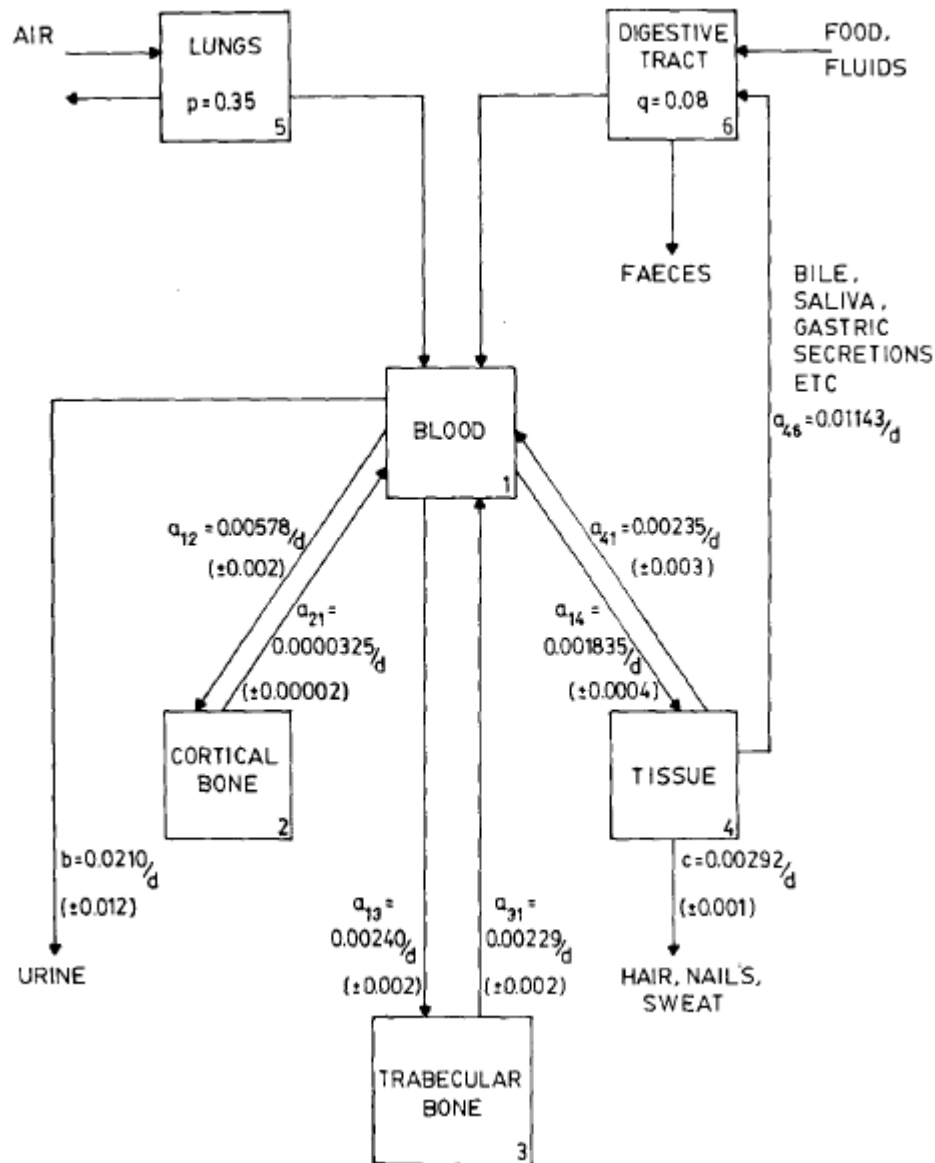


Figure 4: Compartmental model for lead: the transfer coefficients for lead ( $a_{ij}$ ) and the elimination coefficients ( $b$  and  $c$ ) and the fractional absorption of lead in the lungs and digestive tract ( $p$  and  $q$ ) have been shown with their values (Bert *et al.* 1988).

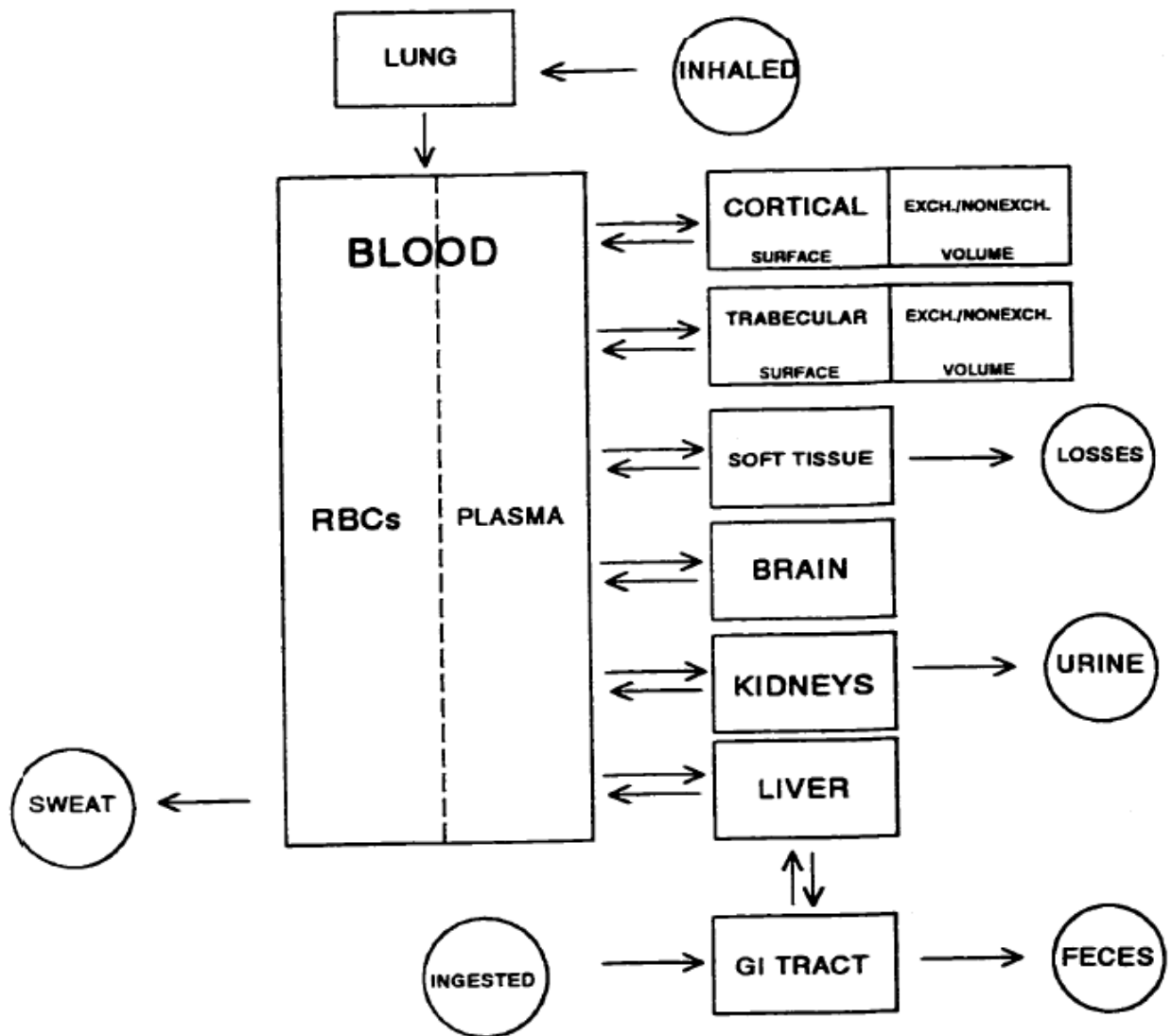


Figure 5: Compartments and paths of movement in the biokinetic model for lead (Leggett 1993). This figure has been taken from David Fleming PhD thesis.

### 1.5 X-Ray Fluorescence

The analysis of major and trace elements (e.g. lead in bone) by using x-ray fluorescence technique is made possible by the behavior of atoms when they interact with radiation.

When materials (e.g. components of bone) are excited with high-energy, short wavelength radiation (e.g.,  $\gamma$ -rays emitted from  $^{109}\text{Cd}$  radioactive source), they can become ionized, when the energy of the radiation is sufficient to remove a tightly-held inner electron, the atom becomes unstable and an outer electron replaces the missing inner electron. As a result of the displacement, energy is released, where the emitted radiation is of lower energy than the primary incident photons and is termed fluorescent radiation. Since the energy of the emitted photon is characteristic of a transition between specific electron orbitals in a particular element, the resulting fluorescent X-rays can be used to detect the abundances of elements that are present in the sample. There are five main processes in which gamma-ray or x-ray photon energy is transferred after interacting with materials. After interacting with an atom, photon energy may either disappear or is partially scattered through a significant angle. Gamma rays energy is lost through five major processes: photoelectric absorption, Compton scattering, pair production, Rayleigh scattering, and photodisintegration (Knoll, 1999; Ermakov *et al.* 2010).

### **1.5.1 Photoelectric Absorption**

In photoelectric absorption, the photon energy is absorbed by the electron, and no more photon energy is scattered after the interaction. Since energy is conserved the relation of energies before and after collision is expressed as

$$E_{\gamma} = E_k + E_b$$

Where  $E_\gamma$ ,  $E_k$  and  $E_b$  are the incident photon energy, kinetic energy of electron, and binding energy of the electron respectively. Providing the photon has sufficient energy, the photoelectric effect is most likely to take place with K shell electrons. Part of the photon energy is required to overcome the binding energy of the target electron and the rest is transferred to the target electron in the form of kinetic energy. The photoelectric effect is most probable for low energy photons interacting with high atomic number elements ( $Z$ ). The linear mass attenuation coefficient for photoelectric absorption coefficient ( $\mu_{ph}$ ) varies with photon energy ( $E_\gamma$ ) and atomic number ( $Z$ ) as (Knoll, 1999)

$$\mu_{ph} \propto \frac{Z^n}{E_\gamma^{3.5}}$$

where the exponent  $n$  varies between 4 and 5 over the gamma-ray energy region of interest.

### **1.5.2 Compton Effect**

When the incident photon interacts with an electron and the photon with a larger value of wavelength (less energy) is released as product of reaction, then the process is explained as Compton Scattering or Compton Effect. With the simplifying assumptions that the target electron is free and at rest, the scattered photon energy is calculated by using the formula below (Knoll, 1999).

$$h\nu' = \frac{h\nu}{1 + \frac{h\nu}{m_0c^2}(1 - \cos\theta)}$$

Where  $h\nu$  is the energy of the incident photon;  $h\nu'$  is the scattered photon energy;  $\theta$  is the scattering angle for the photon, and finally, the energy of the electron before the collision is simply its rest energy  $E_0 = m_0c^2$ .

The probability of Compton scattering per atom depends on the number of electrons in the targets and is directly proportional to the atomic number,  $Z$ . As a result, Compton scattering provides density related information of an atom since the electron density is directly proportional to  $Z$ , and the mass density is proportional to the electron density given that for most elements, except hydrogen, the ratio of atomic number to the mass number is equal to approximately one-half (Ziegler *et al.* 1959).

### 1.5.3 Pair Production

If the incident photon energy exceeds 1.02 MeV (the rest mass energy of the electron is 0.511 MeV), an interaction can take place in the coulomb field of a nucleus by which positron-electron pair is created. By the energy conservation law we have:

$$E_{k-electron} + E_{k-positron} = E_\gamma - 2m_0c^2$$

The positron gradually loses its energy. When the positron has lost most of its kinetic energy ( $E_K$ ), it then combines with an electron through the annihilation process. Pair

production can only happen for photon energies above 1.02 MeV, and it is most likely to occur in high  $Z$  number materials. The probability of pair production occurring is proportional to  $Z^2 + Z$  for energies above 1.02 MeV (Glasstone and Sesonske 1994).

#### 1.5.4 Rayleigh Scattering

Rayleigh scattering, also known as Thomson scattering, coherent, or classical scattering is a type of interaction mechanism, where, virtually, no transfer of energy occurs. After a gamma ray interacts with an atom, the photon is scattered with no change in wavelength (Knoll, 1999). Coherent cross section is calculated by using the formula below (İhlli and Erzeneolu, 2001):

$$\frac{d\sigma_{coh}}{d\Omega} = \frac{d\sigma_T}{d\Omega} [F(x, Z)]^2$$

Thomson cross section is defined as:

$$\frac{d\sigma_T}{d\Omega} = \frac{1}{2} r^2 (1 + \cos^2 \theta)$$

$F(x, Z)$  is the atomic form factor depending on the parameters: photon energy, scattered angle, and atomic number  $Z$ . For the large scattered angle, close to 180 degree and for the energy range of the order of 60-100 keV, the form factor is expressed as a function of  $Z^{5or6}$  (Chettle *et al*, 1991).

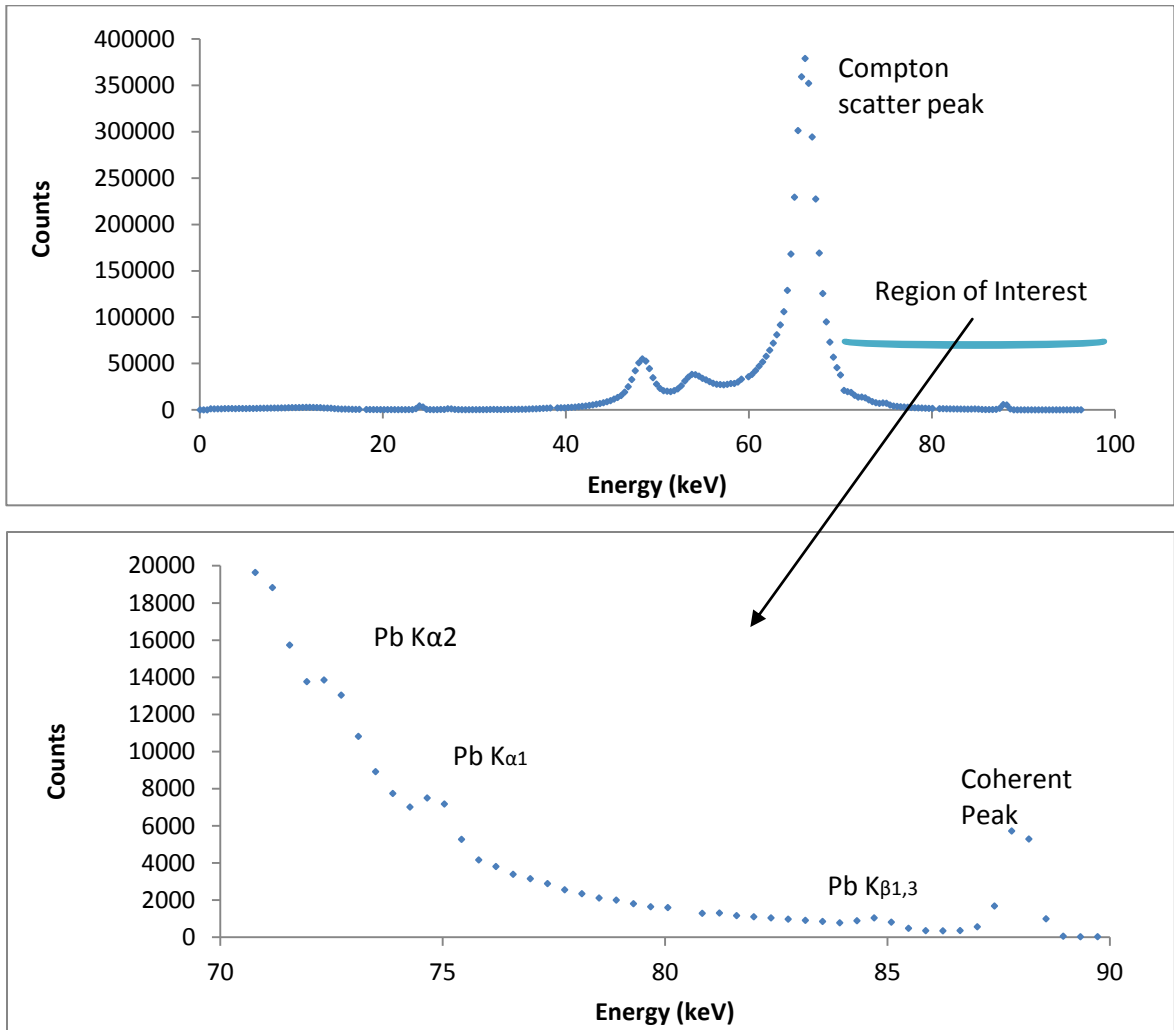
### 1.5.5 Photodisintegration Interaction

Photodisintegration process is a collision of a high energy photon (in general greater than about 10 MeV) with an atomic nucleus, in by which the x-ray photon is captured by the nucleus of the atom with the ejection of a particle from the nucleus (e.g. a neutron, proton, or alpha particle) (Ermakov *et al.* 2010). Two important exceptions are ( $\gamma$ , n) reactions with  $^2\text{H}$  and with  $^9\text{Be}$ .

### 1.5.6 Lead K-Shell X-Ray Characteristic

The principle of x-ray fluorescence has been explained in chapter 2 (page 30) and is not repeated here. Calibrated standards or participants' tibia and calcaneus were irradiated with a radioactive source,  $^{109}\text{Cd}$ , for 20 to 30 minutes. The vacancy created by ejecting an electron from the lead K-shell following an interaction of a  $^{109}\text{Cd}$  gamma-ray with the target electron, may be filled with either L shell or M shell electrons corresponding to the release of  $\text{K}_\alpha$  or  $\text{K}_\beta$  x ray energies respectively. Three main routines contribute to spectrum data analysis. The purpose is to find the number of counts for the coherent region and for lead K x-rays in the alpha and beta regions. Figure 6 is the schematic representation of the spectrum of a phantom measurement with lead concentration of 100  $\mu\text{g-Pb/g-plaster}$  of Paris as a number of counts.





**Figure 6:** Schematic representation of coherent (88.03 keV), K $\alpha$ 1 (74.969 keV), K $\alpha$ 2 (72.804 keV), and K $\beta$ 1 (84.936 keV) and K $\beta$ 3 (84.450 keV) for 100 ppm standard (Behinaein, 2009).

## 1.6 About the Thesis

The main focus of the dissertation research was on two lead exposure groups: occupationally exposed group including 497 smelter employees from New Brunswick, Canada and the environmentally exposed group including 263 residents of Toronto. The

thesis includes eight main chapters. The information presented in chapters two and four have been published in *Physics of Medicine and Biology* and *Environmental Monitoring* journals, and chapters three, five and seven will be published in journal article. The first chapter is a brief introduction about health hazards of lead in humans, sources of lead exposure and concentration in environment, some of the toxicokinetic lead models in humans' body and the principle of X-Ray Fluorescence technique. The second chapter of the thesis, includes examining the effectiveness of a new  $^{109}\text{Cd}$  K-XRF measurement system consisting of four detectors with clover-leaf geometry since after developing the system in 2005, the system was used for the first time in a major survey in 2008 to measure the bone lead level of the smelter employees. Using a clover-leaf geometry detector system improves the MDL (minimum detection limit) significantly. The MDL of the clover-leaf geometry system for calcaneus and tibia measurements was found to be  $4.78 \mu\text{g Pb (g bone mineral)}^{-1}$  and  $3.14 \mu\text{g Pb (g bone mineral)}^{-1}$  respectively, while the MDL for the conventional system for calcaneus measurements was calculated to be  $14.0 \mu\text{g Pb (g bone mineral)}^{-1}$  and  $15.4 \mu\text{g Pb (g bone mineral)}^{-1}$  and for tibia measurements was found to be  $12 \mu\text{g Pb (g bone mineral)}^{-1}$  and  $8.48 \mu\text{g Pb (g bone mineral)}^{-1}$  in the 1999 and 1994 surveys, respectively. The third chapter consists of examining the factors that influence the uncertainties of *in vivo* bone lead measurement besides the choice of radioactive source and the geometry of the detector system. The factors were discussed based on sex, age, body mass index (BMI), and the product of source activity and measurement time by using one-way ANOVA analysis, two-way ANOVA analysis, and multivariate regression model. Results from univariate analyses showed that females have

higher uncertainty compared to males. We observed significant differences for both calcaneus (Cal) and Tibia (Ti) uncertainty measures ( $p < 0.0005$ ) among the age groups, where the uncertainties were highest in the lowest age group ( $< 11$ ). We found that there is no difference between sexes in any age group except for age group (51-65) ( $p < 0.0001$ ), where females had higher levels of uncertainty than males ( $p = 0.0013$ ). Based on the multivariate result analysis, for the predictors including age and tibia lead level, tibia uncertainties for both sexes were found significantly different. Considering the model for calcaneus uncertainty when age and lead are included in the model, the models between sexes were considered statistically similar (coincidence). For smelter employees, tibia uncertainties did not correlate with age and tibia lead level; however, the predictors age and calcaneus lead level correlate with calcaneus uncertainties ( $P = 0.00169$ ). Also, we found that the product of source activity and measurement time influenced the precision of measurements directly, which resulted in having the higher uncertainties for EG than those for smelter employees. In the fourth chapter, the relationship between Cumulative blood lead index (CBLI) and bone lead concentration in smelter employees was examined, where the fact of observing nonlinearity between CBLI and bone lead levels has been used in chapter 4 to establish a function for lead transfer rates from bone to blood with the predictors, CBLI, bone lead level, age, and the years of exposure to lead. So far, in most of the lead kinetic models, the transfer rates of lead between different compartments have been considered to be constant, and in this thesis, for the first time we are exploring the dependency of lead transfer rates to the variables mentioned above. Chapter 5 is about developing a novel method to estimate the rates of lead exchange

between body compartments of smelter employees. In most of the toxicokinetic models of lead, the transport of lead from bone to blood was considered to be constant. In this thesis for the first time, a multivariate model was developed in order to express the transfer rate of lead from tibia and calcaneus into blood stream as a function of employment time, age, body lead contents. We observed a significant decrease in the transfer rate of lead from bone to blood with increasing body lead contents. The model was tested by calculating the bone lead concentration in 1999 and 2008, and by comparing those values with the measured ones. A good agreement was found between the calculated and measured tibia (calcaneus) lead values. Chapter 6 confirms nonlinearity between blood lead and plasma lead concentration. Also, we found that the association of plasma lead concentration and bone lead concentration can be described by a linear multivariate model by having age as one of the predictors. Chapter 7 reflects the relationship between age and body lead levels including tibia, calcaneous, and blood lead levels in different age categories for both sexes, where, we observed the body lead levels were higher in children and elderly, and finally chapter 8 is conclusions and suggestions for future work.

## Chapter 2

### ***In vivo* measurement of lead in the bones using the four-element ‘clover-leaf’ geometry detector system**

#### **2.1 Introduction**

Because of lead's long residence time in bone, it constitutes cumulative dosimeter of lead exposure over many years and may be more reliable to predict the chronic toxicity than recent exposure, which is more reflected by blood lead levels. Also, the dynamic behaviour of lead in bone may release enough stored lead into blood stream, which in turn, can pose a significant threat of delayed toxicity, and finally, even though environmental and occupational lead exposure has largely been controlled in the United States and Canada over the past decades, decades of heavy environmental pollution have resulted in significant accumulation of lead in bone among most members of the general population (Hu 1998).

Because of the extremely small neutron cross sections of lead, X-ray fluorescence is the main technique for *in vivo* lead analysis (Scott and Chettle 1986). The sensitivity of XRF measurement systems depends on the fluorescing source energy and on source-target-detector geometry. *In vivo* measurements of lead in bone have been performed with three XRF methods including fluorescing the K-shell electrons of lead using (i)  $^{57}\text{Co}$  in a  $90^\circ$  geometry, (ii)  $^{109}\text{Cd}$  in a backscatter geometry and (iii) fluorescing the L-shell electrons using  $^{125}\text{I}$  or an X-ray generator. In addition to the radioactive source, the

components of an XRF measurement system consist of a radiation detector, Ge for K XRF, Si (Li) for L XRF, preamplifier, amplifier, analog-to digital converter, multichannel analyzer and computer for data storage and analysis. In all XRF methods, bone lead measurements are non-invasive. The subject must sit in a chair and have the measurement system moved into place, where  $^{109}\text{Cd}$  K XRF measurements are typically performed for approximately 30 min, and L XRF measurements for 16 min (Todd and Chettle 1994). KXRF measures lead approximately 37 mm into bone; whereas, LXRF technique, which has been used only in some pediatric studies, measures lead only 2 to 3 mm into the bone and is highly sensitive to slight variations in thickness of skin overlying the bone (Wielopolski *et al.* 1981).

The validation of K and L XRF methods was confirmed by doing comparison between XRF and atomic absorption spectrophotometry (AAS). Somerville *et al.* (1986) validated  $^{109}\text{Cd}$  K XRF by performing comparison of 80 bare-bone samples including bones from tibia sections, tibia fragments, calcaneus, and metatarsals, where the authors reported the mean difference between  $^{109}\text{Cd}$  K XRF and AAS measurements to be less than  $<0.1 \mu\text{g Pb/g}$  bone mineral. Furthermore, since in the  $^{109}\text{Cd}$  K XRF method, the lead x-rays are normalized to the coherent peak, measurements are independent of fluorescing source to subject distance, overlying tissue thickness, bone size, bone shape, bone geometry, bone density, and minor patient movement (Todd and Chettle 1994).

In order to further improve the precision of  $^{109}\text{Cd}$  K-XRF measurement system, a new  $^{109}\text{Cd}$  K-XRF measurement system consisting of four detectors with clover-leaf

geometry was developed at McMaster in 2005 by Huiling Nie in her Ph.D. thesis. The new system was used in a 2008 survey to measure the bone lead of 497 smelter workers at Belledune, New Brunswick. The main goals followed in this survey were (i) to examine the efficiency of exposure control programs and (ii) to study the effectiveness of new detector system consisting of four germanium detectors with clover-leaf geometry, where the results of analysis were compared with the two previous surveys performed in 1994 and 1998. The details of simulation and comparison results were finalized in an attached paper published in 2011.

## ***In vivo* measurement of lead in the bones of smelter workers using the four-element ‘clover-leaf’ geometry detector system**

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### **Abstract**

A total of 497 smelter employees from New Brunswick participated in a bone lead survey conducted by McMaster University in 2008 to examine the efficiency of lead exposure control programmes and a four-element ‘clover-leaf’ geometry detector system. Nearly 42% of the subjects had participated in both the previous surveys performed in 1994 and 1999. After developing the clover-leaf geometry system in 2006, the reliability of the system based on examining the consistency of four detectors and improving the minimum detection limit (MDL) was tested for the first time in 2008 by measuring lead levels of a large population that was occupationally exposed to lead. The Z test was used to study the distribution of the lead concentration calculated based on  $K_{\alpha}$  and  $K_{\beta}$  lead x-rays, where the results were broadly consistent with a normal distribution criterion, with relatively small means and standard deviations of between 1 and 2. The MDL of the clover-leaf geometry system was improved on average for tibia and calcaneus by a factor of 3.1 compared to the 1999 and 1994 surveys in which a conventional system (one detector) was used. Furthermore, by comparing the results of the three mentioned surveys, the 2008 results were found to represent the highest precision.

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## 1. Introduction

### 1.1. Brief history

Lead has a long residence time in bone, and over 90% of the total body burden of lead in adults is accumulated in bone (Barry 1975). Therefore, previous exposures to lead may be best studied by finding out the lead concentration in bones. Moreover, as lead accumulates (the biological half life of lead in bone is quoted as 5–25 years (Gerhardsson *et al* 2005), bone increasingly acts as a secondary source of lead exposure during its turnover and increases the blood lead level (Carmouche *et al* 2005).

The first *in vivo* bone lead measurement using XRF was developed in the early 1970s at the University of Lund in Sweden. To perform their measurement, the researchers used  $^{57}\text{Co}$  as the radioactive source with a  $90^\circ$  scattering angle. The source emits gamma ray energies at 122 and 136 keV (Ahlgren *et al* 1976). This method was based on the excited K-shell x-ray fluorescence (KXRF) principle, where the hole in the K-shell orbit of lead is filled by an outer electron from either the L shell or M shell orbit corresponding to  $\text{K}_\alpha$  and  $\text{K}_\beta$  characteristic lead x-rays (Knoll 1999). The sensitivity of XRF measurement relies on the choice of the radioactive source, and the source, detector, and target geometry (scattered angle in the Compton effect) (Todd *et al* 1992). Choosing the proper factors minimizes the minimum detection limit (MDL) by maximizing the strength of lead x-ray signals ( $\text{K}_\alpha$  and  $\text{K}_\beta$ ) and the signal to noise (background) ratio (SNR). Hence, by using the source  $^{109}\text{Cd}$  instead of  $^{57}\text{Co}$  and a 16 mm diameter HPGe detector under the same principle, the MDL was decreased from  $50\text{--}60\ \mu\text{g (g bone mineral)}^{-1}$  to  $16\text{--}20\ \mu\text{g (g bone mineral)}^{-1}$  for tibia (Somerville *et al* 1985). What is now termed the conventional system with a 50 mm diameter HPGe detector system was developed by Gordon *et al* (1993), and by using that system an MDL of  $6\ \mu\text{g (g bone mineral)}^{-1}$  for tibia measurement was achieved. The radioactive source,  $^{109}\text{Cd}$ , emits 88.03 keV gamma rays, so the energy is 34 eV above the lead K absorption edge energy. The target is typically irradiated for 30 min and the generated photons are collected as a number of counts by the germanium detector located behind the source in the backscatter geometry with an approximate  $153^\circ$  mean scattering angle. Choosing a near back scatter geometry for  $^{109}\text{Cd}$  avoids having Compton peaks (66.4 keV) interfering with lead alpha x-ray energies (72.804 and 74.969 keV) (Todd *et al* 1992). Lead K-shell x-ray characteristics have been tabulated in table 1 (Lawrence Berkeley Laboratory, Nuclear Science Division 2009).

The intensity ratio of  $\text{K}_{\beta 1}$  x-ray to  $\text{K}_{\alpha 1}$  x-ray is calculated to be 0.232, which is the ratio of their emission probabilities. Therefore, the ratio of the number of net counts for  $\text{K}_{\beta 1}$  x-ray to  $\text{K}_{\alpha 1}$  x-ray is expected to be close to the theoretical value, 0.232, for both phantoms and subjects' measurement.

### 1.2. MDL optimization based on detector–source–target geometry

By choosing an appropriate radioactive source with the energy just above the lead K absorption edge x-ray energy, the yield of the lead XRF per incident photon is maximized. Also, choosing the appropriate source increases the SNR.  $^{57}\text{Co}$ ,  $^{109}\text{Cd}$  and  $^{153}\text{Gd}$  are three possible choices of source to perform KXRF measurement of lead in bone (Somerville *et al* 1985). For the given sources, both the photoelectric absorption coefficient and Compton peak energy are calculated (table 2).

From table 2, the  $^{109}\text{Cd}$   $\gamma$ -ray gives the highest photoelectric absorption cross section for lead. Also, it presents the highest value of the SNR since the Compton peak is well below the lead x-ray spectrum peaks in the  $160^\circ$  geometry. In a  $90^\circ$  geometry, the Compton scatter from the  $^{57}\text{Co}$  is well separated from the Pb K x-rays, but the peak in the Compton distribution

**Table 1.** K-shell x-ray characteristics of lead.

|                      | K <sub>α2</sub> | K <sub>α1</sub> | K <sub>β1</sub> | K <sub>β2</sub> | K <sub>β3</sub> |
|----------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Energy (keV)         | 72.805          | 74.969          | 84.938          | 87.300          | 84.450          |
| Emission probability | 27.7%           | 46.2%           | 10.7%           | 3.91%           | 5.58%           |

**Table 2.** Characteristics of radioactive gamma sources.

| Isotope           | γ-ray energy (keV) | Photoelectric absorption cross section (cm <sup>2</sup> g <sup>-1</sup> ) for lead | Compton peak (keV) |               |
|-------------------|--------------------|--|--------------------|---------------|
|                   |                    |  | 160° Geometry      | 90° Geometry  |
| <sup>109</sup> Cd | 88.035             | 7.12   | 66.00              | 75.097        |
| <sup>57</sup> Co  | 122, 136           | 3.10, 2.60   | 83.38, 89.69       | 98.49, 107.41 |
| <sup>153</sup> Gd | 97, 103            | 5.23, 4.39   | 70.89, 74.049      | 81.52, 85.72  |

is, nevertheless, associated with a continuum of lower energy events which contributes to background under the lead K x-ray peaks. For <sup>153</sup>Gd, the Compton scatter is either close to Pb K<sub>α</sub> x-rays (160° geometry) or close to Pb K<sub>β</sub> x-rays (90° geometry).

### 1.3. MDL optimization based on using a clover-leaf geometry detector system

The four-detector geometry had previously been reported by Nie *et al* (2006), who tested a sample of 20 subjects using an array of 16 mm diameter and 10 mm thick Ge crystals, with a <sup>109</sup>Cd source of activity 2.45 GBq. We report here the use of an array of four 25 mm diameter and 10 mm thick Ge crystals (Fleming and Mills 2007) used to measure lead in the calcaneus of 497 subjects to examine the consistency of results from the four detectors. Also, we compare the uncertainties in the lead concentration results of approximately 209 subjects who had been measured previously using the conventional system to investigate the performance of the clover-leaf geometry detector system versus the conventional system in terms of MDL. A comparison of the lead concentration values made it possible to assess the efficiency of exposure control programmes introduced over a period of years. The source activity at the time of the start of the measurements was calculated to be 3.15 GBq. The last measurements were taken in March 2006, about 52 days later; by that time the source activity had declined to 2.92 GBq. We were also able to use the fact that there are four independent measurements, as well as the two largely independent lead estimates derived from K<sub>α</sub> and K<sub>β</sub> x-rays in each detector, to perform a number of checks on the quality and consistency of the data.

The main purpose of using the clover-leaf geometry is to optimize the efficiency of the HpGe detectors. If we are going to use one detector (conventional system), a larger size of detector crystal must be used in order to achieve a higher efficiency. Increasing the size will however cause poor timing characteristics which in turn decrease the energy resolution. This problem has been solved in clover-leaf geometry (Joshi *et al* 1997). The maximum rate at which nuclear pulses can be processed and recorded in the spectrum sets a limit to the number of counts that can be recorded in a given time and therefore, the precision that can be achieved in that time. The biggest improvement in MDL comes from count rate throughput. With four detectors, four times as much as information can be processed, and the precision can be improved by a factor of 2. The clover-leaf and conventional detector full width at half

**Table 3.** Resolution comparisons between the conventional and the clover-leaf detector system; the results are based on a 30 min phantom measurement.

| Parameters             | Conventional XRF detector system | Clover-leaf system |      |      |      |
|------------------------|----------------------------------|--------------------|------|------|------|
|                        |                                  | Det1               | Det2 | Det3 | Det4 |
| Detector diameter (mm) | 50                               | 25                 | 25   | 25   | 25   |
| FWHM (eV)              | 800                              | 595                | 562  | 515  | 687  |
|                        |                                  | Avg: 590           |      |      |      |

maximum (FWHM) energy resolution was calculated to be 590 and 800 eV respectively at 88 keV peak energy (table 3):

$$\frac{MDL_{\text{clover-leaf}}}{MDL_{\text{Conventional}}} = \sqrt{\frac{Resolution_{\text{clover}}}{Resolution_{\text{conventional}}}} \times \sqrt{\frac{Count_{\text{conventional}}}{Count_{\text{clover}}}} = \sqrt{\frac{590}{800}} \times \frac{1}{2} = 0.429$$

$$\frac{MDL_{\text{Conventional}}}{MDL_{\text{clover-leaf}}} = 2.33.$$

Therefore, the detection limit is expected to be improved by a factor of 0.429 compared to the conventional system.

With the clover-leaf geometry system, low-level environmental lead exposure studies relating to public health are possible. Low-level lead exposure has many adverse health effects, among which are intellectual deficits especially in children (Lanphear *et al* 2005), reading problems, school failure, criminal behaviour, hearing loss, tooth decay, spontaneous abortions, renal disease, and cardiovascular disease (Lanphear *et al* 2005, Borja-Aburto *et al* 1999, Dietrich *et al* 2001, Factor-Litvak *et al* 1999, Lin *et al* 2003, Moss *et al* 1999, Nash *et al* 2003, Needleman *et al* 2002, Schwartz and Otto 1991). Many of these studies cannot be performed by the conventional system due to the relatively high MDL. Also the clover-leaf geometry system can differentiate small differences between the populations with different exposure history, e.g. environmental exposure and occupational low-level exposure (Nie *et al* 2006).

## 2. Methodology

### 2.1. Estimation of underlying linear relationships

In regression equations, there are usually sampling and measurement errors contributing to the predictors and responses. Therefore, it is necessary to formulate a means of estimating the relationship underlying the observed measurement. A set of observations  $x$  and  $y$  are measured for which it is assumed that (Quenouille 1952)

$$y = mx + c \quad (1)$$

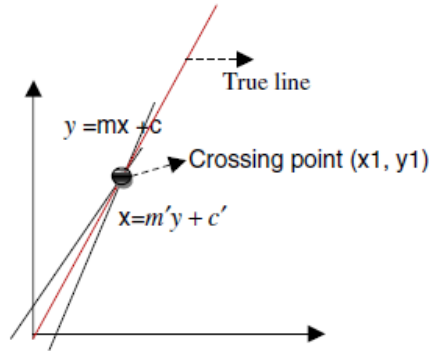
$$x = m'y + c' \quad (2)$$

where in both equations (1) and (2), predictors are treated as variance free, and responses have characteristic variance. For the applications where both variances tend to 0 and the value of the coefficient determination factor ( $R^2$ ) is very close or equal to 1, then

$$m \times m' = 1 \quad \text{and} \quad m = \frac{1}{m'}.$$

However, in actual cases, at least the variable  $y$  has variance and in our case both variables  $x$  and  $y$  have significant variances; therefore

$$m \times m' < 1.$$



**Figure 1.** Schematic representation of the true slope of  $y$  versus  $x$ , where there is variance in both  $y$  and  $x$ .

Hence, it is necessary to estimate the true underlying slope in the situation where

$$m \times m' = R^2 \quad \text{where} \quad R^2 \neq 1.$$

The true slope ( $m_T$ ) can be estimated from ( $m$ ) and ( $\frac{1}{m'}$ ):

$$m_T = \frac{x \text{ as variance free}}{\sigma_x^2} + \frac{y \text{ as variance free}}{\sigma_y^2}$$

$$m_T = \frac{\sigma_y^2 \frac{m}{\sigma_x^2} + \sigma_x^2 \frac{(m')^{-1}}{\sigma_y^2}}{\frac{1}{\sigma_x^2} + \frac{1}{\sigma_y^2}} = \frac{\sigma_y^2 m + \sigma_x^2 (m')^{-1}}{\sigma_y^2 + \sigma_x^2}.$$

In the special case in which  $\sigma_x^2 = \sigma_y^2$ , the value of true slope is calculated to be

$$m_T = \frac{m + (m')^{-1}}{2}. \tag{3}$$

The point  $(x_1, y_1)$ , the crossing point of two lines in figure 1 is located on the true line. The corresponding coordinates to  $(x_1, y_1)$  based on the known variables are calculated easily:

$$x_1 = \frac{m'c + c'}{1 - mm'} \quad \text{and} \quad y_1 = \frac{mc' + c}{1 - mm'}.$$

Therefore, for the true line, the equation parameters are calculated as:

$$y_T = m_T x_T + c_T \quad \text{or} \quad y_T = m_T x_T + (y_1 - m_T x_1). \tag{4}$$

### 2.2. Z-test and normal distribution

Since many physical measurements have actual observed frequency distributions, the normal distribution is perhaps the most important statistic test to study the pattern of distributions (Lapin 1975). The normal distributions are symmetric and have bell-shaped density curves with a single peak and can be completely specified by two parameters: mean and standard deviation (Leabo 1972). All the observations of any normal random variable  $X$  can be

transformed to a new set of observations for a standard normal variable  $Z$  with a mean of zero ( $\mu = 0$ ) and a standard deviation of one ( $\sigma = 1$ ) (Hughes and Grawoig 1971):

$$Z = \frac{(X - \mu)}{\sigma}.$$

To find the lead concentration in participants, related spectrum files were analysed for the elastic peak (coherent) at 88.035 keV, the  $K_{\alpha 1}$  plus  $K_{\alpha 2}$ , and  $K_{\beta 1}$  plus  $K_{\beta 3}$  lead x-ray energies. Peak information from the mentioned spectra was extracted by using the Marquardt algorithm (Marquardt 1963). Then, the results of ( $K_{\alpha}$ /coherent) and ( $K_{\beta}$ /coherent) net counts for all four detector systems were evaluated against their corresponding calibration line equations. The outcome was four sets each having two estimates of lead concentration resulting from  $K_{\alpha}$  and  $K_{\beta}$  lead x-rays. Results are combined by using the inverse/variance weighted mean method (Todd 2000) to find one value of lead concentration ( $\pm$  uncertainty) for each participant. The inverse weighted mean formula is calculated by using formulae 5 and 6, where index 'i' is the detector number (1, 2, 3, and 4);  $Pb_i$  presents the inverse weighted mean of the calculated lead ( $Pb_{\alpha} (\pm \sigma_{Pb_{\alpha}})$ ) and ( $Pb_{\beta} (\pm \sigma_{Pb_{\beta}})$ ) based on  $K_{\alpha}$  and  $K_{\beta}$  lead x-rays respectively, and  $Pb_{\mu}$  is the lead inverse weighted mean of the eight possible estimates:

$$Pb_i = \frac{\frac{Pb_{\alpha}}{\sigma_{Pb_{\alpha}}} + \frac{Pb_{\beta}}{\sigma_{Pb_{\beta}}}}{\frac{1}{\sigma_{Pb_{\alpha}}} + \frac{1}{\sigma_{Pb_{\beta}}}}, \quad \sigma_{Pb_i}^2 = \left( \frac{1}{\sigma_{Pb_{\alpha}}^2} + \frac{1}{\sigma_{Pb_{\beta}}^2} \right)^{-1} \quad (5)$$

$$Pb_{\mu} = \frac{\sum_{i=1}^4 \frac{Pb_i}{\sigma_{Pb_i}^2}}{\sum_{i=1}^4 \frac{1}{\sigma_{Pb_i}^2}}, \quad \sigma_{Pb_{\mu}}^2 = \left( \sum_{i=1}^4 \frac{1}{\sigma_{Pb_i}^2} \right)^{-1}. \quad (6)$$

The Z-test was used to examine the behaviour of each detector based on the  $K_{\alpha}$  and  $K_{\beta}$  lead x-ray estimates. There are eight estimates of lead concentration for each participant. By using formula (7), the distribution of each of the eight estimates is evaluated against the weighted mean value of all of the eight estimates:

$$Z_i = \frac{Pb_{(\alpha \text{ or } \beta)_i} - Pb_{\mu}}{(\sigma_{Pb_{\alpha \text{ or } \beta)_i}^2)^{1/2}}. \quad (7)$$

Also, the distribution of  $K_{\alpha}$  and  $K_{\beta}$  lead x-ray within each detector system was studied. In this case, the Z-value was calculated by using formula (8):

$$Z_{\alpha-\beta} = \frac{Pb_{\alpha} - Pb_{\beta}}{(\sigma_{Pb_{\alpha}}^2 + \sigma_{Pb_{\beta}}^2)^{1/2}} \quad (8)$$

where  $Pb_{\alpha}$  and  $Pb_{\beta}$  address the uncertainties of lead concentration calculated by using the  $K_{\alpha}$  and  $K_{\beta}$  routines for each detector system, respectively.

### 3. Results and discussion

#### 3.1. Phantom measurements

McMaster calibration phantoms are classified from the lowest added lead concentration, 0 ppm to the highest lead concentration 210 ppm. There are ten phantoms available, and, on average, eight repeats were performed for each phantom. Calibration line equations of the ratio of each of lead  $K_{\alpha}$  and lead  $K_{\beta}$  to coherent peak amplitudes were calculated for each of the four detectors. Table 4 shows the slopes, the ratios of  $K_{\beta}$  to  $K_{\alpha}$ , and the coefficients of determination ( $R^2$ ).

**Table 4.** Calibration line equations for McMaster phantoms where ppm corresponds to the known values lead concentration in phantoms.

| Detector no. | $\alpha$ Calibration line equation                       | $\beta$ Calibration line equation                       | ( $K_\beta$ /coh) to ( $K_\alpha$ /coh) | $R^2$  |
|--------------|--|---|---|--------|
| 1            | $\alpha/\text{coh} = (2.98 \pm 0.02) \times 10^{-3}$ ppm | $\beta/\text{coh} = (6.37 \pm 0.06) \times 10^{-4}$ ppm | $0.214 \pm 0.003$                       | 0.9977 |
| 2            | $\alpha/\text{coh} = (2.80 \pm 0.04) \times 10^{-3}$ ppm | $\beta/\text{coh} = (6.04 \pm 0.08) \times 10^{-4}$ ppm | $0.216 \pm 0.004$                       | 0.9974 |
| 3            | $\alpha/\text{coh} = (3.02 \pm 0.03) \times 10^{-3}$ ppm | $\beta/\text{coh} = (6.51 \pm 0.06) \times 10^{-4}$ ppm | $0.216 \pm 0.003$                       | 0.9986 |
| 4            | $\alpha/\text{coh} = (3.06 \pm 0.02) \times 10^{-3}$ ppm | $\beta/\text{coh} = (6.57 \pm 0.05) \times 10^{-4}$ ppm | $0.215 \pm 0.002$                       | 0.9978 |

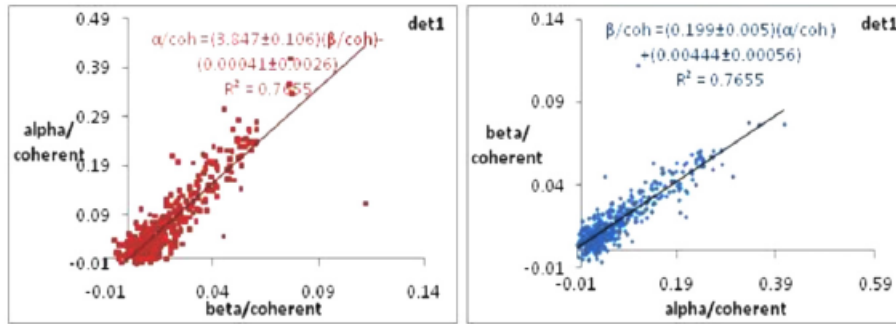
In terms of the slope of the calibration line equations, detectors 1, 3, and 4 are very consistent with each other. However, detector 2 presents the lowest slope for both alpha and beta calibration lines. Nevertheless, the ratio of ( $K_\beta$ /coherent) to ( $K_\alpha$ /coherent) net counts is consistent for all of the four detectors. For phantoms, since the coefficient of determination ( $R^2$ ) is very close to 1, the true slope ( $m_T$ ) (figure 1) is assumed to be equal to the slope values that have been tabulated in table 4.

### 3.2. In vivo measurements

A total of 497 smelter workers in the province of New Brunswick in Canada participated in a lead study during the time interval of 14 January to 6 March 2008. Participants were measured for lead concentration in the left tibia and right calcaneus, each for 30 min. Two separate systems were used to measure the tibia and calcaneus lead concentration of the participants. In this paper, the consistency of the four detectors in one of the systems is examined based on the calcaneus results. This is followed by a brief comparison of calcaneus and tibia results using both systems.

To examine the consistency of the analysis for subjects, the regression equations of ( $K_\alpha$ /coherent) versus ( $K_\beta$ /coherent) net counts for the 497 subjects, measured for calcaneus lead concentration, were derived and compared to that for the phantoms result analysis tabulated in table 4. Subjects' result analyses have been plotted in figure 2 for one of the detectors. For subjects' measurements, unlike the phantoms' measurements, the value of  $R^2$  is not very close to 1. The reason for having more precise values in the phantoms as compared to the people can be related to soft tissue overlying the calcaneus (trabecular bone), which, in turn, increases the background due to increasing Compton and decreases the SNR. Also, the range of values is about twice as large for the phantoms compared to the subjects, and most of the subjects had low lead concentration. As a result of having more background in the subjects' measurements, the uncertainty in slope is increased to approximately 3% of the slope compared to the one for the phantoms, which is close to 1% (tables 4 and 5).

Because relative variances are so similar, equation (3) has been used to estimate the true slope in table 5. The result values of the  $m_T$  are consistent with the slope ratios of table 4 for phantoms. Interestingly, the results for the  $K_{\alpha 1}$  to  $K_{\beta 1}$  ratio in human subjects are even closer to 0.232 (table 1) than are the results for phantoms section, 0.215 (table 4). Since all four detectors measure the lead concentration in approximately the same volume of bone, we are expecting very close values of lead concentration for all of the eight possible estimates of lead concentration. The regression equations among the detectors are shown for Pb- $K_\alpha$  in table 6



**Figure 2.** Relationships between Pb  $K_{\alpha}$  x-ray counts and Pb  $K_{\beta}$  x-ray counts for human calcaneus measurements for one of the four detector elements.

**Table 5.** Analysis of slopes of Pb  $K_{\beta}$  versus Pb  $K_{\alpha}$  x-ray counts for human calcaneus measurements for all four detector elements.

| Detector no. | $m$               | $m'$            | $(\sigma_m/m)^2$ | $(\sigma_{m'}/m')^2$ | True slope $m_T$ |
|--------------|-------------------|-----------------|------------------|----------------------|------------------|
| 1            | $0.199 \pm 0.005$ | $3.85 \pm 0.11$ | 0.000 631        | 0.000 655            | 0.229            |
| 2            | $0.202 \pm 0.006$ | $3.74 \pm 0.11$ | 0.000 882        | 0.000 729            | 0.235            |
| 3            | $0.203 \pm 0.006$ | $3.79 \pm 0.10$ | 0.000 692        | 0.000 692            | 0.233            |
| 4            | $0.200 \pm 0.005$ | $3.85 \pm 0.11$ | 0.000 625        | 0.000 655            | 0.230            |

**Table 6.** Regression equations based on the calcaneus alpha lead concentration values measured by the four detectors; the subscripts 1–4 stand for detector number. Vertical and horizontal entries present responses and predictors, respectively. The relative variances in  $x$  and  $y$  directions are approximately equal.

|                  | Pb $_{\alpha 1}$                  | Pb $_{\alpha 2}$                  | Pb $_{\alpha 3}$                | Pb $_{\alpha 4}$                 |
|------------------|-----------------------------------|-----------------------------------|---------------------------------|----------------------------------|
| Pb $_{\alpha 1}$ |                                   | Slope: 1.04<br>Intercept: 1.10    | Slope: 1.18<br>Intercept: 1.67  | Slope: 1.004<br>Intercept: 0.654 |
| Pb $_{\alpha 2}$ | Slope: 0.973<br>Intercept: -1.40  |                                   | Slope: 1.14<br>Intercept: 0.534 | Slope: 0.987<br>Intercept: -1.06 |
| Pb $_{\alpha 3}$ | Slope: 0.847<br>Intercept: -1.40  | Slope: 0.877<br>Intercept: -0.463 |                                 | Slope: 0.855<br>Intercept: -4.25 |
| Pb $_{\alpha 4}$ | Slope: 0.996<br>Intercept: -0.651 | Slope: 1.013<br>Intercept: 1.08   | Slope: 1.17<br>Intercept: 4.97  |                                  |

and for Pb- $K_{\beta}$  in table 7. The slopes and intercepts shown are estimates of the true values using equations (3) and (4). The slopes are dimensionless, while the intercepts do have the unit  $\mu\text{g Pb (g bone mineral)}^{-1}$ .

Based on the results of tables 6 and 7, regression equations for detectors 1, 2, and 4 are closely similar, and their slopes for both alpha and beta estimates are very close to 1. However, detector 3 behaves differently, and interestingly, the behaviour of the detector is very consistent for both alpha and beta estimates. However, table 4 shows that both  $K_{\alpha}$  and  $K_{\beta}$  calibration lines from phantoms are very similar for detectors 1, 3, and 4, whereas for detector 2 the slopes are about 7% lower. On the other hand, examining the ( $\alpha/\text{coherent}$ )

**Table 7.** Regression equations based on the calcaneus beta lead concentration values measured by the four detectors; the subscripts 1–4 stand for detector number. Vertical and horizontal entries present responses and predictors respectively.

|                  | Pb <sub>β1</sub>                  | Pb <sub>β2</sub>                 | Pb <sub>β3</sub>                | Pb <sub>β4</sub>                   |
|------------------|-----------------------------------|----------------------------------|---------------------------------|------------------------------------|
| Pb <sub>β1</sub> |                                   | Slope: 1.012<br>Intercept: -1.68 | Slope: 1.17<br>Intercept: -3.44 | Slope: 1.002<br>Intercept: -0.0433 |
| Pb <sub>β2</sub> | Slope: 0.988<br>Intercept: 1.66   |                                  | Slope: 1.14<br>Intercept: -1.37 | Slope: 0.986<br>Intercept: 1.72    |
| Pb <sub>β3</sub> | Slope: 0.855<br>Intercept: 2.88   | Slope: 0.877<br>Intercept: 1.19  |                                 | Slope: 0.868<br>Intercept: 2.58    |
| Pb <sub>β4</sub> | Slope: 0.998<br>Intercept: 0.0488 | Slope: 1.014<br>Intercept: -1.75 | Slope: 1.15<br>Intercept: -2.93 |                                    |

**Table 8.** Relationships of estimates of bone Pb concentration derived from Pb K<sub>α</sub> x-rays with those derived from K<sub>β</sub> x-rays for all four detectors.

| Detector no. | Pb <sub>β</sub> versus Pb <sub>α</sub> | Pb <sub>α</sub> versus Pb <sub>β</sub> | $m_T$                                  | $m_T$                                  |
|--------------|--|--|--|--|
|              |  |  | Pb <sub>β</sub> versus Pb <sub>α</sub> | Pb <sub>α</sub> versus Pb <sub>β</sub> |
| 1            | 0.91 ± 0.02                            | 0.88 ± 0.02                            | 1.023                                  | 0.99                                   |
| 2            | 0.92 ± 0.02                            | 0.86 ± 0.02                            | 1.041                                  | 1.01                                   |
| 3            | 0.92 ± 0.02                            | 0.87 ± 0.02                            | 1.035                                  | 0.99                                   |
| 4            | 0.89 ± 0.02                            | 0.85 ± 0.02                            | 1.033                                  | 0.99                                   |

versus ( $\beta$ /coherent) net counts ratios for the subjects shows no evidence for variation between the detectors. The lead concentration results based on all four alphas and all four betas are combined by using equation (6); the two estimates of lead concentration, alpha and beta, are compared (table 8).

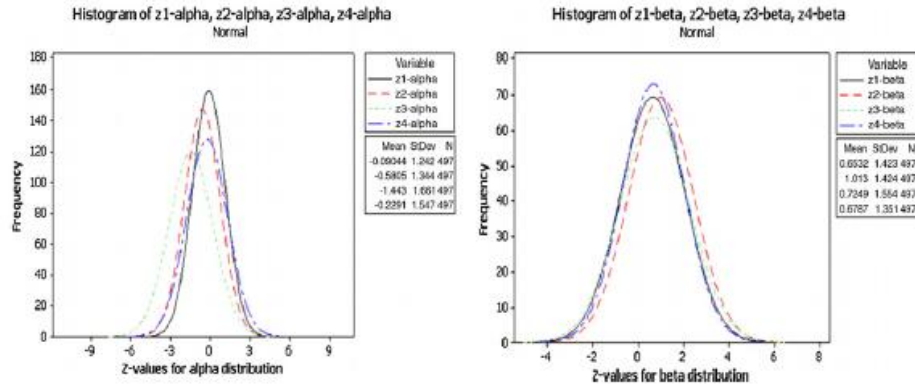
In the next step, the Z-test was used to examine the behaviour of each detector separately based on the K<sub>α</sub> and K<sub>β</sub> lead x-ray estimates. There are eight estimates of lead concentration for each subject. By using formula (7), the distribution of each of the eight estimates is evaluated against the weighted mean value of all of the eight estimates (figure 3).

Also, the distribution of alpha and beta within each detector system was studied. In this case, the Z-value was calculated by using formula (8) (figure 4).

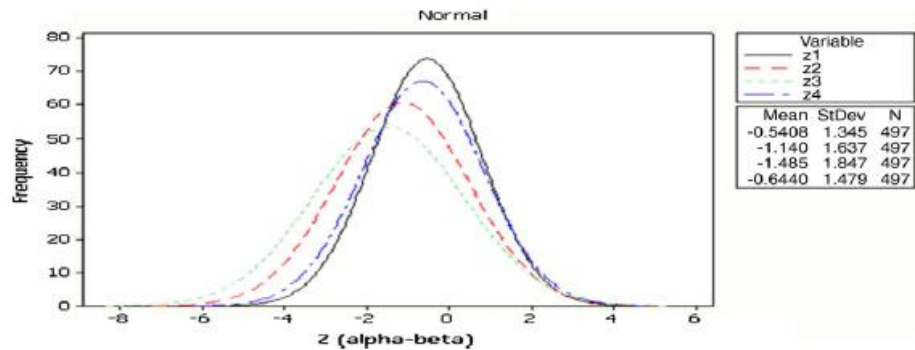
If there is no difference between the lead concentrations measured by each detector, then we expect to get a mean of 0 and an SD of 1 for all estimates (Gordon *et al* 1994). In the results from detectors 2 and 3, the mean of the Z values are different from zero to an extent that is significant, given the large number of measurements, and the standard deviation is greater than 1. For detectors 2 and 3, the mean is more disturbed and the standard deviation is larger. Essentially, improved precision coupled with multiple estimates of the same quantity has revealed these discrepancies for which there are several possible reasons. The data extraction for either K<sub>α</sub> or K<sub>β</sub> regions may need to be improved further. Also, there may not have been sufficient shielding to prevent crosstalk between tibia and calcaneus systems as both systems were located in the same room, albeit about 3.5 m apart. Further, there may have been lead contamination on the floor or walls that may have affected the intercept values.

A preliminary comparison was also made between these calcaneus results and the corresponding tibia lead measurements made with the separate system. The MDL for tibia was lower (0.66) than that for the calcaneus, but not by as large a factor as previously observed.





**Figure 3.** Distributions of the Z-values for Pb concentration estimates compared to the weighted mean of both  $K_{\alpha}$  and  $K_{\beta}$  estimates from all four detectors (eight estimates). Data are from calcaneus Pb measurements in human subjects.



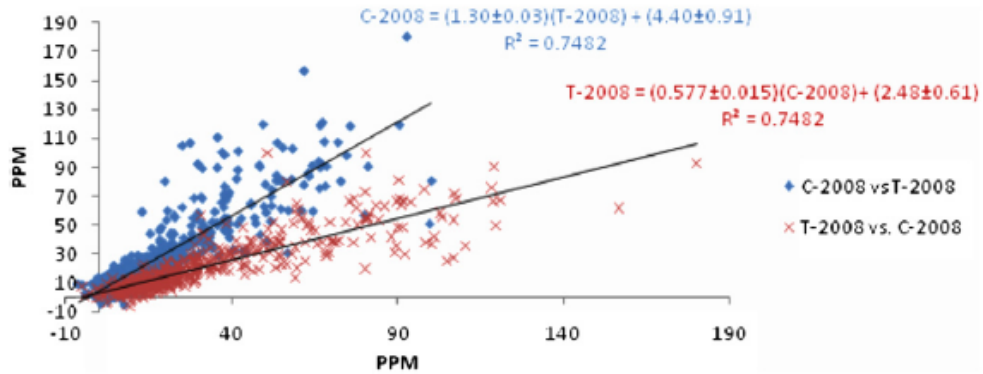
**Figure 4.** Distributions of the differences between Pb  $K_{\alpha}$  and Pb  $K_{\beta}$  bone Pb concentration estimates. Data are shown from all four detectors and the calcaneus measurements in human subjects.

Because of the higher bone mineral density of the tibia (cortical) than the calcaneus (trabecular) a larger mass of bone mineral is sampled in the tibia measurement. However, in this case, the detector elements used for the calcaneus had a larger area ( $491 \text{ mm}^2$ ) than those used for the tibia ( $201 \text{ mm}^2$ ). Linear regression was applied (figure 5) to compare the participants' lead level in their tibia and calcaneus (Fleming *et al* 1997). Using these regression estimates ( $m = 1.3$  and  $c = 4.40$ ) and ( $m' = 0.577$  and  $c' = 2.48$ ), the values of the true slope and intercept by using equation (4) are calculated to be 1.52 and  $-0.242$  for having calcaneus as a response and tibia as a predictor.

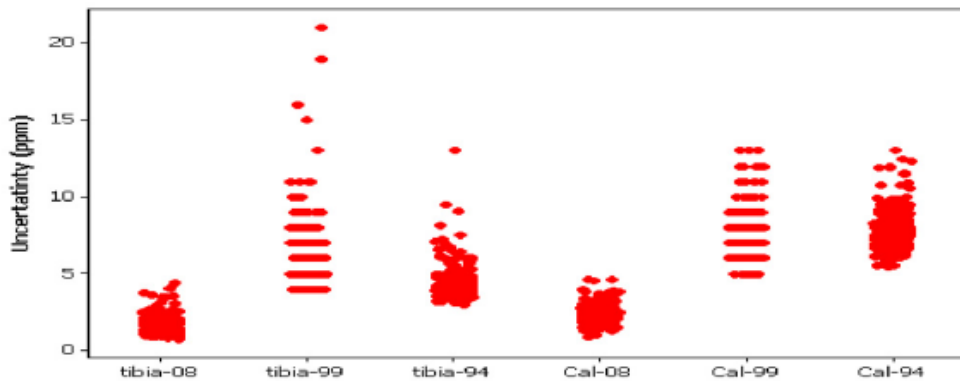
### 3.3. Result analysis comparison of 1994, 1999, and 2008 surveys

Figure 6 illustrates uncertainties from the 43% of the 2008 survey participants who had also participated in both 1994 and 1999 surveys (209 of the 497 participants).

In table 9, the maximum, median, and minimum values of uncertainty have been tabulated. The median has been decreased for the clover-leaf detector system by factors of 2.9 and 3.2



**Figure 5.** Lead concentration regression equations in calcaneus and tibia for 497 subjects. The symbols ‘C’ and ‘T’ stand for calcaneus and tibia, respectively.



**Figure 6.** Uncertainties comparison of calcaneus (Cal) and tibia measurements in 2008, 1999, and 1994.

compared to the 1999 and 1994 surveys respectively for calcaneus and 3.7 and 2.6 compared to the 1999 and 1994 surveys respectively for tibia, which is slightly larger than the value of 2.3 predicted solely from pulse throughput and detector resolution (section 1.3). Defining the MDL as twice the median measurement uncertainty, the MDL is now  $4.8 \mu\text{g Pb (g bone mineral)}^{-1}$  for calcaneus and  $3.2 \mu\text{g Pb (g bone mineral)}^{-1}$  for tibia using the clover-leaf four detector system compared to  $14$  (or  $15.4$ )  $\mu\text{g Pb (g bone mineral)}^{-1}$  for calcaneus and  $12$  (or  $8.5$ )  $\mu\text{g Pb (g bone mineral)}^{-1}$  for tibia using the conventional detector system.

To test the efficiency of the exposure control programmes, statistical parameters (first quartile, median, and third quartile) describing bone lead concentrations are presented in table 10. Between 1994 and 1999 tibia levels were on average unchanged, whereas calcaneus lead levels rose. This was interpreted as indicating that continuing exposure exceeded elimination of lead during that period of time. However, for 1999 to 2008, exposure control programmes were sufficiently effective to indicate an overall reduction in bone lead concentration since 1999. Indeed, the 2008 levels were also lower than the 1994 levels, for calcaneus as well as for tibia measurements.

**Table 9.** Statistical parameters of the calcaneus (Cal) and tibia error measurements for the three surveys.

| Parameter  | Minimum $\mu\text{g Pb}$<br>(g bone mineral) <sup>-1</sup> | Median $\mu\text{g Pb}$<br>(g bone mineral) <sup>-1</sup> | Maximum $\mu\text{g Pb}$<br>(g bone mineral) <sup>-1</sup> |
|------------|--|---|--|
| Tibia 2008 | 0.75   | 1.62  | 4.40   |
| Tibia 1999 | 4.00   | 6.00  | 21.00  |
| Tibia 1994 | 3.00   | 4.24  | 13.05  |
| Cal 2008   | 0.88   | 2.38  | 4.62   |
| Cal 1999   | 5.00   | 7.00  | 13.00  |
| Cal 1994   | 5.50   | 7.70  | 13.04  |

**Table 10.** Statistical analysis (first quartile, median, and third quartile) of the subjects for calcaneus and tibia lead concentrations.

| Parameter  | Q1 $\mu\text{g Pb}$<br>(g bone mineral) <sup>-1</sup> | Median $\mu\text{g Pb}$<br>(g bone mineral) <sup>-1</sup> | Q3 $\mu\text{g Pb}$<br>(g bone mineral) <sup>-1</sup> |
|------------|---|---|---|
| Tibia 2008 | 14.83   | 22.07   | 36.70   |
| Tibia 1999 | 16.00   | 31.00   | 56.00   |
| Tibia 1994 | 15.25   | 31.50   | 51.75   |
| Cal 2008   | 20.19   | 31.61   | 56.25   |
| Cal 1999   | 37.25   | 63.00   | 106.00  |
| Cal 1994   | 17.00   | 44.50   | 99.75   |

#### 4. Conclusion

In clover-leaf geometry, all detectors work almost consistently. The slope values of the regression equations of eight estimates of lead (tables 6 and 7) were close to 1. The reason for nonzero intercepts may be because of having either crosstalk between tibia and calcaneus systems or contamination in the area of measurement.

Using the clover-leaf geometry improved the resolution of the system and maximized the throughput of the system compared to the conventional system. The MDL of the clover-leaf geometry system for calcaneus and tibia measurements was found to be  $4.78 \mu\text{g Pb}$  (g bone mineral)<sup>-1</sup> and  $3.14 \mu\text{g Pb}$  (g bone mineral)<sup>-1</sup> respectively, while the MDL for the conventional system for calcaneus measurements was calculated to be  $14.0 \mu\text{g Pb}$  (g bone mineral)<sup>-1</sup> and  $15.4 \mu\text{g Pb}$  (g bone mineral)<sup>-1</sup> and for tibia measurements was found to be  $12 \mu\text{g Pb}$  (g bone mineral)<sup>-1</sup> and  $8.48 \mu\text{g Pb}$  (g bone mineral)<sup>-1</sup> in the 1999 and 1994 surveys, respectively. Improvement of the detection system makes studies requiring a low MDL possible.

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## 2.2 Conclusion and Future Work

Using the clover-leaf geometry improved the resolution of the system and maximized the throughput of the system compared to the conventional system. The MDL of the clover-leaf geometry system for calcaneus and tibia measurements was found to be  $4.78 \mu\text{g Pb (g bone mineral)}^{-1}$  and  $3.14 \mu\text{g Pb (g bone mineral)}^{-1}$  respectively, while the MDL for the conventional system for calcaneus measurements was calculated to be  $14.0 \mu\text{g Pb (g bone mineral)}^{-1}$  and  $15.4 \mu\text{g Pb (g bone mineral)}^{-1}$  and for tibia measurements was found to be  $12 \mu\text{g Pb (g bone mineral)}^{-1}$  and  $8.48 \mu\text{g Pb (g bone mineral)}^{-1}$  in the 1999 and 1994 surveys, respectively. Therefore, by using the clover-leaf geometry detector system, low-level environmental lead exposure studies relating to public health are possible.

Source activity and measurement time are the main factors that influence the MDL significantly (chapter 3). In the study of the cohort of smelter employees, source activity varied from 3.15 to 2.92 GBq with the measurement time of 1800 seconds real time. Having the same measurement time, by increasing the source activity to 5 GBq, the MDL will be improved by a factor of  $1.26 \left( \sqrt{\frac{5}{3.15}} \right)$ . Therefore, the MDL of the clover-leaf geometry system for calcaneus and tibia measurements is expected to be  $3.79 \mu\text{g Pb (g bone mineral)}^{-1}$  and  $2.49 \mu\text{g Pb (g bone mineral)}^{-1}$  respectively.

Another alternative for improving the MDL can be achieved by using more detectors. For example, by having twelve detectors in cluster geometry, three (12/4) times as much as information can be processed, and the precision can be improved by a factor

of 1.73. Furthermore, by increasing the number of detectors, one can use smaller sizes of detectors, which, in turn, increase the energy resolution and subsequently, improve the MDL.

## Chapter 3

### Factors influencing uncertainties of *in vivo* bone lead measurement using a $^{109}\text{Cd}$ K x-ray fluorescence clover leaf geometry detector system

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#### Abstract

A  $^{109}\text{Cd}$  K-XRF measurement system consisting of four detectors in clover-leaf geometry is a non-invasive, low-radiation-dose method of measuring bone lead concentration. Its high precision in estimating the bone lead content makes it a promising tool for the determination of the low levels of lead currently found in the general population. After developing the clover-leaf geometry system, the system was used for the first time in a major survey in 2008 to measure the lead levels of 497 smelter employees. Since the delivered effective dose is small (50 nSv), the technique can be used to measure the bone lead of sensitive populations such as the elderly and children. This detector system was used from 2009 to 2011, to measure the bone lead concentration of 263 environmentally exposed individuals (EG) residing in Toronto, Ontario. In this paper, the factors that influence uncertainties in lead content in tibia (cortical bone) and calcaneus (trabecular bone) are discussed based on sex, age, and body mass index (BMI) by using one-way ANOVA analysis, two-way ANOVA analysis, and multivariate regression models. Results from univariate analyses showed that females have higher uncertainty compared to males. We observed significant differences for both calcaneus and tibia uncertainty measures ( $p < 0.0005$ ) among different age groups, where the uncertainties were highest in the lowest age group (<11 years). Two-way ANOVA analysis results showed that the interaction between sex and age was not significant for calcaneus uncertainty ( $p = 0.8179$ ), but was for tibia uncertainty ( $p = 0.0198$ ). The interaction between sex and BMI was not a significant predictor of uncertainty for either bone lead measurement. Also, we found that there is no difference between the sexes in any age group except for age group (51-65) ( $p < 0.0001$ ), where females had higher levels of uncertainty than males ( $p = 0.0013$ ). Based on the multivariate analysis, for the predictors including age and tibia lead level, Ti uncertainties for both sexes were found significantly different. Considering the model for Cal uncertainty when age and lead are included in the model, the models between sexes were considered statistically similar (coincidence). For smelter employees, tibia uncertainties did not correlate with age and tibia lead level; however, the predictors age and calcaneus lead level correlate with calcaneus uncertainties ( $p = 0.00169$ ). Also, we

found that the product of source activity and measurement time influenced the precision of measurements directly, which resulted in having the higher uncertainties for EG than those for smelter employees.

## Introduction

Low-level lead exposure has been reported to induce a range of adverse health effects, among which are intellectual deficits (especially in children) <sup>1</sup> and cardiovascular disease.<sup>2</sup> In adults, approximately 90% to 95% of the total lead body burden is in bone.<sup>3</sup> Lead has a long residence time in bone. Therefore, historical exposures to lead are studied by measuring the lead concentration in bone; furthermore, bone acts as a secondary (endogenous) source of lead exposure during its metabolism and contributes to the blood lead level.<sup>4</sup> Hence, since bone is an important physiological compartment in impacting the kinetic behaviour of lead within the body, developing techniques for measuring bone lead is crucial.

The first *in vivo* lead measurement using x-ray fluorescence, used <sup>57</sup>Co as the radioactive source with a 90° scattering angle.<sup>5</sup> Using <sup>109</sup>Cd instead of <sup>57</sup>Co improved the precision significantly since <sup>109</sup>Cd emits 88.03 keV gamma rays which are only 34 eV above the lead K absorption edge energy. The person or calibration standard is typically irradiated for twenty to thirty minutes, and the generated photons are collected as a number of counts by the germanium detector located behind the source in the backscatter geometry with an average 153° scattering angle. Choosing backscatter geometry for <sup>109</sup>Cd is to minimise overlap between Compton scatter and lead K<sub>α</sub> x-rays (at 72.804 keV and 74.969 keV).<sup>6</sup> Developing the four-detector system in clover leaf geometry<sup>7</sup> further improved the precision in determining bone lead contents compared to a conventional (one detector) system. We report here the use of an array of four 25 mm<sup>8</sup> and 16 mm diameter and 10 mm thick Ge crystals used to measure lead in (i) the calcaneus (heel bone) and tibia of 497 subjects respectively,<sup>9</sup> and (ii) the use of 16 mm diameter and 10 mm thick Ge crystals to measure lead in tibia and calcaneus of members of an environmentally exposed group (EG). Besides the choices of radioactive source and geometry of detector system, there are other factors that influence the uncertainty of an *in vivo* K-XRF lead measurement; Hu *et al.*<sup>10</sup> drew attention to some of the factors with which bone Pb measurement uncertainty varies. They pointed out as perhaps most obvious that uncertainty would fall with longer duration of measurement and with greater bone mass. In a multiple linear regression model they found that uncertainty was greater with being female rather than male, with increased body mass and with lower bone lead. The last of these is surprising since, in principle, the opposite trend would be expected from a simple consideration of the variance associated with signal clearly increasing with increasing bone lead, whereas variance associated with background would be independent of bone lead concentration. McNeill *et al.*<sup>11</sup> also observed that uncertainties were greater amongst females than males, pointing out that males tended to have greater bone mass and that the distribution of adipose tissue in females tended to mean that they had more soft tissue overlying the tibia. They also demonstrated a significant bivariate linear regression between the natural logarithm of uncertainty and body mass index



(BMI) for females and males. Ahmed *et al.*<sup>12</sup> also observed that bone lead measurement uncertainty was greater for females than for males and they observed a significant bivariate linear regression between the natural logarithm of uncertainty and BMI. They also pointed out the importance of duration of measurement and used the product of measurement duration and source activity.

## Methods and Materials

### 1. *In vivo* measurements

Bone lead measurements were made in two study populations: smelter employees in New Brunswick and an environmentally exposed group of individuals in Ontario Canada. There were 497 smelter employees who had participated in a survey in 2008 conducted by the McMaster team.<sup>9</sup> Participants were measured for tibia and calcaneus lead concentration, where each bone site was irradiated for 30 minutes. Most of these subjects were male; their dates of birth were between 1923 and 1987. The average and standard deviation (SD) tibia and calcaneus lead concentrations of the employees were 19.90 (18.36) and 30.08 (27.40) ( $\mu\text{g-Pb/g-bone mineral}$ ) with the corresponding uncertainty values of 1.68 (0.59) and 2.43 (0.60) respectively. The source activity at the time of the start of the measurements was calculated to be 3.15 GBq. The last measurements were taken in March 2008, about 52 days later; by that time the source activity had declined to 2.92 GBq.

From September 2009 to February 2011, bone lead measurements were taken on a convenience sample ( $n = 263$ ) of the general population in Toronto, Canada. Participants were measured for 22 minutes each for tibia and calcaneus lead concentration. There were 135 males, ages from 1 to 78 years and 128 females, ages from 3 to 83 years. The average (SD) value of tibia and calcaneus lead for the entire study group of participants was found to be 4.82 (6.32) (uncertainty: 1.75 (2.84)) and 10.22 (22.23) (uncertainty: 1.84 (4.32)) ( $\mu\text{g-Pb/g-bone mineral}$ ) respectively. For this study, the source activity ranged from 1.27 to 0.57 GBq.

### 2. Subjects' analysis results

To find the lead concentration in participants, related spectrum files were analyzed for the coherent,  $\text{PbK}_\alpha$ , and  $\text{PbK}_\beta$  spectral peak areas by mathematically fitting the spectrum. The coherent scatter signal comes mainly from the calcium in bone. The unit of measurement is in micrograms lead/gram bone mineral (or ppm) as the Pb x-ray signal is normalized to the coherent peak. The results of (alpha/coherent) and (beta/coherent) for all four detector systems were evaluated against their corresponding calibration lines. The outcome was four sets each having two estimates of lead concentration resulting from alpha and beta routines for all four detector systems (clover –leaf geometry). Results were combined by using inverse –variance weighted means to find one value of lead concentration (+/-

uncertainty) for tibia and calcaneus for each of the participants. The inverse weighted mean is calculated by using the formula below, where index (i) addresses the detector number (i.e. 1, 2, 3, and 4);  $Pb_i$  presents the inverse weighted mean of the calculated lead ( $Pb_\alpha (\pm\sigma_{Pb\alpha})$ ) and ( $Pb_\beta (\pm\sigma_{Pb\beta})$ ) based on  $PbK_\alpha$  and  $PbK_\beta$  lead x-rays respectively, and  $Pb_\mu$  is the lead inverse weighted mean of the eight possible estimates.

$$Pb_\mu = \frac{\sum_i \frac{Pb_i}{\sigma_{Pb_i}^2}}{\sum_i \frac{1}{\sigma_{Pb_i}^2}} \quad \sigma_{Pb\mu}^2 = \left( \sum_i \frac{1}{\sigma_{Pb_i}^2} \right)^{-1}$$

### 3. Statistical analysis

Summary statistics, including sample size, are reported for levels of Tibia (Ti) and Calcaneus (Cal) uncertainty in each demographic group's age, body mass index (BMI) and sex. The minimum, maximum, median, geometric mean (GM), and 95% confidence interval (CI) of the GM were evaluated for each uncertainty level. The geometric mean and corresponding 95% CI are reported in place of the arithmetic mean and corresponding 95% CI since the uncertainties were log-normally distributed by the Anderson-Darling test for normality. Age and BMI were considered as both continuous variables as well as classification variables in the analysis as described below. The classification groups of age were <11, (11, 19), (20, 35), (36, 50), (51, 65),  $\geq 65$ . Age groups were chosen to capture different developmental stages of males and females, while still being able to compare sex groups. BMI ( $\text{kg/m}^2$ ) was classified as follows: underweight ( $\text{BMI} < 18.5$ ), normal ( $18.5 \leq \text{BMI} < 25$ ), overweight ( $25 \leq \text{BMI} < 30$ ), and obese ( $\text{BMI} \geq 30$ ). The advantage to considering the categorical cases is that large values of either age or BMI can distort the results of the data by being influential in a usual regression model. A description of each type of analysis that was conducted is given below, with corresponding results in Results and analysis section.

#### 3.1 Univariate Analysis of Variance (ANOVA) and Two-Way ANOVA analysis

Analysis of variance (ANOVA) was used to test the null hypothesis: all groups of a demographic variable have similar average uncertainty levels. If the null hypothesis was rejected ( $p < 0.05$ ), then Tukey multiple pair-wise comparison test was used to determine which groups were significantly different from one another. The assumptions of ANOVA were verified using Anderson-Darling test for normality of residuals, and Levene's test for homogeneity of variance between groups. When the assumptions were not satisfied for the log transformed data then non-parametric statistics were used, namely the Kruskal Wallis test. A sensitivity analysis was also carried out to determine the influence of observations with large residuals. This involved rerunning the analysis with

these observations removed from the data set. If the results changed significantly after removing the outliers, then non-parametric results were reported (based on the full data set).

Non-parametric analysis consists of running the analysis on the ranks of the data. The results from non-parametric analysis are more stable than those from parametric analysis, when the assumptions of normality and equal variance are not satisfied. If the results from non-parametric analysis are similar to those from parametric analysis then the assumptions are fairly satisfied and we can keep the results from the parametric analysis. On the other hand, when the results differ from these two analyses and assumptions are not met, then it is recommended that the results from the non-parametric analysis be used since they are more stable. In the case where non-parametric statistics were used in place of parametric statistics, the confidence intervals were based on the data, but the results from multiple pair-wise comparisons (when applied) were based on the ranks of the data.

Considering the two demographic variables sex and age, a two-way ANOVA model the hypothesis would be: does the uncertainty level differ between age groups and is the relationship similar between the two sexes. The assumptions for a two-way ANOVA are similar to those for a simple ANOVA model.<sup>13, 14</sup>

### **3.2 Multivariate Regression Models**

A correlation analysis was carried out to investigate the relationship between the uncertainty measurements with age, BMI and corresponding lead levels. Explanatory variables that were highly correlated were not included in the same model in order to avoid issues of multi-collinearity. Simultaneous regression models were used to investigate the relationship between various explanatory variables with the uncertainty level while also comparing if these relationships were similar for both sexes. An outline of simultaneous regression models is detailed below.

#### **3.2.1 Simultaneous regression models**

The relationship between each variable (age, BMI, lead) was examined by sex group using simultaneous regression analysis. The purpose was to investigate if the relationship between the variable and uncertainty level was similar in the two sexes. If the relationship differed between males and females then the simultaneous regression models

could be used to investigate if the difference was in the intercept (starting values of the models), or the slope (was the rate of change different between sex groups). The simultaneous regression model is as follows:

$$y = a + bx_1 + cx_2 + Z(a_1 + b_1x_1 + c_1x_2)$$

Where,  $y$  is either Ti or Cal uncertainty;  $x_1$  is either age or BMI;  $x_2$  is the corresponding lead level of the outcome variable.  $Z$  is a dummy variable and is defined as follows:  $Z=0$  if male; else  $Z=1$  if female. The regression coefficients are  $a, b, c, a_1, b_1, c_1$ . If the relationship between the variable and  $x_1, x_2$  is similar between males and females then  $a_1 = b_1 = c_1 = 0$  (coincidence). If this hypothesis is rejected, then test if the intercepts are similar ( $a_1 = 0$ ) and/or if the slopes are similar ( $b_1 = 0, c_1 = 0$ ). Tests are Bonferroni corrected for multiple comparisons to ensure that the overall type I error rate remains less than 0.05.<sup>15</sup>

#### 4. Results and analysis

Tables 1-2 report summary statistics for all continuous variables considered in the analysis (Ti lead and corresponding uncertainty level, Cal lead and corresponding uncertainty level, age and BMI) for both studies, environmentally exposed group of participants (EG) and occupationally exposed group of participants (smelter employees), respectively. Since for the latter group of participants, we have not been provided with BMI information, and also as the group of participants are mostly males, age was considered as the only demographic variable that can influence the uncertainty values.

**Table 1: Summary statistics of bone lead levels of EG, corresponding uncertainty, and demographic variables age and BMI**

| Variable            | N   | Median | Min    | Max    | Geometric Mean     | 95% CI             |                    |
|---------------------|-----|--------|--------|--------|--------------------|--------------------|--------------------|
|                     |     |        |        |        |                    | Lower              | Upper              |
| Cal Pb              | 263 | 5.34   | -10.70 | 287.03 | 9.40 <sup>1</sup>  | 6.68 <sup>1</sup>  | 12.13 <sup>1</sup> |
| Cal Un <sup>3</sup> | 263 | 4.86   | 2.97   | 66.94  | 5.16               | 4.95               | 5.38               |
| Ti Pb               | 263 | 2.85   | -18.93 | 19.49  | 1.59 <sup>1</sup>  | 1.95 <sup>1</sup>  | 3.49 <sup>1</sup>  |
| Ti Un <sup>3</sup>  | 263 | 4.06   | 1.88   | 34.18  | 4.39               | 4.17               | 4.62               |
| Age                 | 263 | 33.12  | 1.31   | 83.75  | 32.98 <sup>2</sup> | 30.48 <sup>2</sup> | 35.48 <sup>2</sup> |
| BMI                 | 237 | 23.29  | 10.40  | 85.01  | 23.63 <sup>2</sup> | 22.75 <sup>2</sup> | 24.51 <sup>2</sup> |

<sup>1</sup> For Tibia (Ti) and Calcaneus (Cal) Pb levels the arithmetic mean was reported in place of the geometric mean due to negative values. Ti lead was normally distributed therefore no transformation was required. When Cal lead was used in the analysis then the transformation  $\log(x+11)$  was used to normalize the data.

<sup>2</sup> Arithmetic means and corresponding confidence intervals were reported for age and body mass index (BMI)

<sup>3</sup> Un Stands for uncertainty values

**Table 2: Summary statistics of bone lead levels of smelter employees, corresponding uncertainty, and demographic variable age**

| Variable | N   | Median | Min   | Max    | Geometric Mean     | 95% CI             |                    |
|----------|-----|--------|-------|--------|--------------------|--------------------|--------------------|
|          |     |        |       |        |                    | Lower              | Upper              |
| Cal Pb   | 497 | 19.69  | -4.92 | 180.05 | 30.08 <sup>1</sup> | 27.66 <sup>1</sup> | 32.50 <sup>1</sup> |
| Cal Un   | 497 | 2.40   | 0.880 | 4.62   | 2.35               | 2.30               | 2.40               |
| Ti Pb    | 497 | 13.08  | -6.12 | 100.18 | 19.90 <sup>1</sup> | 18.28 <sup>1</sup> | 21.52 <sup>1</sup> |
| Ti Un    | 497 | 1.57   | 0.640 | 5.29   | 2.72               | 2.67               | 2.77               |
| Age      | 497 | 50.10  | 21.21 | 85.00  | 47.65 <sup>2</sup> | 46.80 <sup>2</sup> | 48.50 <sup>2</sup> |

For Tibia (Ti) and Calcaneus (Cal) Pb levels the arithmetic mean was reported in place of the geometric mean due to negative values. Ti lead and Cal lead were not used in the analysis then the transformation  $\log(x+7)$  was used to normalize the data.

<sup>2</sup> Arithmetic means and corresponding confidence intervals were reported for age

Results from univariate analyses are presented in Tables 3-4. When pair wise comparison tests were carried out in the analysis, groups that were statistically similar have the same letter (adjacent to the confidence interval in the tables presented), and those that are statistically different have different letters (in the tables presented). Since for smelter employees, we have not been provided with BMI information, participants' age was considered as the only demographic variable. Table 5 shows the results from the two-way ANOVA models where the interaction of sex and age as well as sex and BMI were assessed for the EG study. Results for the multivariate regression models relating age or BMI and lead levels to uncertainty levels by sex are presented in Table 6. Columns titled "Males" and "Females" report the regression parameter estimates and standard errors with respect to each independent variable listed in the column titled "Effect". Only when the test of coincidence is rejected are comparisons made between the coefficients between males and females. The advantage of fitting a simultaneous regression model is to ensure that the overall Type I error is less than 0.05, and to account for the variance covariance structure between parameter estimates between the two sex groups. Similarly, the multivariate regression model relating age and calcaneus levels to uncertainty levels of smelter employees has been presented in table 7. Tibia uncertainty values did not correlate significantly with age and tibia lead levels ( $p = 0.619$ ).

**Table 3: Univariate results for calcaneus and tibia uncertainties, for demographic variables sex, age and BMI for the environmentally exposed group of participants**

| Bone Site | Demographic Variable |                | N   | p-value <sup>1</sup> | GM (95%CI) <sup>2</sup> |                   |
|-----------|----------------------|----------------|-----|----------------------|-------------------------|-------------------|
| Calcaneus | Sex                  | Male           | 135 | 0.0717 <sup>a</sup>  |                         | 5.33 (5.03, 5.65) |
|           |                      | Female         | 128 |                      |                         | 4.98 (4.69, 5.29) |
| Tibia     | Sex                  | Male           | 135 | 0.0052 <sup>a</sup>  | B                       | 4.11 (3.82, 4.42) |
|           |                      | Female         | 128 |                      | A                       | 4.70 (4.37, 5.07) |
| Calcaneus | Age                  | <11            | 54  | <0.0001              | A                       | 7.12 (6.57, 7.72) |
|           |                      | 11 ≤age <20    | 44  |                      | CB                      | 4.71 (4.31, 5.15) |
|           |                      | 20 ≤age <36    | 47  |                      | C                       | 4.26 (3.91, 4.65) |
|           |                      | 36 ≤age <51    | 52  |                      | CB                      | 4.82 (4.44, 5.23) |
|           |                      | 51 ≤age <65    | 46  |                      | CB                      | 4.92 (4.51, 5.37) |
|           |                      | ≥65            | 20  |                      | B                       | 5.47 (4.80, 6.24) |
| Tibia     | Age                  | <11            | 54  | 0.0005               | A                       | 5.55 (4.96, 6.20) |
|           |                      | 11 ≤age <20    | 44  |                      | B                       | 4.18 (3.69, 4.74) |
|           |                      | 20 ≤age <36    | 47  |                      | B                       | 4.14 (3.67, 4.66) |
|           |                      | 36 ≤age <51    | 52  |                      | B                       | 3.91 (3.49, 4.38) |
|           |                      | 51 ≤age <65    | 46  |                      | B                       | 4.20 (3.72, 4.74) |
|           |                      | ≥65            | 20  |                      | AB                      | 4.46 (3.71, 5.36) |
| Calcaneus | BMI                  | BMI <18.5      | 46  | <0.0001              | A                       | 5.97 (5.46, 6.52) |
|           |                      | 18.5 ≤BMI < 25 | 99  |                      | C                       | 4.65 (4.38, 4.94) |
|           |                      | 25 ≤BMI < 30   | 66  |                      | BC                      | 4.81 (4.47, 5.18) |
|           |                      | BMI ≥ 30       | 26  |                      | BA                      | 5.57 (4.96, 6.27) |
| Tibia     | BMI                  | BMI <18.5      | 46  | 0.0149               | A                       | 4.82 (4.28, 5.42) |
|           |                      | 18.5 ≤BMI < 25 | 99  |                      | B                       | 3.88 (3.58, 4.20) |
|           |                      | 25 ≤BMI < 30   | 66  |                      | BA                      | 4.45 (4.03, 4.91) |
|           |                      | BMI ≥ 30       | 26  |                      | BA                      | 4.49 (3.84, 5.25) |

Note: Letters adjacent to the confidence intervals indicate the results from pairwise comparisons. Groups with the same letter are statistically similar, whereas groups with different letter are statistically different. <sup>1</sup> p-value for overall group effect

<sup>2</sup> Geometric Mean (GM) and corresponding 95% confidence interval (CI): the 95% CI for the geometric mean were corrected for multiple comparisons using Tukey correction

<sup>a</sup> the analysis is based on the non-parametric Kruskal Wallis test

**Table 4: Univariate results for calcaneus and tibia uncertainties, for demographic variable age for the smelter employees.**

| <b>Bone Site</b> | <b>Demographic Variable</b> |             | <b>N</b> | <b>p-value</b> | <b>GM (95%CI)</b> |                   |
|------------------|-----------------------------|-------------|----------|----------------|-------------------|-------------------|
| Calcaneus        | Age                         | 20 ≤age <36 | 65       | 0.0860         | R                 | 2.42 (2.29, 2.55) |
|                  |                             | 36 ≤age <51 | 193      |                | R                 | 2.34 (2.26, 2.42) |
|                  |                             | 51 ≤age <65 | 202      |                | R                 | 2.32 (2.23, 2.41) |
|                  |                             | ≥65         | 37       |                | R                 | 2.54 (2.34, 2.74) |
| Tibia            | Age                         | 20 ≤age <36 | 65       | 0.273          | D                 | 1.62 (1.46, 1.78) |
|                  |                             | 36 ≤age <51 | 193      |                | D                 | 1.58 (1.50, 1.67) |
|                  |                             | 51 ≤age <65 | 202      |                | D                 | 1.56 (1.49, 1.65) |
|                  |                             | ≥65         | 37       |                | D                 | 1.80 (1.46, 2.14) |

**Table 5: Two way ANOVA results for calcaneus and tibia uncertainties for demographic variables age and BMI by Sex**

| Bone Site    | Demographic Variable | N              | p-value <sup>1</sup> | GM (95%CI) <sup>2</sup> |                   |
|--------------|----------------------|----------------|----------------------|-------------------------|-------------------|
| Calcaneus    | Sex                  |                | 0.2920 <sup>a</sup>  |                         |                   |
|              | Age                  |                | <0.0001 <sup>a</sup> |                         |                   |
|              | Sex*Age              |                | 0.8179 <sup>a</sup>  |                         |                   |
|              | Males:Age            | <11            | 34                   |                         | 7.28 (6.57, 8.06) |
|              |                      | 11 ≤age <20    | 24                   |                         | 4.93 (4.37, 5.57) |
|              |                      | 20 ≤age <36    | 23                   |                         | 4.23 (3.74, 4.79) |
|              |                      | 36 ≤age <51    | 23                   |                         | 4.86 (4.30, 5.50) |
|              |                      | 51 ≤age <65    | 22                   |                         | 5.01 (4.42, 5.69) |
|              |                      | ≥65            | 9                    |                         | 5.42 (4.45, 6.60) |
|              | Females:Age          | <11            | 20                   |                         | 6.86 (6.01, 7.84) |
|              |                      | 11 ≤age <20    | 20                   |                         | 4.46 (3.91, 5.09) |
|              |                      | 20 ≤age <36    | 24                   |                         | 4.30 (3.81, 4.85) |
| 36 ≤age <51  |                      | 29             |                      | 4.79 (4.29, 5.35)       |                   |
| 51 ≤age <65  |                      | 24             |                      | 4.84 (4.29, 5.47)       |                   |
| ≥65          |                      | 11             |                      | 5.51 (4.61, 6.60)       |                   |
| Tibia        | Sex                  |                | 0.0015               |                         |                   |
|              | Age                  |                | 0.0005               |                         |                   |
|              | Sex*Age              |                | 0.0198               |                         |                   |
|              | Males:Age            | <11            | 34                   |                         | 5.68 (4.96, 6.51) |
|              |                      | 11 ≤age <20    | 24                   |                         | 4.03 (3.42, 4.73) |
|              |                      | 20 ≤age <36    | 23                   |                         | 3.69 (3.12, 4.35) |
|              |                      | 36 ≤age <51    | 23                   |                         | 3.68 (3.12, 4.34) |
|              |                      | 51 ≤age <65    | 22                   |                         | 3.21 (2.71, 3.80) |
|              |                      | ≥65            | 9                    |                         | 4.09 (3.14, 5.34) |
|              | Females:Age          | <11            | 20                   |                         | 5.33 (4.46, 6.36) |
|              |                      | 11 ≤age <20    | 20                   |                         | 4.38 (3.67, 5.23) |
|              |                      | 20 ≤age <36    | 24                   |                         | 4.62 (3.93, 5.43) |
| 36 ≤age <51  |                      | 29             |                      | 4.10 (3.54, 4.76)       |                   |
| 51 ≤age <65  |                      | 24             |                      | 5.37 (4.57, 6.32)       |                   |
| ≥65          |                      | 11             |                      | 4.78 (3.76, 6.07)       |                   |
| Calcaneus    | Sex                  |                | 0.1177 <sup>a</sup>  |                         |                   |
|              | BMI                  |                | <0.0001 <sup>a</sup> |                         |                   |
|              | Sex*BMI              |                | 0.3277 <sup>a</sup>  |                         |                   |
|              | Males:BMI            | BMI <18.5      | 27                   |                         | 6.64 (5.92, 7.44) |
|              |                      | 18.5 ≤BMI < 25 | 46                   |                         | 4.75 (4.36, 5.19) |
|              |                      | 25 ≤BMI < 30   | 38                   |                         | 4.94 (4.49, 5.44) |
|              |                      | BMI ≥ 30       | 12                   |                         | 5.50 (4.63, 6.53) |
|              | Females:BMI          | BMI <18.5      | 19                   |                         | 5.13 (4.48, 5.88) |
|              |                      | 18.5 ≤BMI < 25 | 53                   |                         | 4.56 (4.20, 4.95) |
| 25 ≤BMI < 30 |                      | 28             |                      | 4.64 (4.15, 5.19)       |                   |
| BMI ≥ 30     |                      | 14             |                      | 5.64 (4.81, 6.61)       |                   |
| Tibia        | Sex                  |                | 0.0158 <sup>a</sup>  |                         |                   |
|              | BMI                  |                | 0.0044 <sup>a</sup>  |                         |                   |
|              | Sex*BMI              |                | 0.1397 <sup>a</sup>  |                         |                   |
|              | Males:BMI            | BMI <18.5      | 27                   |                         | 5.08 (4.37, 5.91) |
|              |                      | 18.5 ≤BMI < 25 | 46                   |                         | 3.61 (3.21, 4.05) |
|              |                      | 25 ≤BMI < 30   | 38                   |                         | 3.97 (3.50, 4.51) |
|              |                      | BMI ≥ 30       | 12                   |                         | 4.07 (3.25, 5.10) |
|              | Females:BMI          | BMI <18.5      | 19                   |                         | 4.46 (3.73, 5.34) |
|              |                      | 18.5 ≤BMI < 25 | 53                   |                         | 4.14 (3.72, 4.61) |
| 25 ≤BMI < 30 |                      | 28             |                      | 5.19 (4.48, 6.02)       |                   |
| BMI ≥ 30     |                      | 14             |                      | 4.89 (3.96, 6.02)       |                   |

<sup>1</sup> p-value for overall group effect

<sup>2</sup> Geometric Mean (GM) and corresponding 95% confidence interval (CI): the 95% CI for the geometric mean were corrected for multiple comparisons using Tukey correction

<sup>a</sup> the analysis is based on the non-parametric two way ANOVA model



**Table 6: Simultaneous regression models relating age or BMI, and lead levels to uncertainty levels for each sex**

| Effect   | Males<br>PE (SE) <sup>1</sup> | Females<br>PE (SE) <sup>1</sup> | Coincidence $a_1=b_1=c_1=0$ | p-value for equal Effect <sup>2</sup> |
|--|-------------------------------|---------------------------------|-----------------------------|---------------------------------------|
| <b>Dependent Variable: Tibia Uncertainty, Adjusted R<sup>2</sup>=9.3%</b>      |                               |                                 |                             |                                       |
| Intercept  | 1.36 (0.04)****               | 1.58 (0.04)****                 | p < 0.0001                  | 0.0006                                |
| Age  | -0.01 (0.00)****              | 0.00 (0.00)                     |                             | 0.0030                                |
| Lead <sup>3</sup>  | 0.01 (0.01)                   | -0.01 (0.01)*                   |                             | 0.0252                                |
| <b>Dependent Variable: Calcaneus Uncertainty, Adjusted R<sup>2</sup>=29.4%</b> |                               |                                 |                             |                                       |
| Intercept  | 0.98 (0.09)****               | 1.18 (0.09)****                 | 0.0719                      |                                       |
| Age  | -0.01 (0.00)****              | -0.00 (0.00)**                  |                             |                                       |
| Lead <sup>3</sup>  | 0.25 (0.03)****               | 0.16 (0.03)****                 |                             |                                       |
| <b>Dependent Variable: Tibia Uncertainty, Adjusted R<sup>2</sup>=4.8%</b>      |                               |                                 |                             |                                       |
| Intercept  | 1.37 (0.04)****               | 1.53 (0.04)****                 | 0.0126                      | 0.0243                                |
| BMI  | -0.01 (0.01)                  | 0.01 (0.00)                     |                             | 0.1650                                |
| Lead <sup>3</sup>  | 0.01 (0.01)                   | -0.01 (0.01)                    |                             | 0.1848                                |
| <b>Dependent Variable: Calcaneus Uncertainty, Adjusted R<sup>2</sup>=22.6%</b> |                               |                                 |                             |                                       |
| Intercept  | 1.05 (0.09)****               | 1.27 (0.08)****                 | 0.0026                      | 0.2562                                |
| BMI  | -0.01 (0.00)                  | 0.00 (0.00)                     |                             | 0.2901                                |
| Lead <sup>3</sup>  | 0.22 (0.03)****               | 0.11 (0.03)***                  |                             | 0.0414                                |

<sup>1</sup> PE=Parameter Estimate, SE=Standard Error of the parameter estimate

<sup>2</sup> If the test of coincidence is rejected, i.e., the two lines do not coincide, then test for equal intercept and/or equal slopes are carried out, otherwise no further tests are carried out. Bonferroni corrections were applied for multiple tests on the same data set.

<sup>3</sup> Tibia lead levels used in Tibia uncertainty models were not log transformed as they were normally distributed. Calcaneus lead levels used in Calcaneus uncertainty models were transformed using  $\log(x+11)$  to satisfy the normality assumption.

Significance of parameter estimates is as follows: \* p<0.05, \*\* p<0.01, \*\*\* p<0.001, \*\*\*\* p<0.0001

**Table 7: Multivariate regression model relating age and calcaneus lead level for smelter employees.**

| Effect  | PE (SE)             |
|---|---------------------|
| <b>Dependent Variable: Calcaneus Uncertainty, Adjusted R<sup>2</sup>=2.2%</b> |                     |
| Intercept   | 2.13 (0.14)****     |
| Age   | -0.00661 (0.00333)* |
| Lead <sup>1</sup>   | 0.425 (0.134)***    |

<sup>1</sup> Calcaneus lead levels used in Calcaneus uncertainty models were transformed using log(x+7) to satisfy the normality assumption.

\* p<0.05, \*\*\* p<0.01, \*\*\*\* p<0.0001

## Discussion

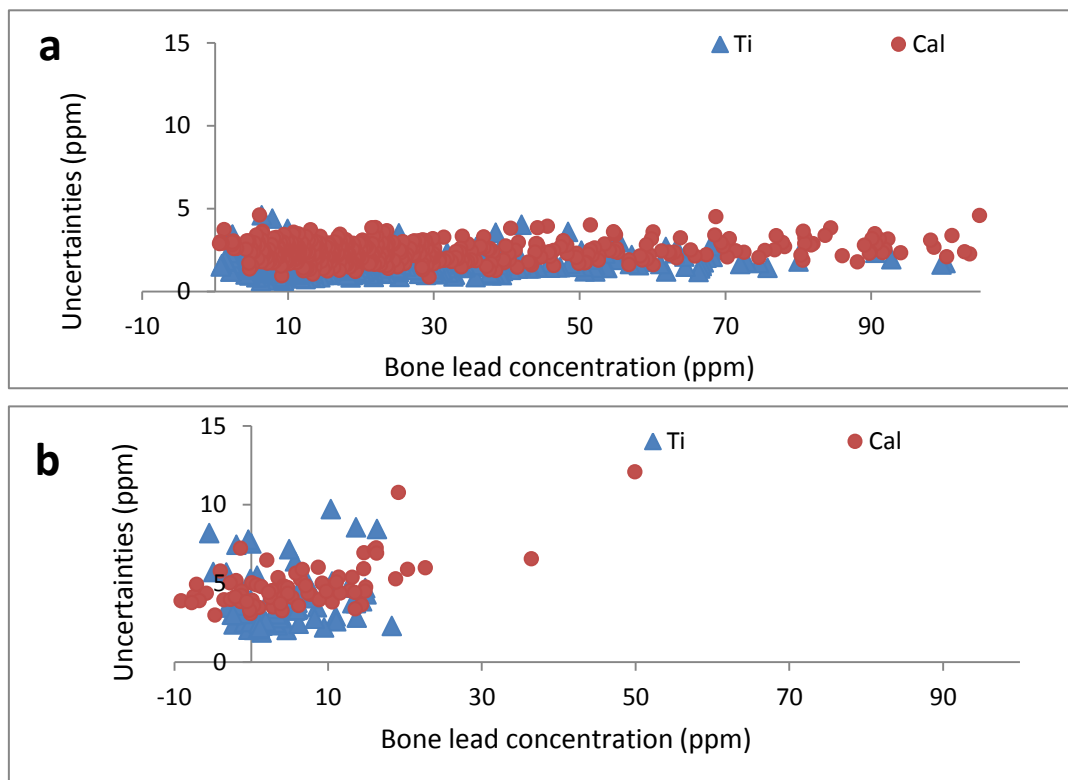
Based on the information presented in table 3, significant sex differences were observed overall in Ti uncertainty measures ( $p=0.0052$ ), where females had higher uncertainty compared to males. Significant differences were observed among the age groups for both Cal and Ti uncertainty measures ( $p<0.0005$ , in both cases). For both Ti and Cal uncertainty, measures were highest in the lowest age group (<11) which can be due to either the imprecision of the measurements, because of the young age of these participants, which meant that they moved a great deal and it was not possible to position them satisfactorily or the presence of smaller bone mass in children. For the Cal, levels monotonically decrease until age group  $20 \leq \text{age} < 36$ , and then begin to increase again. Whereas for the Ti, levels monotonically decrease until age group  $36 \leq \text{age} < 51$ , and then begin to increase again. It is interesting to note that although we see this parabolic pattern in age groups, there is no statistical difference between age groups (11, 19), (20, 35), (36, 50), (51, 65). Table 4 shows that the uncertainty values of calcaneus lead levels are significantly higher than those of tibia lead. However, the differences in neither tibia uncertainty values nor calcaneus uncertainties are statistically significant between the age groups. The value of uncertainties is higher for general population subjects than for smelter employees. As four of the age categories for males are the same between the two groups and there are uncertainties for bone measurements in tibia and calcaneus, we were able to compare these uncertainties in Table 8.

**Table 8: Ratios of uncertainties between EG and smelter smelter for males in different age groups.**

| Age group                                     | $20 \leq \text{age} < 36$ | $36 \leq \text{age} < 51$ | $51 \leq \text{age} < 65$ | $\geq 65$ |
|---|---------------------------|---------------------------|---------------------------|-----------|
| Uncertainty ratio (EG/occupational) tibia     | 2.28                      | 2.33                      | 2.06                      | 2.27      |
| Uncertainty ratio (EG/occupational) calcaneus | 1.75                      | 2.08                      | 2.16                      | 2.13      |

In the environmental exposed group, the activity of source varies between 1.27 to 0.57 GBq, with a measurement time of 1320 seconds real time. Therefore, the average product of measurement time and source activity for the age group presented by figure 1 was

calculated to be  $1.21 \times 10^{12}$  source decays. Whereas, for smelter employees, the variation of source activity is from 3.15 to 2.92 GBq and the measurement time increased to 1800 seconds real time, and the average product of measurement time and source activity was calculated to be  $5.46 \times 10^{12}$  source decays. Therefore, the total number of source photons emitted during the measurement was 4.50 times less for the general population than for the smelter employees. So from this factor alone, the uncertainty for the general population group should be 2.12 times greater than for the smelter employees. The mean of the eight ratios given in Table 8 is 2.13. So this substantial difference in uncertainty for male subjects of the same age groups is well accounted for by the combination of source activity and time of measurement. However, increasing the source activity beyond a certain level may influence dead time losses and pulse pile up.



**Figure 1: Uncertainties in tibia and calcaneus of (a) smelter employees (n=497) and (b) EG (n=77). To make a more fair comparison with the smelter results, females and individuals less than 20 years old were removed from the general population data set.**

Based on the results presented in table 5, the interaction between sex and age was not significant for Cal uncertainty ( $p=0.8179$ ). This indicates that the relationship between Calcaneus uncertainty and age is independent of sex OR, the relationship between Cal uncertainty and age is similar between the two sexes. Also, when the effect

of age is included in the model, there is no significant difference between the sexes ( $p=0.2920$ ). There was a significant difference between age groups ( $p<0.0001$ ). In general age group <11 had the highest uncertainty compared to all other age groups ( $p<0.0001$ ) except age group 65+. As well, age group (20-35) had lower Cal uncertainty measures compared to the two highest age groups, and no statistical difference between the other age groups. These are similar results as we saw in the univariate analyses for age groups in Cal uncertainty. Again, since the interaction between sex and age is not significant, we can conclude that there is a difference between age groups, but this difference does not change by sex. The interaction between sex and age was significant for Ti uncertainty ( $p=0.0198$ ). This indicates that the relationship between Tibia uncertainty and age is not the same between the two sexes i.e., age and sex are not independent of one another. According to Tukey's pair wise tests there is no difference between sexes in any age group except for age group (51-65) ( $p<0.0001$ ), where females had higher levels of uncertainty than males ( $p=0.0013$ ). Also (and more interestingly) there is a significant difference between uncertainty levels and the age groups amongst males ( $p<0.0001$ ), but there is not in females ( $p=0.1337$ ). In the males the pattern is as follows: age group <11 had significantly higher levels of uncertainty compared to age groups (20, 35), (36, 50), (51, 65) (with individual Tukey p-values less than 0.005 in all cases). The interaction between sex and BMI was not significant for Cal uncertainty ( $p=0.3277$ ). This indicates that the relationship between Calcaneus uncertainty and BMI is independent of sex OR, the relationship between Cal uncertainty and BMI is similar between the two sexes. Also, when the effect of BMI is included in the model, there is no significant difference between the sexes ( $p=0.1177$ ). However, there was a significant difference between BMI groups ( $p<0.0001$ ). In general the underweight group had the highest uncertainty compared to those with normal BMI ( $p<0.0001$ ) and overweight BMI ( $p=0.0159$ ). Also, obese participants had higher levels of Cal uncertainty compared to those with normal BMI ( $p=0.0148$ ). Again, since the interaction between sex and BMI is not significant, plus the main effect of sex is not significant, but the main effect of BMI is significant, we can conclude that there is a difference between BMI groups, but this difference does not change by sex. The interaction between sex and BMI was not significant for Ti uncertainty ( $p=0.1397$ ). This indicates that the relationship between Ti uncertainty and BMI is independent of gender OR, the relationship between Ti uncertainty and BMI is similar between the two sexes. However the two main effects sex ( $p=0.0158$ ) and BMI ( $p=0.0044$ ) were found to be significant. Females were found to have higher uncertainty in the Ti compared to males ( $p=0.0158$ ). Underweight people had higher levels of uncertainty compared to normal weight people ( $p=0.0077$ ), and overweight people had marginally higher levels of uncertainty in their Ti measurements compared to normal weight participants ( $p=0.0426$ ). Again, since the interaction between

sex and BMI is not significant, but each main effect is significant, we can conclude that there is a difference between BMI groups, but this difference does not change by sex. A two way ANOVA model was not considered between age and BMI, since these two variables were positively and highly correlated (Pearson correlation coefficient = 0.43,  $p < 0.0001$ ). Furthermore, based on correlation coefficients (which can only reflect linear relationships, see Table 6) further multivariate models would only include age in the model to explain the variability observed in Ti or Cal uncertainty, rather than only BMI, since BMI was not as highly (linearly) correlated with uncertainty as was age.

Based on the results presented in table 6, the first observation to note is that the models for Tibia uncertainty are very poor as they only explain 9.3% (age and lead in the model) and 4.8% (BMI and lead in the model) of the variability found in the Ti uncertainty. Indicating that these variables are poor indicators for Tibia uncertainty or the relationship is not linear. The fit is quite improved for Calcaneus uncertainty where the models explain 29.4% (age and lead in the model) and 22.6% (BMI and lead in the model) of the variability found in Ca uncertainty. As we see in Table 6 Cal uncertainty and corresponding lead levels were highly correlated, both overall and also within each sex group. Considering the model for Ti uncertainty when age and lead are included in the model, the model for both sexes is significantly different with respect to intercept ( $p = 0.0006$ ), age ( $p = 0.0030$ ) and lead levels ( $p = 0.0252$ ). Whereas when BMI and lead are included in the model, the models between sexes differ only in intercept ( $p = 0.0243$ , with females having higher uncertainty regardless of lead levels and age. Considering the model for Cal uncertainty when age and lead are included in the model, the models between sexes were considered statistically similar (coincidence). Whereas when BMI and lead are included in the model, the models between sexes differ only in the slope of lead levels ( $p = 0.0414$ ), with males having a steeper slope (faster increase in uncertainty with respect to lead levels) than females. For smelter employees, tibia uncertainties did not correlate with age and tibia lead level; however, the predictors age and calcaneus lead level correlate with calcaneus uncertainties ( $P = 0.00169$ ).

Table 9 provides a summary of some XRF lead burden studies and compares corresponding uncertainty values; where in all of the cases, measurements were made mid-shaft along the tibia bone, using a  $^{109}\text{Cd}$  radioisotope source and a high purity germanium radiation detector. The activity of the source that we used in the general population study is very close to what has been used by Ahmed et al. in the study population of ages 19.9 to 34.1. By considering the same age range for the current study, the mean (SD) uncertainty of males and females aged 19.9 to 34.1 ( $n = 40$ ) was found to be 4.46 (1.86) which is not very different from what has been reported by Ahmed *et al.*<sup>12</sup> Ahmed *et al* using the cloverleaf with 25mm diameter crystals, compared to the 16mm diameter crystals used here.

**Table 9: Comparison of measurement tibia uncertainty values with previous bone lead studies**

| Study                              | Population    | Sex    | Age range<br>years | N   | Source Activity<br>(GBq) | Measurement<br>Time (min) | Mean (SD)<br>uncertainty/<br>$\mu\text{g Pb g}^{-1}$ |
|------------------------------------|---------------|--------|--------------------|-----|--------------------------|---------------------------|--|
| Hu <i>et al.</i> <sup>10</sup>     | Occupational  | Mixed  |                    | 9   | 3.70                     | 60                        | 3.6 (1.1)  |
| Hu <i>et al.</i> <sup>10</sup>     | Environmental | Mixed  |                    | 34  | 4.63                     | 30                        | 6.1 (1.3)  |
| Ahmed <i>et al.</i> <sup>12</sup>  | Environmental | Mixed  | 19.9-34.1          | 15  | 0.50-1.3                 | 30                        | 4.1  |
| Ahmed <i>et al.</i> <sup>12</sup>  | Environmental | Mixed  | 18.2-30.5          | 19  | 0.39-0.42                | 60                        | 2.6  |
| Hoppin <i>et al.</i> <sup>16</sup> | Environmental | Mixed  | 13-19              | 168 | 1.1                      | 60                        | 3.8  |
| Todd <i>et al.</i> <sup>17</sup>   | Occupational  | Male   |                    | 43  | 0.82                     | 30                        | 3.9  |
| Todd <i>et al.</i> <sup>18</sup>   | Environmental | Female | 11-12.5            | 104 | 1                        | 30                        | 3.2  |
| Todd <i>et al.</i> <sup>18</sup>   | Environmental | Male   | 11-12.5            | 106 | 1                        | 30                        | 2.9  |
| Todd <i>et al.</i> <sup>18</sup>   | Occupational  | Male   |                    | 60  | 1                        | 30                        | 2.8  |
| Present Study                      | Environmental | Female | 3-83               | 128 | 0.57-1.27                | 22                        | 5.15 (2.54)  |
| Present Study                      | Environmental | Male   | 1-78               | 135 | 0.57-1.27                | 22                        | 4.64 (3.30)  |
| Present Study                      | Occupational  | Male   | 21-85              | 497 | 2.92-3.15                | 30                        | 1.68 (0.59)  |

Several previous studies, some conducted decades ago, have evaluated predictors of measurement uncertainty. A more recent study by Ahmed *et al.* (2005)<sup>12</sup> examined linear regressions for natural logarithm of measurement uncertainty in tibia versus BMI for nine females, ages from 18.3 to 30.5 years old and 10 males, ages from 18.3 to 22.5 in a New Brunswick study group with no known history of elevated lead exposure. Their results were as follows:

Females:  $\text{Ln}(\sigma_{\text{Ti}}) = (0.047 \pm 0.009) \text{ BMI} - (0.14 \pm 0.22)$  ( $r^2=0.78$ ;  $n=9$ ;  $P<0.0001$ ).

Males:  $\text{Ln}(\sigma_{\text{Ti}}) = (0.043 \pm 0.015) \text{ BMI} - (0.30 \pm 0.37)$  ( $r^2=0.46$ ;  $n=10$ ;  $P<0.05$ ).

Measurement uncertainty tended to increase with body mass index, and the correlation factor ( $r^2$ ) is stronger in females than males (Ahmed *et al.*, 2005). The authors did not observe any correlation between BMI and uncertainty in measurements for the Vermont group which they attributed to the variation in source activity (from 1.3 GBq down to 0.50 GBq) during the Vermont component of their study. In a study of Idaho and Washington State young adults, McNeill *et al.* (1999)<sup>11</sup> reported a significant correlation between natural logarithm of precision against BMI for males ( $n=276$ ) and females ( $n=254$ ) with the respective slopes of 0.020 and 0.019. An earlier study of American community- and occupationally-exposed subjects reported that larger measurement uncertainty was significantly associated with being female, greater subject weight, and smaller bone lead burdens, but reported the coefficient for age was not significant in the presence of these other factors.<sup>10</sup> For all of the females from the environmental exposed group who provided us with weight and height information ( $n=112$ ), in absence of age and bone lead level, a linear relationship with the respective slope and intercept of  $(0.0129 \pm 0.0060)$  and  $(1.21 \pm 0.15)$  was observed between the variables ( $R^2=0.0361$ ;  $P=0.045$ ). No correlation was observed between tibia lead uncertainties and BMI in females of ages 18.3 to 30.5 as compared to Ahmed *et al.*<sup>12</sup> report.

## Acknowledgements

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## Chapter 4

### Cumulative Blood Lead Index, Bone Lead Concentration and Age Association

#### 4.1 Introduction

Blood lead levels are indicative of recent exposures and nevertheless, correlate significantly with bone lead concentration. Hence, cumulative blood lead index (CBLI)

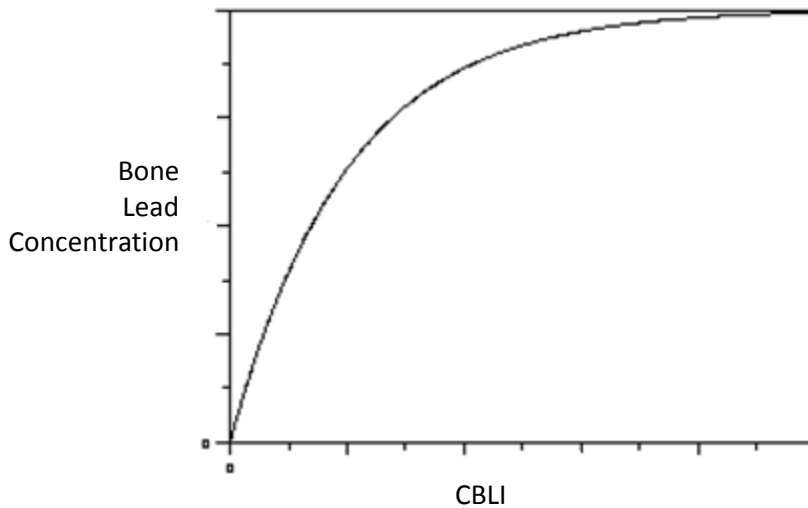
which is defined as  $\int_0^T \text{BL}(t) dt$  (where  $\text{BL}(t)$  is the blood lead level at time  $t$ , and  $T$  is the

total exposure time) can be a good estimate of total exposure. So far, in many of the previous studies, a strong linear correlation was observed between bone lead concentration and CBLI. Two main assumptions were made in deriving the relationship between CBLI and bone lead concentration. In the first assumption, the blood lead contribution from the exchangeable bone fraction compartment was assumed to be negligible. This assumption is reasonable for smelter employees who have been heavily exposed to lead (Somerville *et al.* 1988; Gerhardsson *et al.* 1993). The second assumption indicates a constant transfer rate of lead from blood to bone which results in approximating a linear relationship between bone lead concentration and CBLI. This assumption ignores the influence of some of the potential factors such as age, employment time, and body lead contents including blood and bone lead levels on transfer rates.

Furthermore, if one considers a constant probability of transfer from blood to bone, then the bone lead will be proportional to CBLI in the absence of other factors such as age, employment time, and bone lead concentration, and therefore, transport from one compartment to another is governed by a rate constant (Leggett 1993). The rate constant multiplied by the amount of substance present in the original compartment produces the transfer rate to the new compartment. If all inflow could be stopped and escape to one



new compartment was modelled, the concentration of Pb in the bone would follow the relationship  $C_0 e^{-\lambda t}$ , where  $C_0$  presents the original concentration. Another alternative model to express the association of bone lead concentration and CBLI can be presented by figure 1.



**Figure 1: A schematic presentation of bone lead concentration as a function of CBLI when after a certain level of blood lead concentration, releasing of lead from bone to blood stream becomes dominant.**

According to figure 1, the underlying relationships between bone lead (e.g. Tibia lead (Ti)) and CBLI can be expressed by an exponential function as follows:

$$Ti = C (1 - e^{-A \times CBLI})$$

Where C and A are constants. One may conclude that after a certain level of blood lead concentration, bone becomes a dominant source of lead exposure, and enormous amount of lead is released back into the blood stream.

## 4.2 Previous Studies

Somervaille *et al.* (1988) examined the relationship between tibia lead and CBLI in two occupationally exposed groups of subjects from two factories including lead acid battery manufacture and lead crystal glass manufacture. The former included 88 people (5 females) with an average (standard deviation, SD) age of 45.5 (1.0) years and the hiring years of 14.8 (0.8) years. The latter consisted of 87 people (6 females) with the respective average age (SD) and hiring years (SD) of 27.7 (1.0) and 10.0 (1.1). The underlying relationships between the two variable tibia Pb and CBLI for the first and second factories were expressed respectively by the authors as follows:

$$\text{Tibia Pb} = (0.060 \pm 0.005) \times \text{CBLI} - (1.824 \pm 3.122) \quad (n=88, r = 0.82),$$

$$\text{Tibia Pb} = (0.050 \pm 0.003) \times \text{CBLI} + (7.802 \pm 2.564) \quad (n=79, r = 0.86; 8 \text{ subjects were removed due to not having blood lead history}).$$

Another study was performed by Armstrong *et al.* (1992), where the authors studied the relation between tibia lead concentration and CBLI in a group of workers who were occupationally exposed to lead and had measurements of their tibia lead concentration on two occasions, 5 years apart. In 1983, 15 members of the workforce of a precious metal refiner had been examined for tibia lead concentration for the first time; 5 years after, 11 members were measured for tibia and calcaneus lead, where, 7 of the members were common between both groups. The following equations were used to interpret the association between the tibia lead level and CBLI in 1983 and 1988 respectively.

$$\text{Tibia Pb} = (0.103 \pm 0.016) \times \text{CBLI} + (2.945 \pm 9.621) \quad (n=15, r = 0.87, p<0.001),$$

$$\text{Tibia Pb} = (0.102 \pm 0.015) \times \text{CBLI} - (1.940 \pm 8.214) \quad (n=11, r = 0.91, p<0.001).$$

Gerhardsson *et al.* (1993) studied the association between CBLI and bone lead concentration in 70 active and 30 retired lead smelter workers who had long term exposure to lead. Blood lead levels were available since 1950. The authors reported that the highest bone lead concentration was found among retired lead workers due to higher lead exposure during 1940 to 1960. At the time of study, blood lead correlated well with bone lead concentrations in retired but not in active workers. Calcaneus and tibia lead concentration was studied as a function of blood lead level (BPb), CBLI, age and employment time. Based on the multiple regression analyses, the tibia and calcaneus bone lead concentration was best described by the cumulative blood lead index, where for calcaneus lead level, blood lead level added some significant information ( $r^2 = 0.14$  for CBLI alone;  $r^2 = 0.20$  for BPb and CBLI). The underlying relationships were expressed by the following equation:

$$\text{Ti Pb} = 0.45 \times \text{CBLI} + 5.97 \quad (\text{multiple } r = 0.60, p < 0.001),$$

$$\text{Cal Pb} = 0.87 \times \text{CBLI} + 25.73 \times \text{BPb} + 4.25 \quad (\text{multiple } r = 0.44, p < 0.001).$$

For the retired group, CBLI and bone lead level were not correlated significantly.

Roels *et al.* (1995) studied the relationship between tibia lead and CBLI in 123 workers from a primary lead smelter (age range, 30-61 with the mean of 45 years; duration of employment range, 7-45 with the mean of 20 years). The workers were hired between 1947 and 1985. Participants' blood lead levels were available on a regular basis since 1978. For participants who were hired before 1978, a value of 20  $\mu\text{g/dl}$  was assigned for the date of hiring based on the average blood lead value reported for the males of general population in Belgium (Ducoffre *et al.* 1990), and the blood lead

measurements in 18 representative workers whose blood lead levels were determined through the pre-employment medical examination from 1977 to 1985. The relationship between log (CBLI) and log (tibia Pb) was examined by taking into account age as covariate. Two multivariate models were tested by considering either log (CBLI) or log (tibia Pb) as the dependent variable. Since age contributed a relatively low fraction of the variance to the model, for the sake of simplification and comparison with other studies, the authors considered log (CBLI) and log (tibia Pb) as regression parameters in equations as follow:

$$\text{Log (Ti Pb)} = 1.07186 \times \text{log (CBLI)} - 1.42314 \quad (\text{n}=123, r = 0.80, \text{p}<0.001),$$

$$\text{Log (CBLI)} = 0.58904 \times \text{log (Ti Pb)} + 1.90913 \quad (\text{n}=123, r = 0.80, \text{p}<0.001).$$

Fleming *et al.* (1997) investigated the relationship of CBLI and tibia (calcaneus) lead concentration for our current study group in 1994 for 367 active and 14 retired workers. The authors observed non-linearity in relationships of the variables but to be consistent with previous works, they interpreted the results by linear equations as:

$$\text{Ti} = (0.0556 \pm 0.0020) \text{CBLI} + (2 \pm 2) \quad (\text{N} = 367; r^2 = 0.69).$$

$$\text{Cal} = (0.111 \pm 0.003) \text{CBLI} - (9 \pm 3) \quad (\text{N} = 367; r^2 = 0.77).$$

$$\text{Ti} = (0.0700 \pm 0.0216) \text{CBLI} - (17 \pm 26) \quad (\text{N} = 14; r^2 = 0.47).$$

$$\text{Cal} = (0.144 \pm 0.041) \text{CBLI} - (42 \pm 50) \quad (\text{N} = 14; r^2 = 0.50).$$

The authors also reported that the differences between the two populations (active and retired) are not statistically significant. For the active workers, they found the slopes to be in agreement with Somervaille *et al.* (1988) and Cake (1994). The Cake's study on 53 of

lead active workers reported a slope value of  $0.066 \pm 0.011$  for tibia lead concentration as a function of CBLI.

#### **4.3 Nonlinearity in the relationship between bone lead concentrations and CBLI for lead smelter employees**

The nonlinearity between bone lead content and CBLI of the smelter employees was assessed in longitudinal studies performed in 1994, 1999, and 2008 (figure 2), where the results of 2008 survey has been presented in an attached paper. For the 211 employees common between the three groups, the respective mean (SD) of tibia and calcaneus was 27.62 (18.57) and 41.70 (29.26)  $\mu\text{g Pb / g- bone mineral}$  in 2008; the ones in 1999 were found to be 37.84 (29.68) and 76.07 (49.47), and finally the bone lead values in 1994 were found to be 37.33 (29.04) and 61.25 (54.74)  $\mu\text{g Pb / g- bone mineral}$  respectively. The mean change in CBLI between the two lead measurements in 2008-1999 and 1999-1994 were 151.7 and 110.7  $\mu\text{g yr dl}^{-1}$  respectively. No significant correlation was found between the changes in bone lead concentration and CBLI for 2008-1999 and 1999-1994 due to the large errors in bone lead concentration measurements made in 1999 and 1994 as the conventional system was used to perform the measurements. The underlying relationships between bone lead concentration and employment CBLI has been tabulated in tables 1 and 2 by polynomial and linear regression models respectively. Based on the results presented in tables 1 and 2, the differences between the slopes of tibia (calcaneus) lead concentration and CBLI was not found to be significant between 1994 and 1999. However, results show a significant drop in slope values based on 2008 measurements due to reduction in blood lead levels.

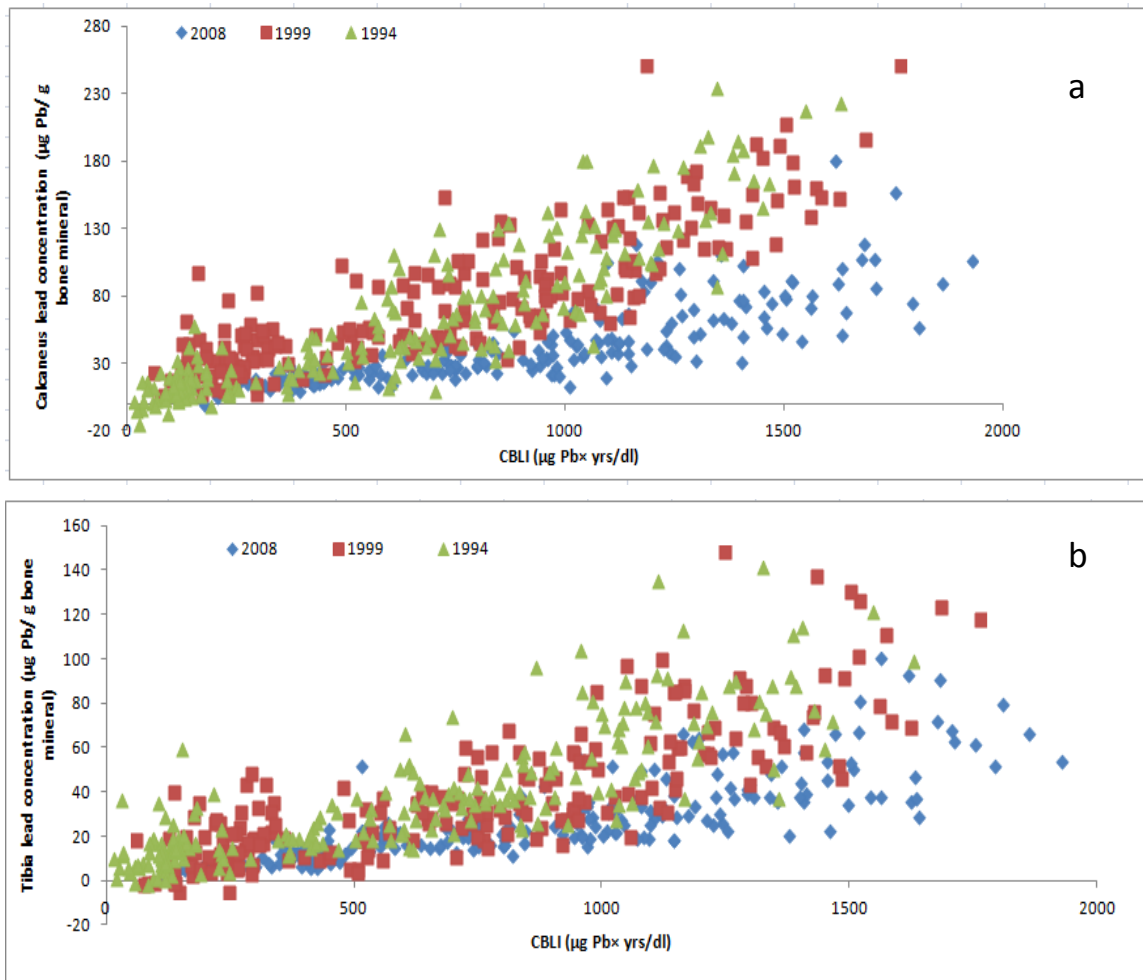
**Table 1: Relation between bone lead concentration and CBLI by applying a polynomial fit**

| Group    | Quadratic term (SE)<br>$\frac{\mu\text{gPb} \times \text{g bone}^{-1}}{\mu\text{gyrdl}^{-1}}$ | Linear term (SE)<br>$\frac{\mu\text{gPb} \times \text{g bone}^{-1}}{\mu\text{gyrdl}^{-1}}$ | Intercept (SE)<br>$\mu\text{gPb} \times \text{g bone}^{-1}$ | $r^2$ | P value |
|----------|---|--|---|-------|---------|
| Ti 2008  | $1.09 (0.38) \times 10^{-5}$  | 0.0139 (0.0073)  | 5.16 (3.06)   | 0.671 | <0.0001 |
| Ti 1999  | $2.64 (0.64) \times 10^{-5}$  | 0.0142 (0.0104)  | 9.03 (3.49)   | 0.690 | <0.0001 |
| Ti 1994  | $2.21 (0.64) \times 10^{-5}$  | 0.0270 (0.0090)  | 8.73 (2.52)   | 0.712 | <0.0001 |
| Cal 2008 | $2.01 (0.61) \times 10^{-5}$  | 0.0161 (0.0116)  | 8.61 (4.85)   | 0.667 | <0.0001 |
| Cal 1999 | $4.15 (0.80) \times 10^{-5}$  | 0.0311 (0.0159)  | 24.51 (5.34)  | 0.738 | <0.0001 |
| Cal 1994 | $5.77 (0.98) \times 10^{-5}$  | 0.0360 (0.0136)  | 7.61 (3.82)   | 0.814 | <0.0001 |

SE Standard Error

**Table 2: Relation between bone lead and CBLI by applying a linear fit.**

| Group    | Slope (SE)<br>$\frac{\mu\text{gPb} \times \text{g bone}^{-1}}{\mu\text{gyrdl}^{-1}}$ | Intercept (SE)<br>$\mu\text{gPb} \times \text{g bone}^{-1}$ | $r^2$ | P value |
|----------|--|---|-------|---------|
| Ti 2008  | 0.0343 (0.0017)  | -2.23 (1.67)  | 0.658 | <0.0001 |
| Ti 1999  | 0.0556 (0.0027)  | -2.07 (2.29)  | 0.664 | <0.0001 |
| Ti 1994  | 0.0566 (0.0026)  | 2.94 (1.92)   | 0.696 | <0.0001 |
| Cal 2008 | 0.0537 (0.0027)  | -5.00 (2.66)  | 0.649 | <0.0001 |
| Cal 1999 | 0.0960 (0.0042)  | 7.05 (3.52)   | 0.715 | <0.0001 |
| Cal 1994 | 0.113 (0.004)  | -7.48 (3.07)  | 0.782 | <0.0001 |



**Figure 2: Scatter plot of tibia (a) and calcaneus (b) lead concentration as a function of CBLI for 211 subjects who had participated in all three surveys in 2008, 1999, and 1994.**

In the attached paper (Behinaein *et al.* 2012), the authors looked at the variation of CBLI and bone lead concentration in four subgroups that were classified based on their hiring year. The retired employees (G1) and the early hired active employees (G2) included elderly subjects, and the recent hired group (G4) consists of younger employees. As a complementary work, the group of employees were classified into five subgroups based on their age and the underlying relationships between the variables were compared with those were classified based on their employment time. Table 3 summarizes the

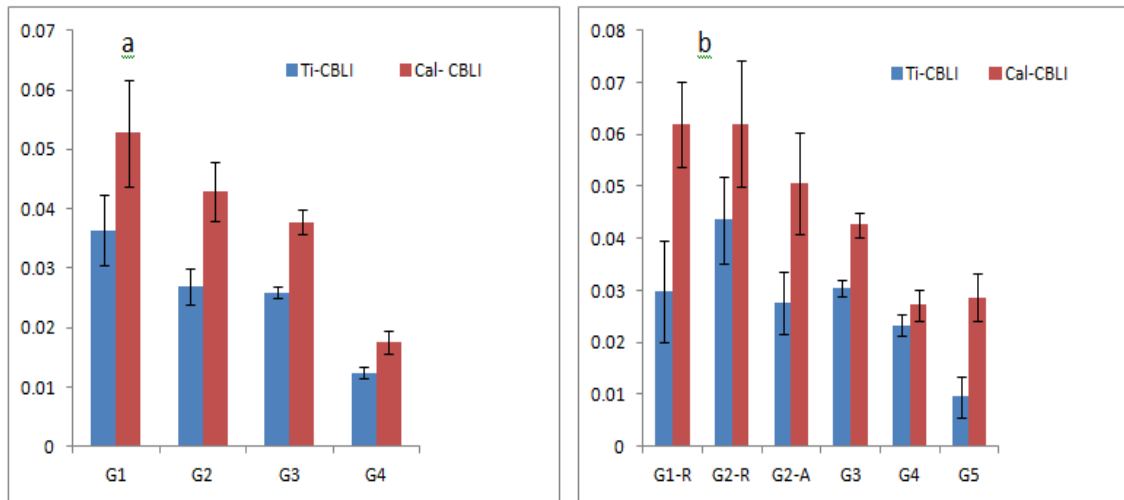
subgroups, and the results have been illustrated by figure 2. Based on the information presented by figure 3, one may conclude that (i) the changes in calcaneus lead level were more reflected by age and hired date than those in tibia; (ii) the greatest value of slope was observed for the retired group, and the smallest value of slope belonged the recent hired group of the youngest ones. (iii) Calcaneus introduced a steeper slope than does tibia.

**Table 3: Subgroups' information divided based on their hiring year and birth year**

| Group | Hiring year /Groups |           |            | Birth Year/Groups |           |
|-------|---------------------|-----------|------------|-------------------|-----------|
|       | N                   | DOB**     | DOH***     | n                 | DOB       |
| G1    | 63 R*               | 1927-1954 | 1966-1976  | 18 R              | 1923-1939 |
| G2    | 89                  | 1946-1957 | 1966-1976  | 85 (45 R)         | 1940-1950 |
| G3    | 157                 | 1942-1971 | 1977-1991  | 167               | 1951-1960 |
| G4    | 185                 | 1945-1985 | After 1992 | 152               | 1961-1971 |
| G5    | -                   | -         | -          | 73                | 1972-1987 |

\*Retired      \*\* Year of Birth      \*\*\* Year of Hiring





**Figure 3:** This figure illustrates the slope values of linear regression equation of tibia (Ti) and calcaneus (Cal) lead concentration as a function of CBLI in subgroups based on their hiring year (a) and birth year (b). ‘R’ and ‘A’ stand for retired and active group respectively.

#### 4.3.1 Age and employment time as continuous variables

Based on the results presented so far, employees hired before 1977 (G1 and G2 groups) demonstrated a steeper slope of lead transfer from blood to bone. The variation in slopes can be caused by employees’ age (i) as the older participants have been exposed into the more lead pollutant environment and (ii) for the earlier employment time, since the technology of estimating blood lead levels was relatively new; the blood lead levels could be accidentally underestimated (Fleming *et al* 1997). While a multivariate regression model was used to study the association of bone lead concentration as dependent variables and CBLI, age, and employment time as predictors, employment time failed to attain statistical significance in the presence of other predictors. The association of bone lead concentration and the predictors CBLI (post employment) and age was assessed for the whole group of participants (n=494) by the following equations:

$$\text{Cal} = (2.14 \pm 0.24) \times 10^{-5} \text{ CBLI}^2 + (0.00975 \pm 0.00409) \text{ CBLI} + (0.166 \pm 0.075) \text{ Age} + (3.47 \pm 3.07) \quad [n = 494; R^2 = 0.780].$$

$$\text{Ti} = (1.08 \pm 0.17) \times 10^{-5} \text{ CBLI}^2 + (0.0121 \pm 0.0028) \text{ CBLI} + (0.0938 \pm 0.0221) \text{ Age} + (1.87 \pm 2.13) \quad [n = 494; R^2 = 0.763].$$

For the retired participants, with the respective average age and CBLI of 65.4 years and 1389  $\mu\text{g.dl}^{-1}\cdot\text{yr}$ , about 62%, 21%, and 17% of calcaneus lead concentration comes from the non-linear part of CBLI, CBLI, and age respectively. Similarly, for tibia lead concentration, the respective values were approximated to be 68%, 13%, and 19%. For the oldest participant with the age of 85 years old and the corresponding CBLI value of 1351.6  $\mu\text{g.dl}^{-1}\cdot\text{yr}$ , the contribution of the aforementioned variables were found to be 63% ( $\text{CBLI}^2$ ), 12% (CBLI), and 25% (age) for tibia lead and the respective percentages were found to be 58%, 20%, and 22% for calcaneus lead concentration. The influence of age on body lead levels has been further discussed in chapter 7 of the thesis.

#### **4.3.2 Retired Population**

As was mentioned before, among the subgroups that were examined for CBLI and bone lead concentration, the retired population demonstrated the most efficient transfer rate of lead from blood to bone. In order to investigate the influence of the years of retirement on slope values, the retired population were classified into three subgroups namely: (i)  $G_{1r}$  includes participants whose retirement date is before 2000 (N=14); (ii)  $G_{2r}$  consists of employees who retired between and including 2000 and 2004 (N=36), and finally (iii)  $G_{3r}$  are participants whose retirement date is between and including 2005 and 2008 (N=14). The slope values of the regression equations of the post employment CBLI and bone lead concentration have been tabulated in table 4. For tibia, the differences in slopes between  $G_{1r}$  and  $G_{2r}$  are not statistically significant (two tailed test  $P = 0.400$ ); whereas, the differences are significant between  $G_{3r}$  and the other two subgroups (two tailed test  $P < 0.001$ ). For calcaneus, the differences in slopes are significant among all of the subgroups (two tailed test  $P < 0.05$ ). The results of table 4 illustrates that for the retired workers the slope of bone lead level and CBLI varies between the subgroups since retired

employees are subjected to endogenous exposures more strongly than exogenous, and because of that employees retired before 2005 demonstrate a more efficient transfer rate of lead from blood to bone.

**Table 4: This table presents the slope values of linear regression equations of bone lead concentration as a function of CBLI in different subgroups of retired workers.**

| CBLI <sub>post</sub> | G <sub>1r</sub> |         | G <sub>2r</sub> |         | G <sub>3r</sub> |         | whole population |         |
|----------------------|-----------------|---------|-----------------|---------|-----------------|---------|------------------|---------|
|                      | Tibia           | Cal     | Tibia           | Cal     | Tibia           | Cal     | Tibia            | Cal     |
|                      | 0.0499±         | 0.0860± | 0.0534±         | 0.0738± | 0.0290±         | 0.0598± | 0.0455±          | 0.0685± |
|                      | 0.0140          | 0.0174  | 0.0090          | 0.0147  | 0.0115          | 0.0120  | 0.0064           | 0.0092  |

## Nonlinearity in the relationship between bone lead concentrations and CBLI for lead smelter employees

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494 smelter employees from New Brunswick participated in a bone lead survey conducted by McMaster University in 2008, using the four element “clover-leaf” geometry germanium detector system. The employees were measured at two different bone sites, tibia and calcaneus, each measurement lasting 30 minutes. Scattered photons, including Pb X-rays, were collected by the germanium detectors located behind the <sup>109</sup>Cd source. A strong positive correlation was observed between tibia and calcaneus lead concentrations. Having been provided with blood lead levels, a cumulative blood lead index (CBLI) was generated. The employees were classified into four groups based on their date of hire, and their CBLI levels were compared to their tibia and calcaneus lead concentrations in the different groups. The slopes of bone Pb *versus* CBLI varied amongst groups, with those hired earliest showing the steepest slopes. This could be taken to imply a non-linearity in the uptake of Pb by bone from blood. In this paper, the association of the bone lead concentrations *versus* CBLI has been expressed by a polynomial function for the whole group of employees.

### Introduction

Lead, a soft, blue-gray and toxic metal, has been used in a wide variety of materials including paint, gasoline, batteries, ceramic glazes, solders, and radiation shielding. It is found in the earth's crust not in the elemental form but mostly in the compound forms of PbS, PbSO<sub>4</sub>, and PbCO<sub>3</sub>, representing the minerals galena, anglesite, and cerussite respectively.<sup>1</sup> Lead has various health hazard effects in humans at varying levels of blood lead (PbB) concentration. It is a neurotoxic metal, and a threshold value of blood lead concentration associated with intellectual deficiencies is poorly defined.<sup>2</sup> The human body is exposed to lead through ingestion and inhalation. Two widely used models

of lead (Pb) metabolism were developed by Leggett<sup>3</sup> and O'Flaherty.<sup>4</sup> These models agree in identifying bone as the tissue compartment in which the overwhelming proportion of the mass of Pb is stored in the body. Since most of the mass of Pb is in bone and the residence time of Pb in bone is much longer than that in other tissues, it is helpful to reduce the number of compartments and associated transfer rates considered in order to focus on the long term aspects of Pb metabolism. These reduced models do not treat Pb in soft tissue and the associated more rapid transfer rates, but do consider three main compartments involved in lead metabolism: blood (as a transfer compartment), cortical bone (tibia), and trabecular bone (calcaneus). There is a bidirectional connection between blood and the other two compartments.<sup>5,6</sup> Lead stays in blood for approximately 30 days.<sup>7</sup> Lead has a long residence time in bone. Therefore, previous exposures to lead are studied by finding out the lead concentration in bones. Moreover, because of the long biological half life (10–50 years) of lead in bone, bone acts as a secondary source of lead exposure during bone turnover and increases the blood lead level.<sup>8</sup>

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### Environmental impact

The non-linearity in relationship of bone lead and CBLI (cumulative blood lead index) is more solid for higher levels of exposure. Also, we observed that the most recently hired employees demonstrated more shallow bone lead–CBLI relations, where, the high lead levels in an elderly population are higher than current best estimates of threshold for adverse health effects of lead exposure. So lead stays as a significant occupational and environmental problem.

More than 90% of lead is accumulated in bone. Hence, developing techniques for bone lead measurement is an essential step in understanding the health effects of lead in the human body.<sup>9</sup> In the K XRF method of bone lead determination,<sup>10</sup> the radioactive source, <sup>109</sup>Cd, emits 88.03 keV gamma rays; so the energy is 34 eV above the lead K absorption edge energy. The volunteer or a calibration standard is typically irradiated for half an hour and the generated photons are collected as a number of counts by the germanium detectors located behind the source in the backscatter geometry.<sup>11</sup> By comparing the signals from volunteers against the signals from calibration standards, the amount of lead in the volunteer's bone can be quantified. The previously described four-detector, clover-leaf geometry<sup>12</sup> was used to detect signals. The efficiency of the new detector system was tested for the first time for the large population in the survey that was conducted in 2008 to measure bone lead concentration of New Brunswick smelter employees.<sup>13</sup>

## Materials and methods

### Cumulative blood lead index

494 of smelter employees in the province of New Brunswick of Canada were measured for lead concentration in left tibia and right calcaneus, each for 30 min.<sup>13</sup> Blood lead level data are available since 1967, where, depending on the exposure history, each employee has been measured for the blood lead level at intervals of between once per month and once per year. Bone lead concentrations can be compared to a cumulative blood lead index (CBLI), where, the CBLI is calculated by using formula (1).<sup>14</sup>

$$\text{CBLI} = \sum_{i=1}^n 0.5 \times (\text{PbB}_{i+1} + \text{PbB}_i) \times (t_{i+1} - t_i) \quad (1)$$

In eqn (1),  $\text{PbB}_i$  and  $\text{PbB}_{i+1}$  represent the blood lead concentrations at two consecutive times  $t_i$  and  $t_{i+1}$  respectively. For each person, the point  $i = 1$  is when they were first hired, and the point  $i = n$  is the time of bone Pb survey which lasted from 14<sup>th</sup> January to 6<sup>th</sup> March 2008. Employees who were hired in 1966 and 1967 had their first blood test in 1968. In order to find the CBLI for the missing span, the rate of change of  $\text{PbB}$  ( $\Delta\text{PbB}$ ) is defined by formula (2) using the specific values for each individual. The blood lead level at the time of hiring is calculated by using formula (3) and the contribution to CBLI from this first time interval is given by formula (4).

$$\Delta\text{PbB} = [\text{PbB}(t_{i+1}) - \text{PbB}(t_i)] / [t_{i+1} - t_i] \quad (2)$$

$$\text{PbB}(t_i) = \text{PbB}(t_{i+1}) - \Delta\text{PbB} \times (t_{i+1} - t_i) \quad (3)$$

$$\text{CBLI}(t_i \text{ to } t_{i+1}) = (\text{PbB}(t_i) + \text{PbB}(t_{i+1})) \times (t_{i+1} - t_i) / 2 \quad (4)$$

### Pre-employment CBLI

Although the  $\text{PbB}$  typically rises sharply when a person begins employment in a lead-related industry, in this case the smelter, there will inevitably have been some Pb exposure prior to the start of their employment. In nearly all cases considered here, this

pre-employment exposure would have been from the general environment. There is no database of  $\text{PbB}$  in the general population that applies to the specific region in which these people lived and to the time span, 1930–2007, of relevance here. However, 166 of these employees had their first blood sample collected for Pb analysis either on the first day they reported for work ( $n = 140$ ) or in the one or two days before they started work ( $n = 26$ ). These  $\text{PbB}$  levels therefore provide an estimate of Pb exposure in the environment in which these people were living prior to their employment. These data are shown in Fig. 1. It can be seen that the average  $\text{PbB}$  fell from 20–25  $\mu\text{g Pb dL}^{-1}$  whole blood in the 1968–1975 period to less than 5  $\mu\text{g Pb dL}^{-1}$  in the most recent years. There are still no explicit data prior to 1968. For subjects born before 1968, the background level of Pb exposure was presumed to result in a  $\text{PbB}$  of 20.21  $\mu\text{g Pb dL}^{-1}$ , the mean of the annual mean levels up to 1974. We used this mean estimate because if one were to extrapolate the regression line shown in Fig. 1 back to 1930, approximately the earliest date of birth amongst this cohort, the projected  $\text{PbB}$  would be about 40  $\mu\text{g Pb dL}^{-1}$ , which is implausibly high. The pre-employment CBLI is then estimated in two stages, as illustrated in Fig. 2. First, from birth to 18 years of age, the year by year estimated  $\text{PbB}$  is summed. This sum is then divided by two to allow for smaller body mass through childhood.<sup>22</sup> The sum of  $\text{PbB}$  for each year between the age of 18 years and start of employment is then calculated. These two terms constitute the pre-employment CBLI. This was added algebraically to the post-employment CBLI calculated by using formula (1), and then the relationship of bone lead concentrations was examined both as a function of the full CBLI and for that part of the CBLI dating from the start of employment.

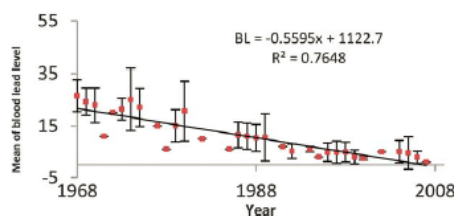


Fig. 1 Mean of the blood lead level as a function of the year of blood test.

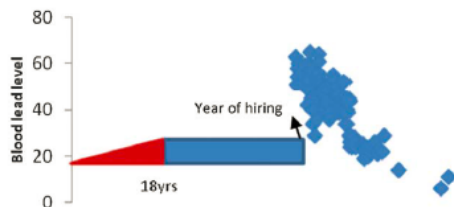


Fig. 2 This figure presents the pre-employment blood lead level which was used to calculate background CBLI; the calculated value was added to the post-employment CBLI.

### Variation of PbB over time

The average of PbB measurements made year by year is plotted in Fig. 3. This illustrates that PbB in the smelter started decreasing over time. There are two significant points of change. During 1976 to 1977 major improvements were made in occupational hygiene practices and these resulted in a significant fall in PbB. Secondly, there was a 10 month strike at the smelter from July 1990 to May 1991. The strike was in part about occupational health and there were improvements in health monitoring and health maintenance, as well as the interruption in occupational exposure. The group of participants was divided into four subgroups depending on their date of hire by taking to consideration both the points of change noted above and the current employment status of the subjects. The first group (G1) was hired between 1966 and 1976, and they had also retired by 2008; the second group (G2) was hired between 1966 and 1976, but they were active workers in 2008; the third group (G3) had active workers hired between 1977 and 1991, and finally, the fourth group (G4) was hired after 1992 (post-strike employees). A similar pattern was reported by Fleming *et al.*,<sup>15</sup> who presented the mean blood lead levels of the employees of the same plant reported here from 1968 to 1994.

### Function used for regression

When Somerville *et al.*,<sup>14</sup> reported the relationship of tibia Pb with CBLI, they used a straight line to describe that relationship and subsequent studies have followed this pattern. When Fleming *et al.* reported the bone Pb–CBLI relationship amongst workers at this smelter<sup>15</sup> they pointed out that there was an apparent upward curvature, so that bone Pb values were higher than predicted by a straight line at high CBLI values. Subsequently Chettle<sup>16</sup> suggested that the slope between bone Pb and CBLI was steeper in surveys for which the mean bone Pb was higher. In 2007, Schwartz and Hu<sup>17</sup> published recommendations about maximum tibia Pb in the occupational setting based on the relationship between tibia Pb and CBLI presented by Hu *et al.* in 2007.<sup>18</sup> Responding to this recommendation, Healey *et al.*<sup>19</sup> suggested that the tibia Pb *versus* CBLI relationship should be seen as varying, depending on the level of tibia or CBLI. This suggestion was then rejected by Schwartz *et al.*<sup>20</sup> If indeed there is an upward curvature in the bone Pb–CBLI relationship, then some function other than a straight line should result in a better fit to the data. The two simplest alternatives to a straight line are perhaps a quadratic function or an exponential function. The approach taken here was to explore the fit of a quadratic function

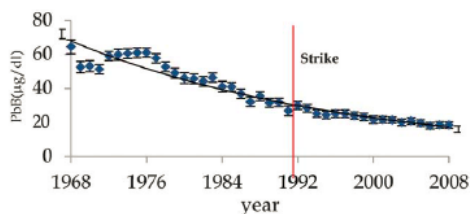


Fig. 3 Mean blood lead level as a function of test years. Error bars are standard deviation of mean values at any particular test date.

or a parabolic model. A straight line is still part of this model and, in principle at least, the quadratic term could be positive, insignificant or negative. There is, therefore, an open possibility of examining whether the bone Pb–CBLI relationship is indeed better described by a model other than a straight line. A parabolic model is written in the form:<sup>21</sup>

$$\mu_{Y|X} = \beta_0 + \beta_1 X + \beta_2 X^2$$

or

$$Y = \beta_0 + \beta_1 X + \beta_2 X^2 + E$$

where,  $Y$ 's and  $X$ 's represent statistical variables; the coefficients  $\beta_0$ ,  $\beta_1$ , and  $\beta_2$  stand for the unknown parameters which are called regression coefficients;  $\mu_{Y|X}$  presents the mean of  $Y$  at a given  $X$ , and  $E$  stands for the error component, which is the difference between the observed response  $Y$  at  $X$  and the true average response  $\mu_{Y|X}$  at  $X$ . Similar to the straight-line case, the best parabolic fit can be determined by employing the least-squares method. The minimum sum of squares achieved by using the least-squares parabola is defined as:

$$SSE = \sum_{i=1}^n (Y_i - \hat{Y}_i)^2 = \sum_{i=1}^n (Y_i - \hat{\beta}_0 - \hat{\beta}_1 X_i - \hat{\beta}_2 X_i^2)^2$$

The total sum of squares is calculated as:

$$SSY = \sum_{i=1}^n (Y_i - \bar{Y})^2$$

$$F = \frac{\text{Regression MS}}{\text{Residual MS}} = \frac{(SSY - SSE)/k}{(SSE)/(n - k - 1)} \quad (5)$$

There are two basic questions that are associated with second order polynomial regression. Firstly, assuming  $\beta_1$  and  $\beta_2$  to be  $X$  and  $X^2$  coefficients, the null hypothesis ( $H_0$ ), whether there is no significant overall regression using  $X$  and  $X^2$  (i.e.,  $\beta_1 = \beta_2 = 0$ ), is tested by computing the  $F$  values; where  $F$  is calculated by using formula (5). In eqn (5),  $n$  and  $k$  represent sample size and regression degree of freedom respectively; then the value of the computed  $F$  is compared with an appropriate critical point of the  $F_{k, n-k-1, 1-\alpha}$  distribution ( $1 - \alpha$  presents the confidence level where the confidence level of 0.999 is generally considered in our cases). Secondly, the polynomial fit is tested to see if the second-order model provides significantly better fit than the straight line model. In this case, the partial  $F$  statistic is used to assess whether the addition of any specific independent variable significantly contributes to the prediction of  $Y$ . The partial  $F$  statistic (formula (6)) is computed, and the result is compared to an appropriate  $F$  value.

$$F = \frac{(\text{Extra sum of squares due to adding } X^2)/1}{\text{Residual of MS for the second order model}} \quad (6)$$

### Results

There is a strong correlation between bone lead concentrations in two different bone sites, tibia and calcaneus (Fig. 4). In a variety

of studies,<sup>15,23,24</sup> a linear relationship has been applied to a comparison of calcaneus and tibia bone lead concentrations. The relationship for the data reported here is  $\text{Cal} = (1.30 \pm 0.03) \text{Ti} + (4.40 \pm 0.91)$ . This can be compared to the equivalent relationship reported to be  $\text{Cal} = (1.70 \pm 0.04) \text{Ti} + (0.6 \pm 2.2)$  for 367 New Brunswick smelter employees who had participated in a 1994 survey reported by Fleming *et al.*<sup>15</sup> Further investigation can test whether the polynomial function is a better fit to express the underlying relationships (eqn (7a) and (7b)).

$$\text{Cal} = (-0.0057 \pm 0.0013) \text{Ti}^2 + (1.69 \pm 0.09) \text{Ti} + (0.69 \pm 1.21) \quad R^2 = 0.758 \quad (7a)$$

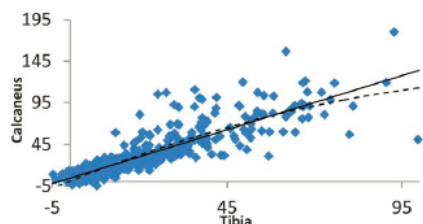
$$\text{Ti} = (-0.0023 \pm 0.0003) \text{Cal}^2 + (0.822 \pm 0.040) \text{Cal} - (1.16 \pm 0.82) \quad R^2 = 0.768 \quad (7b)$$

The ANOVA table for parabolic fit to the calcaneus-tibia bone lead concentrations for the whole population of employees is summarized in Table 1.

By looking at the ANOVA table, we can decide whether adding the  $X^2$  can improve the prediction significantly. By using formula (6), the partial  $F$  statistic is calculated to be 20.08, which exceeds  $F_{1, 491, 0.999} = 10.95$ . Therefore, adding the  $X^2$  term to the model improves the prediction significantly. The linear part of eqn (7a) is very similar to the one that has been reported by Fleming *et al.*<sup>15</sup>

#### Looking at CBLI in the whole group of employees

Tibia and calcaneus lead concentrations of employees were plotted *versus* CBLI values for the whole group and subgroups of participants. So far, based on previous studies, a linear regression has been reported to express the relationships of bone lead



**Fig. 4** In this figure the relationship of bone lead concentrations in tibia (Ti) and calcaneus (Cal) for the  $n = 494$  of employees has been illustrated.

**Table 1** Regression ANOVA table for the quadratic model weight gain data

| Source         | DF  | SS      | MS = SS/DF | $F$                  |
|----------------|-----|---------|------------|----------------------|
| Regression $X$ | 1   | 279.278 | 1461.82    | 1461.82 <sup>a</sup> |
| $X^2$          | 1   | 3671    | 19.96      | 20.08                |
| Residual       | 491 | 90.325  | 183.96     |                      |

<sup>a</sup>  $F = (279.278)/(3671 + 90.325)/(491 + 1)$ . DF: degree of freedom; SS: sum squares; MS: mean squares.

concentrations in different bone sites against CBLI.<sup>15,16,18,25-31</sup> However, what was tested here was whether the relationship between bone Pb and CBLI was better explained when a quadratic term was added to an existing linear model.

First, a linear and polynomial regression was applied to compare tibia and calcaneus lead levels of all employees ( $n = 494$ ) as a function of post- and pre-employment CBLIs (Fig. 5 and 6). In this classification, post-employment refers to the CBLI calculated only from the start of employment, whereas post-pre employment refers to CBLI estimated from birth and throughout the person's life to the date of the survey. The least squares method was used to find the best linear and polynomial fit, where the resultant equations are expressed as:

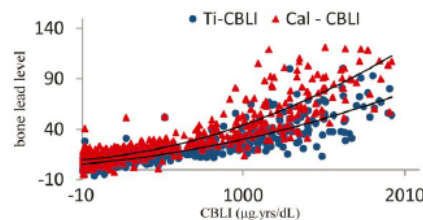
$$\text{Ti} = (0.0300 \pm 0.0008) \text{CBLI}_{\text{post}} + (2.73 \pm 0.62) \quad [n = 494; R^2 = 0.7418; p < 0.001]. \quad (8a)$$

$$\text{Cal} = (0.0449 \pm 0.0012) \text{CBLI}_{\text{post}} + (4.43 \pm 0.92) \quad [n = 494; R^2 = 0.7409; p < 0.001]. \quad (8b)$$

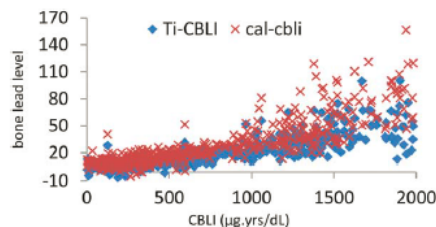
$$\text{Ti} = (1.04 \pm 0.16) \times 10^{-5} \text{CBLI}_{\text{post}}^2 + (0.0142 \pm 0.0026) \text{CBLI}_{\text{post}} + (5.47 \pm 0.74) \quad [n = 494; R^2 = 0.7614; p < 0.001]. \quad (8c)$$

$$\text{Cal} = (2.07 \pm 0.24) \times 10^{-5} \text{CBLI}_{\text{post}}^2 + (0.0135 \pm 0.0038) \text{CBLI}_{\text{post}} + (9.86 \pm 1.06) \quad [n = 494; R^2 = 0.7778; p < 0.001]. \quad (8d)$$

The ANOVA table for the parabolic fit of the tibia-CBLI and the calcaneus-CBLI for the whole population of employees is summarized in Tables 2 and 3.



**Fig. 5** Linear and polynomial regressions of bone lead concentration as a function of post-employment CBLI for the whole group of employees.



**Fig. 6** Linear and polynomial regressions of bone lead concentration as a function of pre-employment CBLI for the whole group of employees.

**Table 2** Regression ANOVA table for the quadratic model weight gain data for tibia – post-employment CBLI

| Source         | DF  | SS      | MS = SS/DF | F       |
|----------------|-----|---------|------------|---------|
| Regression $X$ | 1   | 123 238 | 123 238    | 1413.84 |
| $X^2 X$        | 1   | 3245    | 3245       | 40.20   |
| Residual       | 491 | 39 640  | 80.73      |         |

**Table 3** Regression ANOVA table for the quadratic model weight gain data for calcaneus – post-employment CBLI

| Source         | DF  | SS      | MS = SS/DF | F       |
|----------------|-----|---------|------------|---------|
| Regression $X$ | 1   | 275 102 | 275 102    | 1423.90 |
| $X^2 X$        | 1   | 12 797  | 12 797     | 76.38   |
| Residual       | 491 | 82 259  | 168        |         |

By looking at the ANOVA tables, one can decide whether adding the  $X^2$  can improve the prediction significantly. In other words, we can investigate whether the increase in  $R^2$  of 0.0196 for tibia and of 0.0369 for calcaneus obtained by including the  $X^2$  term significantly improves the fit. By using formula 6, the partial  $F$  statistic is calculated to be 40.2 and 76.38 for tibia and calcaneus respectively, where the result exceeds  $F_{1, 491, 0.999} = 10.95$ . Therefore, adding the  $X^2$  term to the model improves the prediction significantly. The steeper linear slope for calcaneus implies a different behaviour of lead in cortical and trabecular bones which is consistent with previous studies.<sup>15,25,26</sup> In the next step, the pre-employment CBLI (Fig. 2) is added into the post-employment CBLI. The resulting equations are expressed as:

$$Ti = (0.0261 \pm 0.0007) CBLI_{post+pre} - (0.41 \pm 0.71) [n = 494; R^2 = 0.726; p < 0.001].$$

$$Cal = (0.0390 \pm 0.0011) CBLI_{post+pre} - (0.32 \pm 1.05) [n = 494; R^2 = 0.7299; p < 0.001].$$

$$Ti = (5.67 \pm 1.20) \times 10^{-6} CBLI_{post+pre}^2 + (0.0149 \pm 0.0024) CBLI_{post+pre} + (2.83 \pm 0.98) [n = 494; R^2 = 0.7379; p < 0.001].$$

$$Cal = (1.24 \pm 0.17) \times 10^{-5} CBLI_{post+pre}^2 + (0.0145 \pm 0.0036) CBLI_{post+pre} + (6.76 \pm 1.41) [n = 494; R^2 = 0.7553; p < 0.001].$$

Similarly, the ANOVA table for a parabolic fit of calcaneus–tibia bone lead concentrations *versus* CBLI for the whole population of employees is summarized in Tables 4 and 5.

In both cases, the partial  $F$  statistic, 22.22 for tibia and 51.05 for calcaneus, exceeds  $F_{1, 491, 0.999} = 10.95$ . Therefore, adding the  $X^2$  term to the model improves the prediction significantly.

**Table 4** Regression ANOVA table for the quadratic model weight gain data for tibia – post- and pre-employment CBLI

| Source         | DF  | SS      | MS = SS/DF | F       |
|----------------|-----|---------|------------|---------|
| Regression $X$ | 1   | 120 620 | 120 620    | 1304.19 |
| $X^2 X$        | 1   | 1970    | 1970       | 22.22   |
| Residual       | 491 | 43 533  | 259.13     |         |

**Table 5** Regression ANOVA table for the quadratic model weight gain data for calcaneus – post- and pre-employment CBLI

| Source          | DF  | SS      | MS = SS/DF | F       |
|-----------------|-----|---------|------------|---------|
| Regression: $X$ | 1   | 270 179 | 270 179    | 1329.56 |
| $X^2 X$         | 1   | 9417    | 9417       | 51.05   |
| Residual        | 491 | 90 562  | 184.44     |         |

### Looking at CBLI in the subgroups

In the subgroups G1, G2, G3, and G4, it was found that a quadratic term could not produce a significantly better fit, at the  $p > 0.999$  level, than linear regression to express the association of bone lead concentration as a function of CBLI. The results of linear regression for both post- and pre-employment CBLI have been summarized in Tables 6 and 7. However, at  $P > 0.950$ , a polynomial fit produced a better fit for calcaneus in sub-groups G2 and G3 (Tables 8 and 9). Group G1, a retired group whose hired date is before 1977, has demonstrated a more efficient transfer of lead to bone. Oppositely, group G4, the most recently hired group, has demonstrated a shallower transfer of lead to bone. The calculated post-employment CBLI is an underestimate of their lifetime CBLI where, underestimation could produce a steeper bone–CBLI relation. The differences between the slopes in different subgroups except for groups G2 and G3 are significant.

**Table 6** Linear regression equations of bone lead concentration as a function of post-employment CBLI in different subgroups

| G | Tibia vs. CBLI   | Calcaneus vs. CBLI  |
|---|--|---|
| 1 | Ti = (0.0455 ± 0.0064)<br>CBLI – (16.90 ± 9.29);<br>$n = 63, R^2 = 0.451, T = 7.08$  | Cal = (0.0685 ± 0.0092)<br>CBLI – (24.39 ± 13.31);<br>$R^2 = 0.475, T = 7.43$ |
| 2 | Ti = (0.0293 ± 0.0033)<br>CBLI + (1.37 ± 3.66);<br>$n = 89, R^2 = 0.468, T = 8.75$   | Cal = (0.0447 ± 0.0053)<br>CBLI + (1.46 ± 5.77);<br>$R^2 = 0.451, T = 8.45$   |
| 3 | Ti = (0.0311 ± 0.0021)<br>CBLI + (0.90 ± 1.26);<br>$n = 157, R^2 = 0.588, T = 14.86$ | Cal = (0.0450 ± 0.0030)<br>CBLI + (1.07 ± 1.80);<br>$R^2 = 0.597, T = 15.14$  |
| 4 | Ti = (0.0160 ± 0.0029)<br>CBLI – (5.41 ± 0.44);<br>$n = 185, R^2 = 0.142, T = 5.49$  | Cal = (0.0249 ± 0.0037)<br>CBLI + (9.10 ± 0.56);<br>$R^2 = 0.199, T = 6.76$   |

**Table 7** Linear regression equations of bone lead concentration as a function of post- + pre-employment CBLI in different subgroups

| G | Tibia vs. CBLI   | Calcaneus vs. CBLI  |
|---|--|---|
| 1 | Ti = (0.0364 ± 0.0062)<br>CBLI – (17.53 ± 11.18);<br>$n = 63, R^2 = 0.364, T = 5.91$ | Cal = (0.0528 ± 0.0091)<br>CBLI – (21.80 ± 16.49);<br>$R^2 = 0.356, T = 5.81$ |
| 2 | Ti = (0.0269 ± 0.0033)<br>CBLI – (1.69 ± 4.22);<br>$n = 89, R^2 = 0.438, T = 8.24$   | Cal = (0.0429 ± 0.0049)<br>CBLI – (5.58 ± 6.41);<br>$R^2 = 0.463, T = 8.66$   |
| 3 | Ti = (0.0259 ± 0.0018)<br>CBLI – (1.75 ± 1.49);<br>$n = 157, R^2 = 0.565, T = 14.20$ | Cal = (0.0378 ± 0.0026)<br>CBLI – (2.94 ± 2.10);<br>$R^2 = 0.581, T = 14.65$  |
| 4 | Ti = (0.0124 ± 0.0017)<br>CBLI – (3.92 ± 0.53);<br>$n = 185, R^2 = 0.218, T = 7.15$  | Cal = (0.0175 ± 0.0022)<br>CBLI + (7.24 ± 0.68);<br>$R^2 = 0.253, T = 7.88$   |



**Table 8** Quadratic regression equations of bone lead concentration as a function of post-employment CBLI

| G | Calcaneus vs. CBLI   |
|---|--|
| 2 | Cal = $(3.82 \pm 1.06) \times 10^{-5} \text{CBLI}^2 - (0.0209 \pm 0.0189) \text{CBLI} + (22.77 \pm 8.01)$ ; $R^2 = 0.525$ , $T = 3.60$ |
| 3 | Cal = $(3.62 \pm 0.87) \times 10^{-5} \text{CBLI}^2 + (0.0011 \pm 0.0109) \text{CBLI} + (11.49 \pm 3.04)$ ; $R^2 = 0.637$ , $T = 4.14$ |

**Table 9** Quadratic regression equations of bone lead concentration as a function of CBLI post- + pre-employment

| G | Calcaneus vs. CBLI  |
|---|---|
| 2 | Cal = $(2.80 \pm 0.85) \times 10^{-5} \text{CBLI}^2 - (0.0177 \pm 0.0189) \text{CBLI} + (21.74 \pm 10.27)$ ; $R^2 = 0.524$ , $T = 3.29$ |
| 3 | Cal = $(1.47 \pm 0.70) \times 10^{-5} \text{CBLI}^2 - (0.0133 \pm 0.0120) \text{CBLI} + (5.61 \pm 4.59)$ ; $R^2 = 0.592$ , $T = 2.09$   |

By excluding the retired group (G1) and merging groups G2, G3, and G4 as an active group, the behaviour of bone lead as a function of CBLI is studied in the active group, and the results can be compared with those of previous studies (Fig. 7 and 8). The ANOVA tables for a parabolic fit for the post-employment CBLI-tibia and calcaneus bone lead concentrations are summarized in Tables 10 and 11.

The partial  $F$  statistic was calculated to be 14.34 and 51.86 for tibia and calcaneus respectively, which exceeds  $F_{1, 428, 0.999} = 10.99$ . Therefore, adding the  $X^2$  term to the model improves the prediction. Similarly, Tables 12 and 13 present the ANOVA tables for the pre- and post-employment CBLI.

The partial  $F$  statistic was calculated to be 39.97 and 61.65 for tibia and calcaneus respectively, which are larger than  $F_{1, 428, 0.999} = 10.99$ . Therefore, adding the  $X^2$  term to the model improves the prediction. The regression equations, to express the association of bone lead concentration as a function of CBLI in the active group of employees, have been formulated as:

Active group:

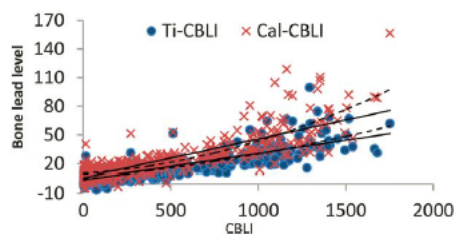
$$\text{Ti} = (7.82 \pm 2.06) \times 10^{-6} \text{CBLI}_{\text{post}}^2 + (0.017 \pm 0.003) \text{CBLI}_{\text{post}} + (5.14 \pm 0.65); R^2 = 0.707$$

**Table 10** Regression ANOVA table for the quadratic model weight gain data for tibia – post-employment CBLI

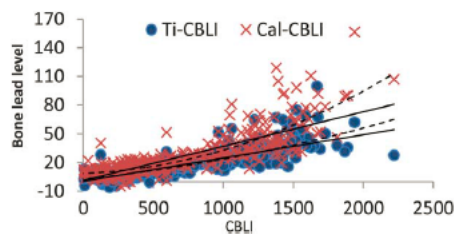
| Source          | DF  | SS       | MS = SS/DF | F      |
|-----------------|-----|----------|------------|--------|
| Regression: $X$ | 1   | 59 172.4 | 59 172.4   | 989.38 |
| $X^2 X$         | 1   | 832.0    | 832.0      | 14.34  |
| Residual        | 428 | 24 825.4 | 58.0       |        |

**Table 11** Regression ANOVA table for the quadratic model weight gain data for calcaneus – post-employment CBLI

| Source          | DF  | SS      | MS = SS/DF | F      |
|-----------------|-----|---------|------------|--------|
| Regression: $X$ | 1   | 126 044 | 126 044    | 937.52 |
| $X^2 X$         | 1   | 6233    | 6233       | 51.86  |
| Residual        | 428 | 51 444  | 120.2      |        |



**Fig. 7** Linear and polynomial regressions of bone lead concentration as a function of post-employment CBLI for the active workers.



**Fig. 8** Linear and polynomial regressions of bone lead concentration as a function of pre- and post-employment CBLI for the active workers.

$$\text{Ti} = (0.0271 \pm 0.0009) \text{CBLI}_{\text{post}} + (3.68 \pm 0.54); R^2 = 0.690$$

$$\text{Cal} = (2.14 \pm 0.30) \times 10^{-5} \text{CBLI}_{\text{post}}^2 + (0.012 \pm 0.004) \text{CBLI}_{\text{post}} + (10.08 \pm 0.94); R^2 = 0.720$$

$$\text{Cal} = (0.0396 \pm 0.0013) \text{CBLI}_{\text{post}} + (6.09 \pm 0.81); R^2 = 0.686$$

$$\text{Ti} = (5.96 \pm 1.64) \times 10^{-6} \text{CBLI}_{\text{post+pre}}^2 + (0.015 \pm 0.003) \text{CBLI}_{\text{post+pre}} + (2.86 \pm 0.90); R^2 = 0.690$$

$$\text{Ti} = (0.0243 \pm 0.0008) \text{CBLI}_{\text{post+pre}} + (0.521 \pm 0.637); R^2 = 0.680$$

**Table 12** Regression ANOVA table for the quadratic model weight gain data for tibia – post- + pre-CBLI

| Source          | DF  | SS       | MS = SS/DF | F      |
|-----------------|-----|----------|------------|--------|
| Regression: $X$ | 1   | 588 00.7 | 58 800.7   | 962.87 |
| $X^2 X$         | 1   | 2236.5   | 2236.5     | 39.97  |
| Residual        | 425 | 23 778.6 | 55.9       |        |

**Table 13** Regression ANOVA table for the quadratic model weight gain data for calcaneus – post- + pre-CBLI

| Source          | DF  | SS      | MS = SS/DF | F      |
|-----------------|-----|---------|------------|--------|
| Regression: $X$ | 1   | 117 478 | 117 478    | 848.12 |
| $X^2 X$         | 1   | 7475    | 7475       | 61.65  |
| Residual        | 425 | 51 533  | 121.3      |        |

$$\text{Cal} = (1.87 \pm 0.23) \times 10^{-5} \text{CBLI}_{\text{post+pre}}^2 + (0.005 \pm 0.004) \text{CBLI}_{\text{post+pre}} + (8.70 \pm 1.26); R^2 = 0.720$$

$$\text{Cal} = (0.0356 \pm 0.0012) \text{CBLI}_{\text{post+pre}} + (1.35 \pm 0.94); R^2 = 0.676$$

## Discussion

Cake *et al.*,<sup>24</sup> Fleming *et al.*,<sup>15</sup> and Somervaille *et al.*<sup>23</sup> have reported a strong correlation between lead concentration in calcaneus and tibia with the slope values of  $1.17 \pm 0.16$  ( $n = 49$ ),  $1.70 \pm 0.04$  ( $n = 367$ ), and  $2.03$  ( $n = 120$ ) of an occupationally exposed group of participants respectively. In this report, the slope for the linear regression equation was found to be  $1.30 \pm 0.03$ . For the polynomial regression equations the rate of changes of lead in calcaneus with respect to that in tibia can be estimated by taking the derivative of calcaneus Pb with respect to tibia Pb ( $\partial\text{cal}/\partial\text{ti}$ ) from eqn (7a), which yields the result  $-0.0114 \text{Ti} + 1.69$ . Similarly, the rate of changes of lead in tibia with respect to that in calcaneus was found to be  $-0.0046 \text{Cal} + 0.822$ , which indicates that the rate of changes of lead in one bone site depends on the exposure level of another bone site. All results indicate that the turnover of lead in trabecular bone is faster than that in cortical bone.

In all of the aforementioned equations of bone lead concentration *versus* CBLI, calcaneus introduces a steeper slope than does tibia. In the polynomial fitting equation the release of lead from blood to calcaneus is almost twice that of tibia. For the active groups of workers, G2 and G3, there is a wide range of CBLIs (roughly 100–2200  $\mu\text{g years/dL}$ ); whereas, for G4, CBLI roughly ranges from 1 to 500  $\mu\text{g years/dL}$ ; therefore, in groups G2 and G3, calcaneus lead concentration can be expressed as a polynomial function of CBLI since a wider range of CBLIs and bone lead concentration in calcaneus (*vs.* tibia) are observed for these groups of employees. The reduction in transferring lead from blood to bone sites in sub-groups G1 to G4 strongly supports that an increase in the exposure level to lead increases the rate of exchange of lead from blood to bone. According to O'Flaherty,<sup>4</sup> the rate of transfer of lead out of the blood is related to the concentration of lead in plasma, where the relationship between plasma lead and blood lead is determined by a capacity limited lead binding associated with the red blood cells. The partitioning of lead between plasma and red cells shifts with the level of exposure; plasma lead is a smaller fraction of total blood lead at low blood lead concentrations than at high blood lead levels. At higher levels of lead exposure, the concentration of lead in plasma, and consequently the level of lead in bone sites increase. Also, the reason for expecting the nonlinear relationship between CBLI and bone lead concentration is a saturation of the red blood cells.<sup>32</sup> Hence, in this study, for the early hired smelter workers, one should expect a more rapid transfer rate of lead from blood to bone (refer to subgroups G1 to G3) since they may have been beyond the saturation point. However, for the most recent hired employees (group G4), one should expect the blood lead levels to be below the saturation point as a slow transfer rate of lead from blood to bone has been observed in the results. The same pattern was observed and reported by Fleming *et al.*<sup>15</sup> Also, there is a significant increase of the value of  $R^2$  (Tables 6 and 7) in subgroup G4 which can be due to the relatively higher contribution from environmental exposure than from occupational exposure.

Using exponential functions over polynomial functions to interpret the underlying relationships of bone lead concentration and tibia reduces the degrees of freedom by two (*vs.* three for the polynomial function and two for the linear function), which, in turn, makes an increase in  $F$  values. However, there are no significant differences between  $R^2$  and residuals in polynomial and exponential functions. Fleming *et al.*<sup>15</sup> observed a similar range of CBLI, tibia and calcaneus lead values as those reported here, in their study in 1994 of the relationships of the CBLI and bone lead concentrations for the same industry in New Brunswick. Fleming introduced a linear function to fit the data for active and retired population; where for active population, he reported:

$$\text{Ti} = (0.0556 \pm 0.0020) \text{CBLI}_{\text{post}} + (2 \pm 2) [n = 367; R^2 = 0.69; p < 0.001].$$

$$\text{Cal} = (0.111 \pm 0.003) \text{CBLI}_{\text{post}} - (9 \pm 3) [n = 367; R^2 = 0.77; p < 0.01].$$

Similarly, for the retired population:

$$\text{Ti} = (0.0700 \pm 0.0216) \text{CBLI}_{\text{post}} - (17 \pm 26) [n = 14; R^2 = 0.47; p < 0.01].$$

$$\text{Cal} = (0.144 \pm 0.041) \text{CBLI}_{\text{post}} - (42 \pm 50) [n = 14; R^2 = 0.50; p < 0.01].$$

For the retired group, G1 (Table 6), the slopes of tibia and calcaneus as a function of CBLI were found to be 65% and 47.6% of those that have been reported by Fleming *et al.* respectively. Also, for the active population, the slopes for calcaneus and tibia were found to be 48.7% and 35.6% respectively of the ones reported by Fleming *et al.* Moreover, ongoing exposure to lead causes the transfer rate of lead from blood to both bone sites to be approximately the same. Also, Fleming *et al.*<sup>15</sup> considered the pre-employment CBLI, where, the blood lead background which was used to calculate pre-employment CBLI was established to be 10  $\mu\text{g years/dL}$  in 1991 and after, 15  $\mu\text{g years/dL}$  in 1978 and 20  $\mu\text{g years/dL}$  in 1972. Then, a linear fit was used to express the tibia and calcaneus lead *versus* CBLI data for those hired before 1977 and after 1977. For the former the resultant equations are:

$$\text{Ti} = (0.0584 \pm 0.0048) \text{CBLI}_{\text{post+pre}} - (24 \pm 7) [n = 209; R^2 = 0.42];$$

$$\text{Cal} = (0.127 \pm 0.007) \text{CBLI}_{\text{post+pre}} - (76 \pm 11) [n = 209; R^2 = 0.58].$$

Similarly, for the latter, the best fit linear equations relating the same variables were reported to be:

$$\text{Ti} = (0.0406 \pm 0.0029) \text{CBLI}_{\text{post+pre}} - (7 \pm 2) [n = 158; R^2 = 0.56];$$

$$\text{Cal} = (0.0842 \pm 0.0054) \text{CBLI}_{\text{post+pre}} + (26 \pm 4) [n = 158; R^2 = 0.61].$$

In the current study, the groups were arranged in a comparable way with those reported by Fleming *et al.* The linear fits to the data for active workers hired before 1977 (G2) resulted in the following equations:

$$\text{Ti} = (0.0269 \pm 0.0033) \text{CBLI}_{\text{post+pre}} - (1.69 \pm 4.22) [n = 89; R^2 = 0.438];$$

$$\text{Cal} = (0.0429 \pm 0.0049) \text{CBLI}_{\text{post+pre}} - (5.58 \pm 6.41) [n = 89; R^2 = 0.463];$$

Similarly, when the same linear fitting routine is applied to the workers hired after 1977 (G3 + G4), the following regression equations are produced:

$$\text{Ti} = (0.0219 \pm 0.0009) \text{CBLI}_{\text{post+pre}} + (1.42 \pm 0.55) [n = 342; R^2 = 0.623];$$

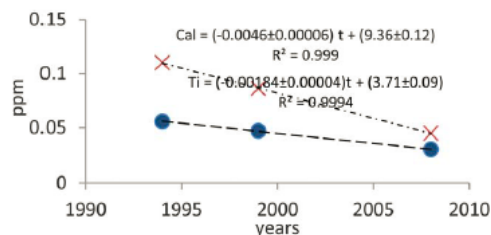
$$\text{Cal} = (0.0300 \pm 0.0013) \text{CBLI}_{\text{post+pre}} + (3.51 \pm 0.77) [n = 342; R^2 = 0.617].$$

The slopes of tibia as a function of CBLI for the former and latter groups of active workers were found to be 46% and 54% and those of calcaneus were calculated to be 33% and 36% of those that have been reported by Fleming *et al.* In both studies, the differences between the two groups of active workers are significant, and individuals whose hired date is more recent (after 1977) demonstrate a more shallow bone lead–CBLI relationship. Furthermore, Brito J<sup>33</sup> reported the association between bone lead concentration and post-employment CBLI in his PhD thesis for the same group of study participants back in 1999 as follows:

$$\text{Ti} = 0.047 \text{CBLI}_{\text{post}} - 18.340 [n = 313; R^2 = 0.577];$$

$$\text{Cal} = 0.086 \text{CBLI}_{\text{post}} - 23.550 [n = 313; R^2 = 0.656].$$

Fig. 9 illustrates the changes of bone lead concentration–CBLI slopes in different years of measurement (*i.e.* 1994, 1999, and 2008). The change in the slope of (bone Pb vs. CBLI) vs. time is a rate of change of input function. It may well reflect at least two separate phenomena: (i) decline in input rates as blood Pb declines due to improvements in hygiene and (ii) loss of Pb from bone.



**Fig. 9** The rate of change of bone lead concentration–CBLI based on a linear relationship of bone lead level–CBLI association as a function of time.

## Conclusion

The non-linearity in relationship is more solid for higher levels of exposure. Also, we observed that the most recently hired employees demonstrated more shallow bone lead–CBLI relationships. However, retired workers, in turn, presented the steepest slopes in bone lead–CBLI relationships. The active workers excluding the most recently hired groups (Group G4) demonstrated a non-linear behavior in their calcaneus–CBLI relationship. There are two consequences of the lower rate of uptake of lead to bone from blood for lower levels of blood lead concentration. One is that workers hired since 1991 will never acquire bone lead levels close to those seen in older workers both because the exposure levels are lower and because the transfer rates are lower. Secondly, it is probably not valid to use transfer rates (slope of bone lead vs. CBLI) derived from studies of occupational exposure to estimate transfer rates for the usually lower exposure conditions experienced in the general environment. Moreover, the high lead levels in an elderly population are higher than current best estimates for a threshold (if there is one) for adverse health effects of lead exposure; so lead stays as a significant occupational and environmental problem. The advantages or disadvantages of using exponential function over polynomial function should be investigated further in future work.

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#### **4.4 Conclusion**

The non-linearity in relationship of bone lead concentration and CBLI is more solid for higher levels of exposure. We observed that the most recently hired employees demonstrated more shallow bone lead–CBLI relations. However, retired workers, in turn, presented the steepest slopes in bone lead–CBLI relationships. The results can be used by authorities to set regulations regarding the employment time and the retirement age.

In principle, the pattern shown in figure 1 (page 65) could have been brought about if the company had cheated and deliberately reported values lower than the true blood lead levels in order to appear to meet the requirements of the regulations. However, this work shows that there is a physiological explanation for the observed pattern, so there is no evidence for such under reporting.

## Chapter 5

### The estimation of the rates of lead exchange between body compartments of smelter employees

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#### Abstract:

The overwhelming proportion of the mass of lead (Pb) is stored in bone and the residence time of Pb in bone is much longer than that in other tissues. Hence, in a metabolic model that we used to solve the differential equations governing the transfer of lead between body compartments, three main compartments are involved: blood (as a transfer compartment), cortical bone (tibia), and trabecular bone (calcaneus). There is a bidirectional connection between blood and the other two compartments. A grid search chi-squared minimization method was used to estimate the initial values of lead transfer rate values from tibia ( $\lambda_{TB}$ ) and calcaneus ( $\lambda_{CB}$ ) to blood of 209 smelter employees whose bone lead measurements are available from 1994, 1999, and 2008, and their blood lead level from 1967 onwards (depending on exposure history from once per month to once per year), and then the initial values of kinematic parameters were used to develop multivariate models in order to express  $\lambda_{TB}$  and  $\lambda_{CB}$  as a function of employment time, age, body lead contents and their interaction. We observed a significant decrease in the transfer rate of lead from bone to blood with increasing body lead contents. The model was tested by calculating the bone lead concentration in 1999 and 2008, and by comparing those values with the measured ones. A good agreement was found between the calculated and measured tibia /calcaneus lead values. Also, we found that the transfer rate of lead from tibia to blood can be expressed solely as a function of cumulative blood lead index.

#### 1. Introduction:

Most of the toxicokinetic models of lead have employed compartmental analysis<sup>1-6</sup> in which transport of lead from one compartment to another has governed by a rate constant.

By multiplying the rate constant by the amount of lead present in the original compartment, the mass of lead transferred to the new compartment per unit time is found. The rate constants were first initiated by Rabinowitz *et al.*<sup>1</sup> in five healthy men. Participants' daily intake was supplemented with 79-204  $\mu\text{g}$   $^{204}\text{Pb}$  for 1-124 days (average 367  $\mu\text{g}/\text{day}$ ). Three main compartments labeled with  $^{204}\text{Pb}$  and  $^{207}\text{Pb}$  tracers included, blood that contained 1.7-2.0 mg of lead with a mean life of 35 days; soft tissue that contained 0.3-0.9 mg of lead, and had an approximate mean life of 40 days, and finally the last compartment was skeleton which enclosed the vast majority of bone lead with a very long mean life. Leggett's work<sup>6</sup> consists of a very detailed compartmental model of lead metabolism, where he believes distribution of lead throughout the body depends on the concentration of lead in diffusible plasma compartment. Lead distribution among the compartments has been assumed to be linear as long as the concentration of lead in blood stays below a threshold value of about 25  $\mu\text{g}/\text{dl}$  for adults. Lead transfers from plasma first to the surface layer of bone; 50% of this lead will move to the exchangeable bone volume, and from there 20% of the amount will eventually make a transfer to the non-exchangeable volume. When lead reaches the non-exchangeable volume, it must wait for bone resorption in order to return to plasma. In the Leggett model, turnover rates of 3% per year and 18% per year are assigned to cortical and trabecular bone, respectively. O'Flaherty<sup>5</sup> considered a physiologically based toxicokinetic model in the growing rat, where the model was capable of integrating exposure over time by considering growth, development, and aging. In this model, age was considered as a main factor that controls lead distribution to bone and soft tissue, lead absorption from the gastrointestinal tract, lead elimination, and transfers of lead between plasma and bone. In the O'Flaherty model<sup>5</sup>, turnover rates were fixed at 4.4% per year for cortical bone and 32.5% per year for trabecular bone. Jose Brito's work<sup>7</sup> for the first time showed that the rates of lead removal from cortical bone decrease significantly with increasing exposure, where the trend was observed to be less obvious for the rate of lead removal from trabecular bone.

The goal of this work is to study the variations of lead transfer rates with the level of occupational exposure to lead including tibia and calcaneus lead level and CBLI, age, and employment time by taking into consideration (i) tibia and calcaneus lead values as independent variables and (ii) bone lead concentrations as intermediate dependent variables. To validate our observation, we found the function for the transfer rates of lead from bone to blood based on 1994 results, and then the transfer rates were used to estimate the bone lead concentration values in 1999 and 2008, and finally, the calculated and measured values of bone lead concentrations were compared.

## **2. Methodology:**

### **2.1 Study Groups**

The group of study consisted of the smelter employees from New Brunswick who have participated in longitudinal studies performed in 1994, 1999, and 2008.<sup>8-10</sup> For the 211

employees who were common between the three groups, the respective mean (SD) of tibia and calcaneus was 27.62 (18.57) and 41.70 (29.26)  $\mu\text{g Pb / g- bone mineral}$  in 2008; the ones in 1999 were found to be 37.84 (29.68) and 76.07 (49.47), and finally the bone lead values in 1994 were found to be 37.33 (29.04) and 61.25 (54.74)  $\mu\text{g Pb / g- bone mineral}$  respectively. The mean change in CBLI between the two lead measurements in 2008-1999 and 1999-1994 were 151.7 and 110.7  $\mu\text{g yr dl}^{-1}$  respectively. For those participants who have taken part in all three studies two bone lead uncertainty values in 1999 and 1994 were missing; therefore, the modeling study was performed for 209 of employees, most of whom were males, with their year of birth between 1923 and 1971.

In all three studies the concentration of lead in tibia and calcaneus was measured using the in vivo  $^{109}\text{Cd}$  K X-ray fluorescence technique.<sup>11</sup> The volunteer or a calibration standard is typically irradiated for half an hour and the generated photons are collected as a number of counts by the germanium detectors located behind the source in the backscatter geometry.<sup>12</sup> In the 2008 study since the clover-leaf geometry system<sup>13</sup> was used to perform bone measurements, the MDL was improved on average for tibia and calcaneus by a factor of 3.1 compared to the 1999 and 1994 surveys in which a conventional system (one detector) was used.<sup>10</sup> Also blood lead levels are available from 1967 onward, depending on exposure history from once per month to once per year. The blood lead levels were used to calculate cumulative blood index (CBLI) which is defined as integration of blood levels at time  $t$  over the total exposure time. The results of 2008 study indicated a non-linearity in the uptake of Pb by bone from blood with those hired earliest showing the steepest slopes.<sup>14</sup>

## 2.2 Reduced Model

Lead has a long residence time in bone and up to 90% (adults) to 70% (children) of the absorbed lead accumulates in bone.<sup>23</sup> Two widely used models of lead (Pb) metabolism which were developed by Leggett<sup>6</sup> and O'Flaherty<sup>5</sup> agree in identifying bone as the tissue compartment in which the overwhelming proportion of the mass of Pb is stored in the body. Therefore, in this study we used a reduced model including three compartments i.e. blood (B: as transfer compartment), cortical bone represented by tibia (Ti) and trabecular bone represented by calcaneus (Cal) to represent the kinetics of lead in the human body (Figure 1). In this model, lead is absorbed by blood from external sources through ingestion and inhalation. Blood then exchanges the material with bones and eliminates a significant percentage of lead through the excreta. Similar to the most widely used physiologically based models for lead in humans, the rate of change of the mass of lead in blood ( $\text{dB}/\text{dt}$ ), trabecular bone ( $\text{dCal}/\text{dt}$ ), and cortical bone ( $\text{dTi}/\text{dt}$ ) in mg per unit time is expressed by the first-order kinetic equations as follows:

$$\begin{aligned}\frac{\text{dB}}{\text{dt}} &= (\text{I} + \lambda_{\text{TB}}\text{Ti} + \lambda_{\text{CB}}\text{Cal}) - (\lambda_{\text{BT}}\text{B} + \lambda_{\text{BC}}\text{B} + \lambda_{\text{0}}\text{B}) = (\text{I} + \lambda_{\text{TB}}\text{Ti} + \lambda_{\text{CB}}\text{Cal}) - \lambda_{\text{B}}\text{B} \\ \frac{\text{dTi}}{\text{dt}} &= \lambda_{\text{BT}}\text{B} - \lambda_{\text{TB}}\text{Ti} \\ \frac{\text{dCal}}{\text{dt}} &= \lambda_{\text{BC}}\text{B} - \lambda_{\text{CB}}\text{Cal}\end{aligned}$$



Where, the parameters  $I$ ,  $\lambda_0$ ,  $\lambda_{BT}$  ( $\lambda_{BC}$ ),  $\lambda_{TB}$  ( $\lambda_{CB}$ ) present lead intake ( $\text{mg}\cdot\text{day}^{-1}$ ), lead removal from blood to the excreta and soft tissue ( $\text{day}^{-1}$ ), the fractional removal of lead from blood to tibia (calcaneus), and the fractional removal of lead from tibia (calcaneus) to blood respectively ( $\text{day}^{-1}$ ). Also,  $B$ ,  $Ti$ , and  $Cal$  are the masses of lead in blood, tibia representing cortical bone, and calcaneus representing trabecular bone respectively (mg). The total bone lead level (mg) can be calculated by assuming an average of 2000g cortical bone, 500 g trabecular bone<sup>15</sup> and 54 dl blood in an adult human body.<sup>16</sup>

Euler's method<sup>17</sup> was adopted to estimate the transfer rate parameters. The equations were imported to Matlab to estimate the initial values of kinematic parameters by using a grid search chi-squared minimization method.

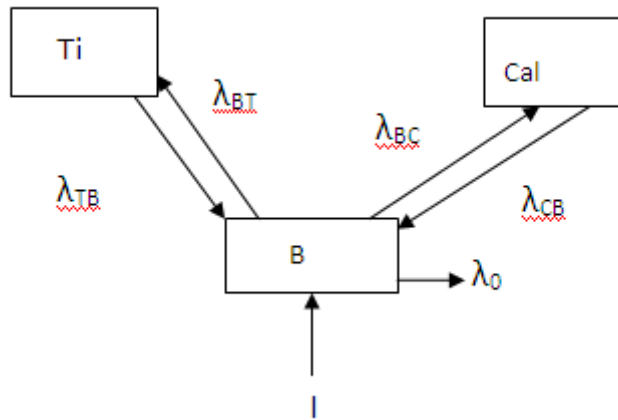


Figure 1: Metabolic model for lead and its pathways: exchanges between blood and bone

## 2.3 Statistical Methods

### 2.3.1 Mallows' $C_p$ statistic test

Mallows'  $C_p$  statistic test is used to assess the goodness of a fit. For a subset model with  $p$  explanatory variables,  $C_p$  is defined as:

$$C_p = (\text{SSE}_p/s^2) - (n-2p)$$

Where  $s^2 = \text{MSE}$  for the whole model, and  $\text{SSE}_p$  is the residuals sum of squares for the subset model containing  $p$  explanatory variables including the intercept. Usually  $C_p$  is plotted against  $p$  for the collection of subset models of various sizes under consideration. Acceptable models in the sense of minimizing the total bias of the predicted values are

those models for which  $Cp$  approaches the value  $p$ , where  $p$  is defined as a number of variables plus one for intercept.<sup>18</sup>

### 2.3.2 Euler's Method

Euler's method<sup>17</sup> is one of the simplest techniques for solving a differential equation. For a first order differential equation as:

$$x'(t) = g(x(t)), \quad x(t_0) = x_0, \quad t_0 \leq t \leq b.$$

By dividing the interval  $[t_0, b]$  into  $N$  equal subintervals, the size of each subinterval (step size) is defined as:

$h = (b - t_0) / N$ . This step size defines the node points  $t_0, t_1, t_2, \dots, t_N$ , where  $t_j = t_0 + jh$ . By substituting the step size value into the first order differential equation, we have:  $x(t + h) = x(t) + hg(x(t))$ , where, Euler's method approximates  $x'(t)$  by  $(x(t + h) - x(t)) / h$ . For  $t = t_0 + n \times h$ , we obtain:  
 $x[t_0 + (n + 1)h] = x(t_0 + n \times h) + hg[x(t_0 + n \times h)]$

for  $n = 0, 1, 2, \dots, N-1$ , by replacing  $x(t_0 + n \times h)$  by  $x(n)$  we have:

$$x(n+1) = x(n) + hg[x(n)].$$

In this study, Euler's model can be used to solve the first order differential equations set out in section 2.2. Considering three measurement time in 1994, 1999, and 2008, the differential equations can be written as (e.g. tibia (mg)):

$$Ti_{2008} = Ti_{1999} + (2008 - 1999) \times 365 \times (\lambda_{BT}B - \lambda_{TB}Ti)_{1999}$$

$$Ti_{1999} = Ti_{1994} + (1999 - 1994) \times 365 \times (\lambda_{BT}B - \lambda_{TB}Ti)_{1994}$$

### 2.3.3 Grid Search Method

In the grid search method that we applied to initiate the kinematic parameters, a measure of goodness of fit is determined by evaluating the chi-square ( $\chi^2$ ), which is defined as:<sup>19</sup>

$$\chi^2 = \sum \{(B_i - B(x_i)) / \sigma_i\}^2$$

Where  $B_i$  is the measured blood lead level and  $B(x_i)$  is the calculated blood lead level which depend on 6 variables ( $a_j$ )  $\lambda_{TB}$ ,  $\lambda_{CB}$ ,  $\lambda_{BT}$ ,  $\lambda_{BC}$ ,  $I$ , and  $\lambda_0$ . Also,  $\sigma_i$  are the

uncertainties in the data points  $B_i$ . In the grid search method for minimizing  $\chi^2$  the procedure is as follow:

- (i) One of the variables  $a_j$  is incremented by a quantity  $\Delta a_j$  in a way that  $\chi^2$  decreases.
- (ii) The parameter  $a_j$  is incremented by the same step size until  $\chi^2$  starts to increase.
- (iii) Assuming the variation of  $\chi^2$  near the minimum can be described in terms of a parabolic function of the parameter  $a_j$ , the minimum of the parabola is assigned for the parameter  $a_j$ .
- (iv)  $\chi^2$  is minimized for each parameter in turn.
- (v) The above procedure is repeated until the last iteration yields a negligibly small decrease in  $\chi^2$ .

In the case that we have here, since we are dealing with 6D kinetic parameters, it is important to determine a good range of values with which the grid search of such parameters should start.

The nature of the measures of uncertainty can greatly influence the sensitivity of the grid search method to define the goodness of the fit to data. For tibia and calcaneus lead concentration, the uncertainty values are originally derived as a variance of the number of counts of the Pb  $K_\alpha$  x-rays, the Pb  $K_\beta$  x-rays and the 88 keV  $\gamma$ -rays that have been coherently scattered back from the sample into the detector. However, for blood lead levels, the uncertainty values have not been assigned as clearly as for bone lead concentration and have been considered as the standard deviation of the blood lead masses for the whole group of participants ( $9.35 \mu\text{g dl}^{-1}$ ).

### 2.3.4 Two-Way ANOVA

The relationship between bone lead concentration and CBLI relies on exposure level, that implies, the higher the value of CBLIs are the higher the transfer rate of lead from blood to bone is.<sup>14</sup> Hence, the kinematic parameters can rely on body lead content (BLC). BLC is defined as a sum of tibia, calcaneus, and blood lead masses. Table 1 presents the subgroups of the study where Euler's model was modified based on 2008/1999, 1999/1994, and 2008/1994 (assuming that no measurement had been done in 1999). In order to investigate how kinematic parameter are influenced by BLC, and also, to find out how they are affected by these three time points (time intervals carry the information related to participants' age and employment time), a two-way ANOVA technique was used. The main advantages of using a two-way ANOVA method is the model's efficiency which means that with a simultaneous analysis of the two variables, we carry out two separate research studies concurrently. Also, the model enables us to investigate the interaction of independent variables presented in the model. The parameters of two-way ANOVA are defined as:<sup>20</sup>

- **Main Effect** which involves in the mean of the predictors at a same time, where the interaction is ignored for this part.

- **Interaction Effect** which is the effect that one factor has on the other one (BLC  $\times$  time intervals).

By defining the BLC as the rows variable (typically identified as J), and time intervals as the column variable (typically identified as K), the null hypotheses of the two ways ANOVA are summarized as following:

- The population means of the row factors (BLC) are equal  
H0:  $\mu_1 = \mu_2 = \dots = \mu_J$ .
- The population means of the column factor (time intervals) are equal.  
H0:  $\mu_{.1} = \mu_{.2} = \dots = \mu_{.K}$
- There is no interaction between the two factors.  
H0: all  $(\mu_{JK} - \mu_{.J} - \mu_{.K} + \mu) = 0$

The assumptions underlying the two-way ANOVA are summarized as:

- Assumptions of independence: the samples are independent, random samples from defined populations.
- Assumptions of normality: the scores on the dependent variable are normally distributed in the population.
- The assumption of homogeneity of variance: the population variances in all cells of the factorial design are equal.
- The general case for two way ANOVA is summarized in the table below:

### Summary ANOVA: The General Case for Two-way ANOVA

| Source of Variation | Sum of Squares (SS) | Degrees of Freedom (df) | Variance Estimate (Mean Square, MS)        | F Ratio                |
|---------------------|---------------------|-------------------------|--|------------------------|
| Rows                | $SS_J$              | $J - 1$                 | $MS_J = \frac{SS_J}{J - 1}$                | $\frac{MS_J}{MS_W}$    |
| Columns             | $SS_K$              | $K - 1$                 | $MS_K = \frac{SS_K}{K - 1}$                | $\frac{MS_K}{MS_W}$    |
| Interaction         | $SS_{JK}$           | $(J - 1)(K - 1)$        | $MS_{JK} = \frac{SS_{JK}}{(J - 1)(K - 1)}$ | $\frac{MS_{JK}}{MS_W}$ |
| Within-Cells        | $SS_W$              | $JK(n - 1)$             | $MS_W = \frac{SS_W}{JK(n - 1)}$            |                        |
| Total               | $SS_T$              | $N - 1$                 |  |                        |

<http://classweb.gmu.edu/dwilsonb/handouts/Understanding%20the%20Two-way%20ANOVA.pdf><sup>21</sup>

### 3. Results and Analysis

#### 3.1. Developing the model for $\lambda_{TB}$ based on employment time, age, body content, and their interaction

Based on the information presented in section 2.3.4, for each one of the time intervals, three levels for body lead content were considered including: high (BLC<sub>1</sub>), moderate (BLC<sub>2</sub>), and low (BLC<sub>3</sub>), where for each level two samples exists (table 1). The main difference between the two samples is related to the participants' year of hiring (retired employees; employees who were hired before 1976; employees with the hiring year between 1977 and 1991, and the ones who were hired after 1992<sup>10</sup>). The average  $\pm$  standard deviation of the first sample\* (second sample\*\*) of BLC<sub>1</sub> based on 2008, 1999, 1994 measurements were calculated to be  $163.23 \pm 48.52$  ( $129.67 \pm 37.29$ ),  $240.10 \pm 71.66$  ( $205.51 \pm 65.24$ ), and  $236.33 \pm 75.50$  ( $203.40 \pm 61.10$ ) mg respectively, and those of BLC<sub>2</sub> were found to be  $73.67 \pm 16.42$  ( $67.26 \pm 15.96$ ),  $119.70 \pm 42.67$  ( $90.47 \pm 34.18$ ), and  $114.57 \pm 41.1$  ( $96.79 \pm 33.14$ ) mg, and finally, those for BLC<sub>3</sub> were established to be  $39.90 \pm 10.83$  ( $29.62 \pm 9.67$ ),  $46.15 \pm 11.67$  ( $41.48 \pm 24.18$ ), and  $37.47 \pm 18.59$  ( $28.27 \pm 21.52$ ) mg respectively. Table 2 shows the results for two – way ANOVA, which indicates that  $\lambda_{TB}$  and  $\lambda_{CB}$  are influenced by BLCs and time interval but not their interaction.

**Table 1: This table shows the values of  $\lambda_{TB}$  ( $\lambda_{CB}$ ) for three time points namely T<sub>1</sub> (2008/1999), T<sub>2</sub> (2008/1994), T<sub>3</sub> (1999/1994). For each time point and its corresponding BLC two samples exists in which participants have been distributed approximately uniformly based on their age and BLC.**

| Intervals | BLC <sub>1</sub>                  | BLC <sub>2</sub>                | BLC <sub>3</sub>                |
|-----------|-----------------------------------|---------------------------------|---------------------------------|
| T1        | 4.60E-05 (1.00E-04)*<br>(n = 27)  | 6.80E-05 (1.37E-04)<br>(n = 40) | 6.70E-05 (1.56E-04)<br>(n = 39) |
|           | 5.60E-05 (1.07E-04)**<br>(n = 29) | 7.31E-05 (1.51E-04)<br>(n = 33) | 6.20E-05 (1.37E-04)<br>(n = 41) |
| T2        | 6.80E-05 (1.05E-04)<br>(n = 27)   | 9.60E-05 (1.58E-04)<br>(n = 40) | 1.09E-04 (2.12E-04)<br>(n = 39) |
|           | 8.30E-05 (1.07E-04)<br>(n = 29)   | 1.17E-04 (1.59E-04)<br>(n = 33) | 1.71E-04 (3.66E-04)<br>(n = 41) |
| T3        | 3.70E-05 (5.70E-05)<br>(n = 27)   | 3.00E-05 (1.38E-04)<br>(n = 40) | 5.80E-05 (2.06E-04)<br>(n = 39) |
|           | 3.40E-05 (5.80E-05)<br>(n = 29)   | 5.60E-05 (1.02E-04)<br>(n = 33) | 6.70E-05 (1.75E-04)<br>(n = 41) |

**Table 2: ANOVA table for  $\lambda_{TB}$  and  $\lambda_{CB}$** 

| Source of Variance | df | $\lambda_{TB}$ |          | $\lambda_{CB}$ |         |
|--------------------|----|----------------|----------|----------------|---------|
|                    |    | F              | P-Value  | F              | P-Value |
| Rows               | 2  | 19.63          | 0.000522 | 4.55           | 0.0430  |
| Columns            | 2  | 6.12           | 0.0210   | 14.63          | 0.00148 |
| Interaction        | 4  | 1.37           | 0.318    | 2.09           | 0.165   |
| Within – cells     | 9  |                |          |                |         |
| Total              | 17 |                |          |                |         |

The information of table 1 was considered as initial estimates of the kinematic parameters, and in the next step, the association of  $\lambda_{TB}$  ( $\lambda_{CB}$ ) and the related predictors were investigated. In the model, employment time ( $\Delta t$ ) is defined as the difference between hiring year and the year of bone lead measurement (i.e. 2008, and 1999). The influence of BLCs on transfer rates were studied exclusively as tibia lead (mg), calcaneus lead (mg), and CBLI (mg  $\times$  year). The model was tested, by calculating tibia lead levels in 1999 and 2008 and comparing those values with the measured values. The possible predictors of the multivariate model with their efficiency ( $R^2$  and  $C_p$ ) for  $\lambda_{TB}$  have been tabulated in table 3 (age and employment time have been considered and body lead levels are based on the measurements performed in 1994). Based on the results of table 3, the model with five variables was found out to be our best option since (i) the value of  $C_p$  (5.6) is close enough to  $p$  (= 6) (ii) the value of the coefficient of determination is also another indicator for choosing a good fit, and finally (iii) the number of predictors is as small as possible which keeps the model simple. The summary output of the selected model has been shown in table 4. Other alternatives to find a fitting equation for  $\lambda_{TB}$  were managed by considering different time intervals (i.e. 2008/1999 and 1999/1994) and by finding the body lead levels in different possible time points (i.e. 2008, 1999, and 1994) (table 5).

**Table 3: This table presents the best fit subsets for  $\lambda_{TB}$  based on 1994 measurements.**

| Var | R <sup>2</sup><br>(%) | C <sub>p</sub> | A<br>g<br>e | Δt | Age×<br>(Ti+Cal) | Δt ×CBLI | Ti | Age×Δt | CBLI <sup>2</sup> | Cal | CBLI | Ti <sup>2</sup> | Age×<br>CBLI | Cal <sup>2</sup> |
|-----|-----------------------|----------------|-------------|----|------------------|----------|----|--------|-------------------|-----|------|-----------------|--------------|------------------|
| 1   | 66.4                  | 96.0           |             | X  |                  |          |    |        |                   |     |      |                 |              |                  |
| 1   | 62.8                  | 128.2          |             |    |                  |          |    |        |                   |     | X    |                 |              |                  |
| 2   | 71.4                  | 52.8           |             | X  |                  |          | X  |        |                   |     |      |                 |              |                  |
| 2   | 70.9                  | 57.7           |             | X  |                  | X        | X  |        |                   |     | X    |                 |              |                  |
| 3   | 74.3                  | 29.1           |             | X  |                  |          |    |        |                   | X   | X    |                 |              |                  |
| 3   | 72.4                  | 45.7           |             | X  | X                | X        |    |        |                   | X   |      |                 |              |                  |
| 4   | 77.0                  | 6.6            | X           | X  | X                |          |    |        |                   | X   | X    |                 |              |                  |
| 4   | 76.6                  | 10.9           |             | X  |                  | X        |    |        |                   | X   | X    |                 |              |                  |
| 5   | 77.4                  | 5.6            |             | X  | X                | X        |    |        |                   |     | X    | X               |              |                  |
| 5   | 77.1                  | 7.6            |             | X  | X                | X        | X  |        |                   |     | X    |                 |              |                  |
| 6   | 77.7                  | 5.0            | X           | X  | X                | X        |    | X      |                   |     |      |                 | X            |                  |
| 6   | 77.5                  | 6.4            | X           | X  | X                | X        | X  |        | X                 |     | X    | X               |              |                  |
| 7   | 78.0                  | 3.9            | X           | X  |                  |          | X  |        | X                 | X   |      | X               | X            |                  |
| 7   | 77.7                  | 6.3            | X           | X  | X                | X        |    | X      |                   |     | X    |                 | X            |                  |
| 8   | 78.1                  | 5.4            | X           | X  | X                | X        |    | X      |                   |     | X    | X               | X            |                  |
| 8   | 78.0                  | 5.8            | X           | X  | X                | X        |    | X      |                   | X   |      | X               | X            |                  |
| 9   | 78.1                  | 7.1            | X           | X  | X                | X        | X  | X      |                   |     | X    | X               | X            |                  |
| 9   | 78.1                  | 7.2            | X           | X  | X                | X        | X  | X      |                   |     | X    | X               | X            |                  |
| 10  | 78.1                  | 9.0            | X           | X  | X                | X        | X  | X      | X                 |     | X    | X               | X            | X                |
| 10  | 78.1                  | 9.0            | X           | X  | X                | X        | X  | X      | X                 |     | X    | X               | X            | X                |
| 11  | 78.1                  | 11.0           | X           | X  | X                | X        | X  | X      | X                 |     | X    | X               | X            | X                |
| 11  | 78.1                  | 11.0           | X           | X  | X                | X        | X  | X      | X                 | X   | X    | X               | X            | X                |
| 12  | 78.1                  | 13.0           | X           | X  | X                | X        | X  | X      | X                 | X   | X    | X               | X            | X                |

\*The unit for Ti, Cal is in mg and for CBLI is in mg.yr

\* Age is in yrs

\*Δt is in yrs (2008- year of hiring)

**Table 4: Summary output between the variables  $\lambda_{TB}$  and the predictors. According to the P values all of the predictors are statistically significant.**

| <i>Regression Statistics</i> |                     |                       |                 |                |
|------------------------------|---------------------|-----------------------|-----------------|----------------|
| Multiple R                   | 0.880               |                       |                 |                |
| R Square                     | 0.774               |                       |                 |                |
| Adjusted R Square            | 0.769               |                       |                 |                |
| Standard Error               | 1.60E-05            |                       |                 |                |
| Observations                 | 209                 |                       |                 |                |
|                              | <i>df</i>           | <i>SS</i>             | <i>MS</i>       | <i>F</i>       |
| Regression                   | 5                   | 1.79E-05              | 3.57E-08        | 139.30         |
| Residual                     | 203                 | 5.20E-08              | 2.56E-10        |                |
| Total                        | 208                 | 2.31E-07              |                 |                |
|                              | <i>Coefficients</i> | <i>Standard Error</i> | <i> t  Stat</i> | <i>P-value</i> |
| Intercept                    | 2.33E-04            | 7.57E-06              | 30.80           | <0.0001        |
| $\Delta t$                   | -3.80E-06           | 3.39E-07              | 11.16           | <0.0001        |
| $Ti^2$                       | 5.23E-10            | 2.79E-10              | 1.98            | 0.0491         |
| $\Delta t \times CBLI$       | 4.11E-08            | 5.39E-09              | 7.636           | <0.0001        |
| CBLI                         | -1.20E-06           | 2.00E-07              | 5.92            | <0.0001        |
| age (Ti+Cal)                 | -4.00E-09           | 1.05E-9               | 3.79            | 0.000196       |

**Table 5: This table presents the coefficient of the multivariate regression model of  $\lambda_{TB}$  for all of the possible alternatives**

| Time intervals                            | 2008/1994                         | 2008/1994                         | 2008/1999                         | 2008/1999                         | 1999/1994                         | 1999/1994                         |
|---|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| Body lead level<br>year of<br>measurement | 1994                              | 2008                              | 1999                              | 2008                              | 1999                              | 1994                              |
| $R^2$                                     | 0.774                             | 0.793                             | 0.601                             | 0.652                             | 0.705                             | 0.706                             |
| Intercept                                 | $(2.33 \pm 0.76) \times 10^{-4}$  | $(2.53 \pm 0.86) \times 10^{-4}$  | $(5.36 \pm 0.27) \times 10^{-5}$  | $(4.90 \pm 0.30) \times 10^{-5}$  | $(8.51 \pm 0.26) \times 10^{-5}$  | $(8.31 \pm 0.24) \times 10^{-5}$  |
| $\Delta t$                                | $-(3.80 \pm 0.34) \times 10^{-6}$ | $-(4.35 \pm 0.36) \times 10^{-6}$ | $(6.29 \pm 1.27) \times 10^{-7}$  | $(6.91 \pm 1.24) \times 10^{-7}$  | $-(1.50 \pm 0.18) \times 10^{-6}$ | $-(1.50 \pm 0.60) \times 10^{-6}$ |
| age (Ti+Cal)                              | $-(4.0 \pm 1.1) \times 10^{-9}$   | $-(7.0 \pm 1.6) \times 10^{-9}$   | $-(1.30 \pm 0.33) \times 10^{-9}$ | $-(2.60 \pm 0.55) \times 10^{-9}$ | $-(1.34 \pm 0.53) \times 10^{-9}$ | $-(1.50 \pm 0.60) \times 10^{-9}$ |
| $\Delta t \times CBLI$                    | $(4.11 \pm 0.54) \times 10^{-8}$  | $(4.08 \pm 0.50) \times 10^{-8}$  | $-(1.03 \pm 0.17) \times 10^{-8}$ | $-(1.10 \pm 0.17) \times 10^{-8}$ | $(1.33 \pm 0.26) \times 10^{-8}$  | $(1.44 \pm 0.27) \times 10^{-8}$  |
| CBLI                                      | $-(1.20 \pm 0.20) \times 10^{-6}$ | $-(1.14 \pm 0.19) \times 10^{-6}$ | $(3.74 \pm 0.67) \times 10^{-7}$  | $(4.39 \pm 0.64) \times 10^{-7}$  | $-(3.41 \pm 0.79) \times 10^{-7}$ | $-(3.40 \pm 0.82) \times 10^{-7}$ |
| $Ti^2$                                    | $(5.23 \pm 2.79) \times 10^{-10}$ | $(1.10 \pm 0.58) \times 10^{-9}$  | $(3.64 \pm 8.18) \times 10^{-11}$ | $(1.96 \pm 1.99) \times 10^{-10}$ | $(1.95 \pm 1.11) \times 10^{-10}$ | $(2.08 \pm 1.33) \times 10^{-10}$ |



### 3.1.1 Testing the proposed model for $\lambda_{TB}$ :

To test the model, tibia, calcaneus, and blood lead levels in 1994 were assumed to be known, and then by using the Euler's equation (eq. 1) the tibia lead levels in any required intermediate year (t) were calculated. The measured lead values in 1994 were employed into the equation in (mg), and at the end to compare the results with the measured lead values in 1999 and 2008 the values were transferred back into  $\mu\text{g-Pb/g-bone mineral}$  (figure 2).

$$Ti_t = Ti_{1994} + (t - 1994)(\lambda_{BT}B_{1994} - \lambda_{TB}Ti_{1994}) \quad \text{eq. 1}$$

The association of the measured tibia lead concentration ( $Ti_m$ ) and the calculated lead level ( $Ti_c$ ) has been expressed by the following equations:

$$Ti_{c(1999)} = (0.806 \pm 0.022) Ti_{m(1999)} + (1.56 \pm 1.08) \quad (R^2 = 0.860; n = 209)$$

$$Ti_{c(2008)} = (0.988 \pm 0.037) Ti_{m(1999)} - (2.24 \pm 1.23) \quad (R^2 = 0.775; n = 209)$$

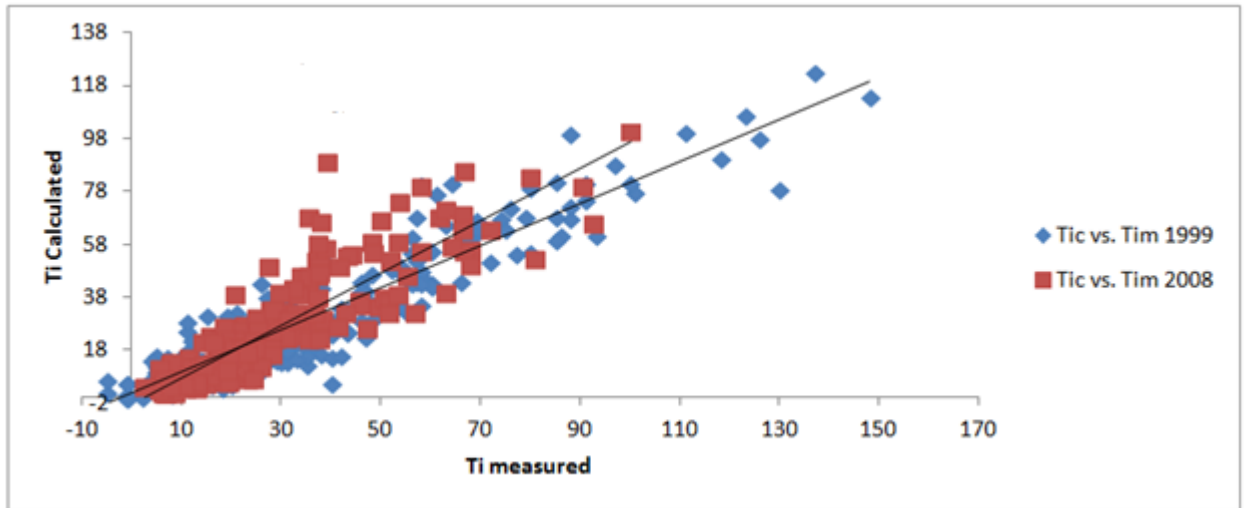


Figure 2: This figure shows a comparison between lead values and calculated ( $Ti_c$ ) and measured concentrations ( $Ti_m$ ) in 2008 and 1999.

### 3.2. Developing the model for $\lambda_{CB}$ based on employment time, age, body lead levels, and their interaction

A similar approach that was taken to develop a model for  $\lambda_{TB}$  was considered to find the predictors that can best associate with the transfer rate of lead from calcaneus to blood ( $\lambda_{CB}$ ). Table 6 shows the summary output of the best subset that was considered for  $\lambda_{CB}$  based on 1994 body lead contents measurement. For other alternatives time interval, the regression equations have been presented in table 7.

**Table 6: Summary output between the variables  $\lambda_{CB}$  and the predictors with  $C_p$  value of 10.4 (close enough to  $p=10$ ).**

| <i>Regression Statistics</i> |                     |                       |                 |                |
|------------------------------|---------------------|-----------------------|-----------------|----------------|
| Multiple R                   |                     | 0.878                 |                 |                |
| R Square                     |                     | 0.771                 |                 |                |
| Adjusted R Square            |                     | 0.761                 |                 |                |
| Standard Error               |                     | 4.49E-05              |                 |                |
| Observations                 |                     | 209                   |                 |                |
| <i>ANOVA</i>                 |                     |                       |                 |                |
|                              | <i>df</i>           | <i>SS</i>             | <i>MS</i>       | <i>F</i>       |
| Regression                   | 9                   | 1.35E-06              | 1.50E-07        | 74.52          |
| Residual                     | 199                 | 4.01E-07              | 2.01E-09        |                |
| Total                        | 208                 | 1.75E-06              |                 |                |
|                              | <i>Coefficients</i> | <i>Standard Error</i> | <i> t  Stat</i> | <i>P-value</i> |
| Intercept                    | 1.05E-03            | 1.12E-04              | 9.39            | <0.0001        |
| Age                          | -1.09E-05           | 2.24E-06              | 4.88            | <0.0001        |
| $\Delta t$                   | -4.11E-05           | 6.05E-06              | 6.80            | <0.0001        |
| Age $\times$ $\Delta t$      | 6.63E-07            | 1.02E-07              | 6.49            | <0.0001        |
| Ti                           | -7.29E-07           | 2.70E-07              | 2.71            | 0.00741        |
| CBLI                         | 6.84E-06            | 1.92E-06              | 3.56            | <0.001         |
| Cal                          | -5.89E-07           | 3.38E-07              | 1.75            | 0.08252        |
| Ti <sup>2</sup>              | 1.85E-09            | 9.27E-10              | 2.00            | 0.0468         |
| CBLI <sup>2</sup>            | 3.79E-08            | 1.21E-08              | 3.12            | 0.00205        |
| Age $\times$ CBLI            | -1.90E-07           | 4.31E-08              | 4.41            | <0.0001        |

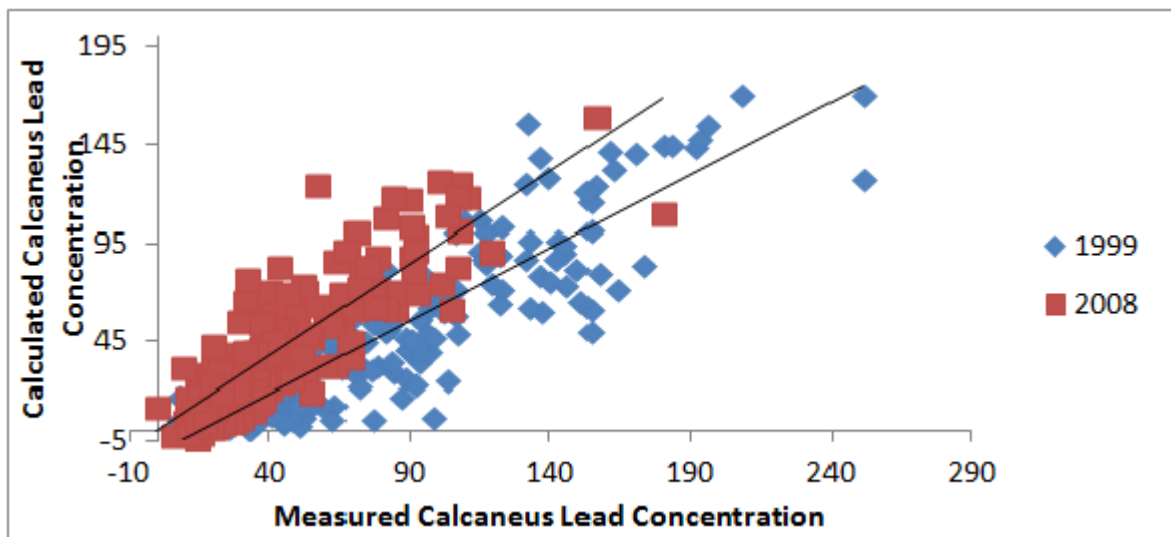
By using the information presented in table 7 for the time interval 2008/1994 (measured body lead levels in 1994), the value of calcaneus lead levels were calculated for 2008 and 1999 by using the Euler's equation, and the values were compared with the measured calcaneus lead values (figure 3). The relationship between the measured ( $Cal_m$ ) and calculated calcaneus lead ( $Cal_c$ ) concentration were defined by the following equations which show a significant agreement between the measured and calculated lead values.

$$Cal_{c\ 2008} = (0.938 \pm 0.038) Cal_{m\ 2008} - (0.139 \pm 1.930) \quad (R^2 = 0.749; n = 209)$$

$$Cal_{c\ 1999} = (0.743 \pm 0.025) Cal_{m\ 1999} - (11.6 \pm 2.3) \quad (R^2 = 0.810; n = 209)$$

**Table 7: This table presents the coefficient of the multivariate regression model of  $\lambda_{CB}$  for different time intervals**

| Time intervals                    | 2008-1994                         | 2008-1994                         | 2008-1999                         | 2008-1999                         | 1999-1994                         | 1999-1994                         |
|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| Body lead level measurement point | 1994                              | 2008                              | 1999                              | 2008                              | 1999                              | 1994                              |
| $R^2$                             | 0.771                             | 0.795                             | 0.680                             | 0.718                             | 0.673                             | 0.756                             |
| Intercept                         | $(1.05 \pm 0.11) \times 10^{-3}$  | $(9.25 \pm 0.77) \times 10^{-4}$  | $(2.48 \pm 2.59) \times 10^{-5}$  | $(7.04 \pm 2.09) \times 10^{-5}$  | $(4.45 \pm 7.29) \times 10^{-5}$  | $(8.55 \pm 8.27) \times 10^{-5}$  |
| Age                               | $-(1.09 \pm 0.22) \times 10^{-5}$ | $-(7.40 \pm 1.79) \times 10^{-5}$ | $(2.24 \pm 0.55) \times 10^{-6}$  | $(1.25 \pm 0.49) \times 10^{-6}$  | $(3.68 \pm 1.55) \times 10^{-6}$  | $(2.19 \pm 1.66) \times 10^{-6}$  |
| $\Delta t$                        | $-(4.11 \pm 0.60) \times 10^{-5}$ | $-(3.70 \pm 0.50) \times 10^{-5}$ | $(7.13 \pm 1.52) \times 10^{-5}$  | $(5.65 \pm 1.36) \times 10^{-6}$  | $(5.85 \pm 4.29) \times 10^{-6}$  | $(3.76 \pm 4.48) \times 10^{-6}$  |
| Age $\times$ $\Delta t$           | $(6.63 \pm 1.02) \times 10^{-7}$  | $(5.87 \pm 0.85) \times 10^{-7}$  | $-(1.20 \pm 0.26) \times 10^{-7}$ | $-(9.85 \pm 2.32) \times 10^{-8}$ | $-(7.10 \pm 7.26) \times 10^{-8}$ | $-(3.40 \pm 7.56) \times 10^{-8}$ |
| Ti                                | $-(7.29 \pm 2.70) \times 10^{-7}$ | $-(2.70 \pm 0.40) \times 10^{-6}$ | $-(2.71 \pm 0.56) \times 10^{-7}$ | $-(6.59 \pm 1.09) \times 10^{-7}$ | $-(8.00 \pm 1.59) \times 10^{-7}$ | $-(5.70 \pm 2.0) \times 10^{-7}$  |
| CBLI                              | $(6.84 \pm 1.92) \times 10^{-6}$  | $(5.82 \pm 1.47) \times 10^{-6}$  | $-(1.40 \pm 0.46) \times 10^{-6}$ | $-(5.80 \pm 3.99) \times 10^{-7}$ | $-(2.50 \pm 1.29) \times 10^{-6}$ | $-(1.10 \pm 1.43) \times 10^{-6}$ |
| Cal                               | $-(5.89 \pm 3.38) \times 10^{-7}$ | $-(5.70 \pm 3.81) \times 10^{-7}$ | $-(2.04 \pm 0.72) \times 10^{-7}$ | $-(4.40 \pm 1.04) \times 10^{-7}$ | $-(4.50 \pm 2.02) \times 10^{-7}$ | $-(1.11 \pm 0.25) \times 10^{-6}$ |
| Ti <sup>2</sup>                   | $(1.85 \pm 0.93) \times 10^{-9}$  | $(1.06 \pm 0.20) \times 10^{-8}$  | $(5.18 \pm 1.95) \times 10^{-10}$ | $(2.29 \pm 0.54) \times 10^{-9}$  | $(1.65 \pm 0.55) \times 10^{-9}$  | $(1.47 \pm 0.69) \times 10^{-9}$  |
| CBLI <sup>2</sup>                 | $(3.79 \pm 1.21) \times 10^{-8}$  | $(2.74 \pm 0.85) \times 10^{-8}$  | $-(5.02 \pm 2.82) \times 10^{-9}$ | $-(7.90 \pm 2.31) \times 10^{-9}$ | $(1.86 \pm 0.80) \times 10^{-8}$  | $(2.05 \pm 0.90) \times 10^{-8}$  |
| Age $\times$ CBLI                 | $-(1.90 \pm 0.43) \times 10^{-7}$ | $-(1.60 \pm 0.52) \times 10^{-7}$ | $(3.19 \pm 1.08) \times 10^{-8}$  | $(2.88 \pm 0.96) \times 10^{-8}$  | $-(1.41 \pm 3.04) \times 10^{-8}$ | $-(2.61 \pm 3.19) \times 10^{-8}$ |



**Figure 3: The relationship between the calculated and measured calcaneus lead concentration.**

## Discussion

Lead intoxication directly and indirectly can influence many aspects of bone cell function. The indirect effect of lead on bone cell function is through changes in the circulating levels of some hormones, particularly 1,25-dihydroxyvitamin D3, which is responsible for adjusting bone cell function. The direct influence of lead on bone cell function is by disturbing the ability of bone cells to respond to hormonal regulation. For instance, the 1,25-dihydroxyvitamin D3-stimulated synthesis of osteocalcin, which is defined as a calcium binding protein synthesized by osteoblastic bone cells, is repressed by low levels of lead. Impaired osteocalcin production may slow down new bone formation, as well as the functional coupling of osteoblasts and osteoclasts.<sup>22</sup> Therefore, at the higher bone lead concentration the formation of new bone may be delayed due to the presence of lead, and because of that lead stays in bone longer.

In the proposed models for  $\lambda_{TB}$  and  $\lambda_{CB}$ , tibia and calcaneus lead levels were considered as independent variables, and the model was developed for the applicable predictors. Setting up the K-XRF lead measurement system is very expensive and time consuming as it takes 20 to 30 minutes for each bone site to be measured. Also, for performing measurements and data analysis well trained experts are required. Therefore, it is important, if the model can be conveyed in a simpler way. For the same population of the study, the relationship between CBLI and bone lead concentration was found to be best expressed by a polynomial function.<sup>12</sup> Therefore, by defining the transfer rates of lead from bone to blood as a function of CBLI, one may consider the tibia and calcaneus lead concentration as intermediate dependent variables (figures 5-6). To do so, the values of  $\lambda_{TB}$  and  $\lambda_{CB}$  were calculated by using the information presented in tables 4-7 for each of the participants, and then the relationship of the transfer rates were investigated against CBLI (table 8). After estimating  $\lambda_{TB}$  values by using the CBLI values as a predictor, we are able to estimate tibia and calcaneus lead concentration in 2008 and 1999 having known the bone lead concentration in 1994 (eq.1). A comparison between the calculated and the measured lead values may confirm how confident we can be in using CBLI values to compute  $\lambda_{TB}$  and  $\lambda_{CB}$  values. The result of comparison has been shown in figure 4, and the relationship between the calculated and measured tibia lead concentration have been shown by the following equations:

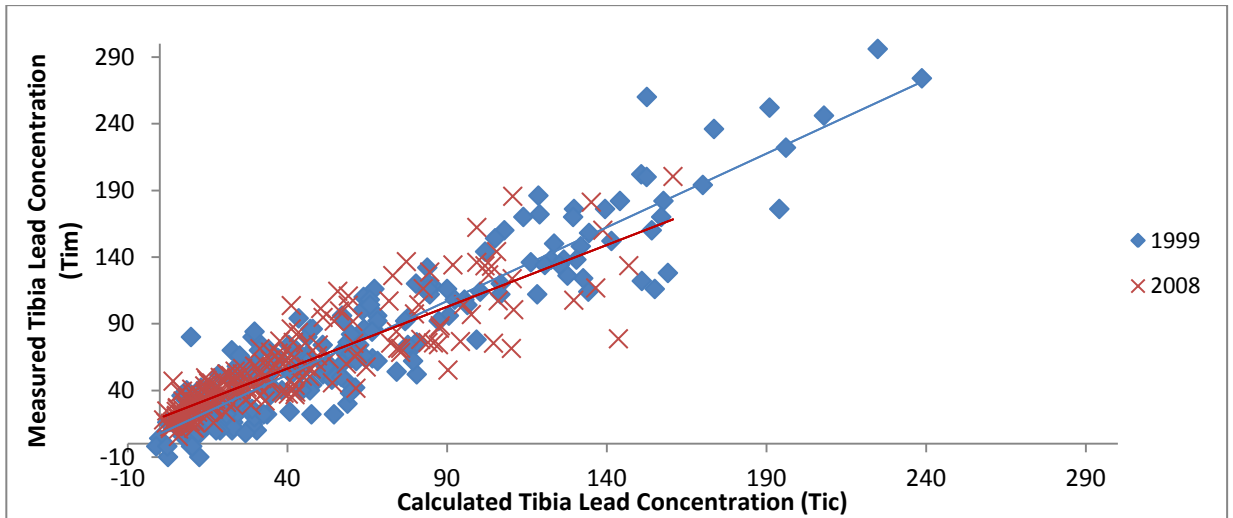
$$Ti_{C\ 2008} = (0.833 \pm 0.031) Ti_{m\ 2008} - (7.25 \pm 2.10) \quad (R^2 = 0.772; \text{units } \mu\text{g} - \text{Pb/ g- bone mineral})$$

$$Ti_{C\ 1999} = (0.800 \pm 0.022) Ti_{m\ 1999+} + (2.88 \pm 2.10) \quad (R^2 = 0.860; \text{units } \mu\text{g} - \text{Pb/ g- bone mineral})$$

**Table 8: Result of regression equations of  $\lambda_{TB}$  and  $\lambda_{CB}$  as a function of CBLI (mg.yr) for different years in 1994, 1999, and 2008**

|                | 1994   | 1999   | 2008   |
|----------------|--|--|--|
| $\lambda_{TB}$ | $(1.08 \pm 0.08) \times 10^{-8} \text{CBLI}^2 - (2.5 \pm 0.08) \times 10^{-6} \text{CBLI} + (0.000228 \pm 0.000002)$<br>$R^2 = 0.953$  | $(1.01 \pm 0.08) \times 10^{-8} \text{CBLI}^2 - (2.4 \pm 0.1) \times 10^{-6} \text{CBLI} + (0.000221 \pm 0.000002)$<br>$R^2 = 0.932$   | $(8.7 \pm 0.9) \times 10^{-9} \text{CBLI}^2 - (2.1 \pm 0.1) \times 10^{-6} \text{CBLI} + (0.000198 \pm 0.000004)$<br>$R^2 = 0.865$     |
| $\lambda_{CB}$ | $(6.35 \pm 0.56) \times 10^{-8} \text{CBLI}^2 - (1.2 \pm 0.06) \times 10^{-5} \text{CBLI} + (0.000764 \pm 0.0000014)$<br>$R^2 = 0.849$ | $(5.31 \pm 0.42) \times 10^{-8} \text{CBLI}^2 - (1.03 \pm 0.05) \times 10^{-5} \text{CBLI} + (0.000651 \pm 0.000001)$<br>$R^2 = 0.867$ | $(3.27 \pm 0.24) \times 10^{-8} \text{CBLI}^2 - (6.75 \pm 0.33) \times 10^{-6} \text{CBLI} + (0.000463 \pm 0.000009)$<br>$R^2 = 0.863$ |

We did not observe a good agreement between the measured and calculated calcaneus lead concentrations (about 50% difference between the measured and calculated lead values), while  $\lambda_{CB}$  was taken to the consideration as a function of CBLI.



**Figure 4: This figure shows the measured tibia lead level as a function of calculated one having known the measured tibia lead levels in 1994.**

Brito *et al.*<sup>7</sup> compiled the subgroups under the study according to the concentration of lead in tibia (PbT) since tibia is the best biological marker of long-term lead exposure including: subgroup A (N = 45; age: 44[27–65]; mean PbT: 34.2  $\mu\text{g Pb (g-bone mineral)}^{-1}$ ), in the battery recycling plant in Montreal. Subgroups B (N = 45; age: 38[29–55]; mean PbT: 8.9  $\mu\text{g Pb (g-bone mineral)}^{-1}$ ) and C (N = 44; age: 51[38–60]; mean PbT: 89.0  $\mu\text{g Pb (g-bone mineral)}^{-1}$ ), and  $\lambda_{TB}$  values in subgroups A, B, and C were found

to be  $(14 \pm 1) \times 10^{-5}$ ,  $(29 \pm 3) \times 10^{-5}$ , and  $(4.0 \pm 0.2) \times 10^{-5}$ , and the respective values for  $\lambda_{CB}$  were found to be  $(20 \pm 1) \times 10^{-5}$ ,  $(28 \pm 3) \times 10^{-5}$ , and  $(18.0 \pm 0.4) \times 10^{-5}$ . In our study, participants were classified based on their average tibia lead close to Brito's study. For the corresponding group to subgroup B (N = 43; mean PbTi = 9.08  $\mu\text{g Pb (g-bone mineral)}^{-1}$ ),  $\lambda_{TB}$  and  $\lambda_{CB}$  values (Avg (SD)) by using the multivariate model were found to be  $(14.8 \pm 1.6) \times 10^{-5}$  and  $(30.6 \pm 4.6) \times 10^{-5}$  respectively; those values for the equivalent group to A (N = 46; mean PbTi = 34.61  $\mu\text{g Pb (g-bone mineral)}^{-1}$ ) were found to be  $(8.78 \pm 1.02) \times 10^{-5}$  and  $(13.7 \pm 2.5) \times 10^{-5}$  respectively, and finally, the comparable ones with subgroup C (N = 8; mean PbTi = 81.60  $\mu\text{g Pb (g-bone mineral)}^{-1}$ ) were found to be  $(7.04 \pm 0.44) \times 10^{-5}$  and  $(11.1 \pm 8.2) \times 10^{-5}$  ( table 8). In both studies, the order of magnitude of lead transfer rates are the same, but in the recent study the transfer rates were found to be on average 60% lower (excluding subgroup C for  $\lambda_{TB}$  and subgroup B for  $\lambda_{CB}$ ) than those in Brito's study. The reason of discrepancy can be due to using the multivariate model to find the transfer rates of lead from bone to blood for every of the participants by considering participants' age, employment time, and body lead contents. Whereas, Brito et al. used grid search method to find the transfer rates in subgroups and studied the influence of exposure levels on transfer rates. The pattern that was suggested by Brito et al. was supported in this study as well. Figures 5-6 show that by increasing the BLC, the transfer rates are decreasing significantly.

**Table 8: Comparing the transfer rates in the current study with those in Brito's work.**

| Subgroups | Brito's Study |                                |                                 | Current Study |                                  |                                 |
|-----------|---------------|--------------------------------|---------------------------------|---------------|----------------------------------|---------------------------------|
|           | Ti (#n)       | $\lambda_{TB}$                 | $\lambda_{CB}$                  | Ti (#n)       | $\lambda_{TB}$                   | $\lambda_{CB}$                  |
| A         | 34.2 (44)     | $(14 \pm 1) \times 10^{-5}$    | $(20 \pm 1) \times 10^{-5}$     | 34.61(46)     | $(8.78 \pm 1.02) \times 10^{-5}$ | $(13.7 \pm 2.5) \times 10^{-5}$ |
| B         | 8.9 (45)      | $(29 \pm 3) \times 10^{-5}$    | $(28 \pm 3) \times 10^{-5}$     | 9.08 (43)     | $(14.8 \pm 1.6) \times 10^{-5}$  | $(30.6 \pm 4.6) \times 10^{-5}$ |
| C         | 89 (44)       | $(4.0 \pm 0.2) \times 10^{-5}$ | $(18.0 \pm 0.4) \times 10^{-5}$ | 81.60 (8)     | $(7.04 \pm 0.44) \times 10^{-5}$ | $(11.1 \pm 8.2) \times 10^{-5}$ |

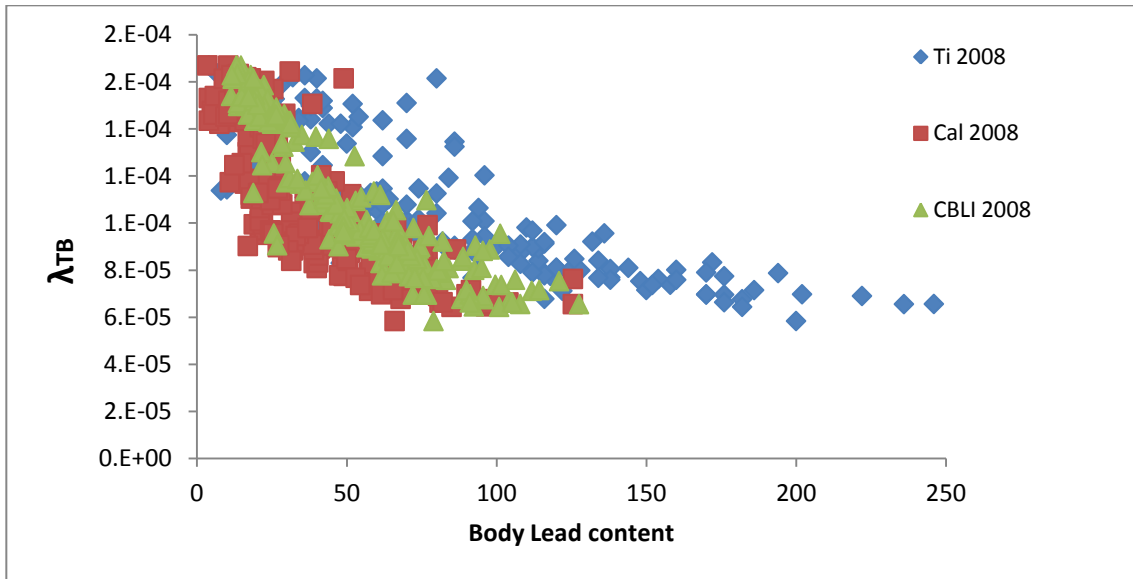


Figure 5:  $\lambda_{TB}$  as function of tibia lead (mg), calcaneus lead (mg), and CBLI (mg $\times$ year)

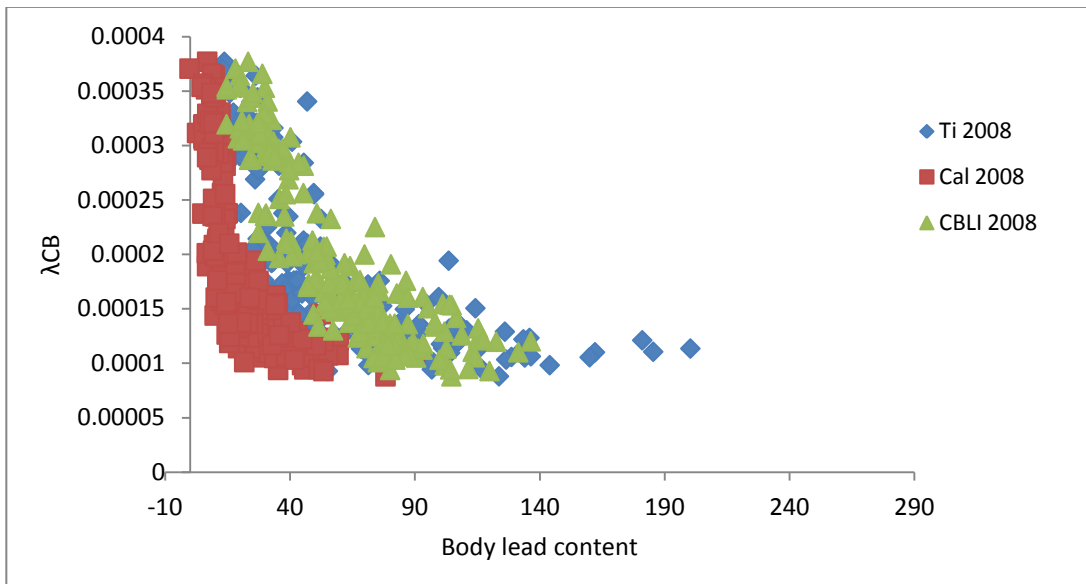
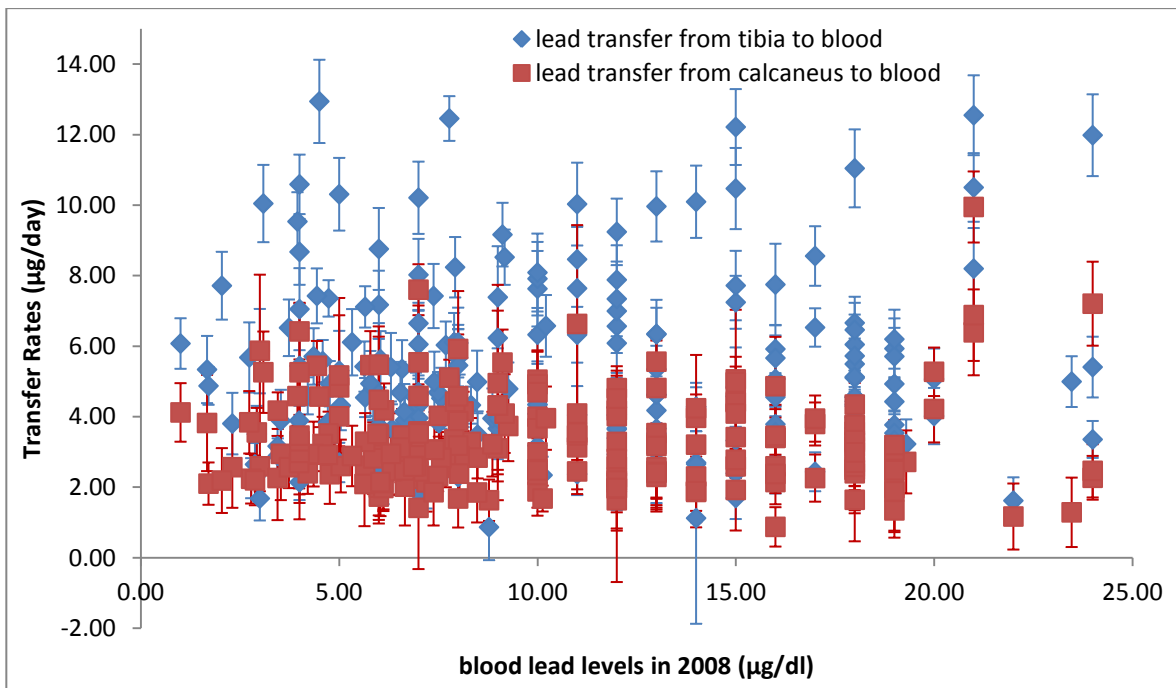


Figure 6:  $\lambda_{CB}$  as function of tibia lead (mg), calcaneus lead (mg), and CBLI (mg $\times$ year)

An overview of Rabinowitz *et al.*<sup>1</sup> was given in the introduction. The authors' study was designed for five subjects, A, B, C, D, and E. The average blood lead levels (BLL) of the subjects were reported to be 25, 18, 17, 20, and 17 $\mu$ g/dl, and the subjects were studied in the metabolic unit for 150, 210, 10, 190, and 108 days respectively. The authors considered cortical and trabecular bones as one compartment, and they found the value of

7  $\mu\text{g}/\text{day}$  for the transfer rate of lead from bone to blood. For the sake of comparison, the transfer rate values that were calculated by multivariate model were multiplied by tibia and calcaneus lead concentration, and then the resulting values were multiplied by the masses of cortical bone and trabecular bone to find the units of transfer rates in  $\mu\text{g}/\text{day}$ . The result has been shown by figure 7. The mean (SD) of the lead transfer rate from tibia and calcaneus to blood ( $\mu\text{g}/\text{day}$ ) were found to be 5.26 (2.39) and 3.34 (1.30) respectively, which values are close to the ones reported by Rabinowitz et al. Table 9 presents more detailed comparison between the two studies.



**Figure 7:** The calculated mass transfer rate values of lead in  $\mu\text{g}/\text{day}$  in 2008 as a function of blood lead levels measured in 2008.

**Table 9:** This table presents the transfer rates corresponding to the blood lead levels that were reported by Rabinowits et al.

| BLL<br>$\mu\text{g}/\text{dl}$ | n  | Mean transfer rate from tibia to blood<br>(SD)<br>$\mu\text{g}/\text{d}$ | Mean transfer rate from calcaneus to blood<br>(SD)<br>$\mu\text{g}/\text{d}$ |
|--------------------------------|----|--|--|
| 24                             | 3  | 6.92 (4.51)  | 3.98 (2.80)  |
| 20                             | 2  | 4.55 (0.74)  | 4.75 (0.75)  |
| 18                             | 15 | 5.44 (1.79)  | 3.05 (0.68)  |
| 17                             | 3  | 5.84 (3.11)  | 3.34 (0.93)  |



In conclusion, these observations reinforce the need to implement adjustments in previous studies according to the level of long term exposure, age, and employment time. The authors' next goal is to apply the model to the general population.

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## Chapter 6

### **Plasma lead, Blood lead and Bone lead concentration in the study of a cohort of the general population**

#### **6.1 Introduction**

Most of the recent studies of chronic lead exposure using K-XRF technology have mainly been performed on occupationally exposed group of workers, most of whom were male (Fleming *et al.* 1997; Brito *et al.* 2005; Gerhardsson *et al.* 2005; Behinaein *et al.* 2011). For the general population, Roy *et al.* (1996) reported tibia and calcaneus lead concentration using in vivo fluorescence excitation in 90 females and 59 males aged between 6 and 81. Also, Gamblin *et al.* (1994) reported tibia lead measurement of 73 women aged 19 – 81 and 38 men, aged 23-70 of non-occupationally exposed subjects, where nineteen of the non-occupationally exposed women also had calcaneus lead measurement.

Due to increasing scientific evidence of a wide range of potentially adverse effects of lead on human health at low levels of exposure, Health Canada initiated a rigorous review of the current toxicological and toxicokinetic data on lead in 2005. Also, as part of a comprehensive lead risk management strategy, the Government of Canada has implemented a wide range of regulatory and non-regulatory initiatives in collaboration with provincial and territorial governments, industry and other stakeholders (Risk Management Strategy for Lead 2013). Some of the examples of these regulations are included: *Secondary Lead Smelter Release Regulation* (1991) which “limit the concentration of particulate matter (PM) containing lead emitted into the ambient air”

(CEPA 1999); *Metal Mining Effluent Regulations* (updated 2006) which “establish a discharge limit of 0.2 mg/L as the maximum authorized monthly mean concentration of lead in mine effluents that discharge to waters frequented by fish” (Fisheries Act); *Gasoline Regulations* (1990) which “ limit the lead and phosphorous content of gasoline that is produced, imported or sold in Canada. Gasoline is now 99.8% lead free” (CEPA 1999); *Lead Risk Reduction Strategy for Consumer Products* which “Proposes further limits on the lead content of consumer products with which children are most likely to interact. Action has already been taken under this strategy in the form of the *Consumer Products Containing Lead (Contact with Mouth) Regulations* (2010), the *Children’s Jewellery Regulations* and amendments to the *Surface Coating Materials Regulations* (2010)”. In 2008, scientists at Health Canada (the Government of Canada’s Chemicals Management Plan at Health Canada) identified that after introducing the regulations, the extent of the presumed reduction in chronic exposure to lead was not known. In addition, there were limited data on long term or chronic lead exposure as assessed by bone lead measurements back to more than fifteen years ago. Therefore, it was important to measure the levels of current environmental exposure and to assess levels in sensitive populations, i.e., women and children. Therefore, Health Canada funded a feasibility study to measure bone, plasma and whole blood lead levels among healthy Canadian volunteers across the lifespan who were non-industrially-exposed to lead with the following age categories: pre-school (1-5 years), pre-puberty (6-10), adolescent (11-19), prime reproductive ages (20-35), ranges that in women would capture the perimenopausal

(36-50), and post-menopausal (51-64) and seniors (65+) from September 2009 to January 2011 in Toronto, Canada.

A total of 263 of individuals participated in this pilot study of bone and blood lead measurements supported by Health Canada's Chemical Management Plan. The study was conducted in collaboration with McMaster University and St. Joseph's Health Centre located in Toronto. Participants were measured for bone lead (calcaneus and tibia) concentration and whole blood and serum lead levels. Among participants 17 (6.5%) of them were 1 to 5 years old; 37 (14%) of them 6 to 10; 44 (17%) of them 11 to 19; 47 (18%) of them 20 to 35; 36 (14%) of them 36 to 50; 46 (17.5%) of them 51 to 64, and finally 19 (7%) of them belonged to age range of 65 to 85 years old. Calcaneus and tibia lead concentration were measured using a  $^{109}\text{Cd}$  source in a backscattering geometry. The source was positioned in a four-detector geometry system, each detector with 16 mm diameter, owned by McMaster University; each measurement lasted about 22 min. During the time of measurement, participants were asked to complete a questionnaire. Adults were asked socioeconomic questions such as level of education and household income; age of their house, renovation of house; use of traditional medicines and foodstuffs. Women were asked about use of oral contraceptive, menopausal status and number of pregnancies. For children over age 7, the questionnaire was similar to adults, except the questions that were non-relevant to children were excluded, finally for children under the age of 7, parents were asked to identify the possible sources of lead exposure such as putting soil or paint chips in their mouth (pica).

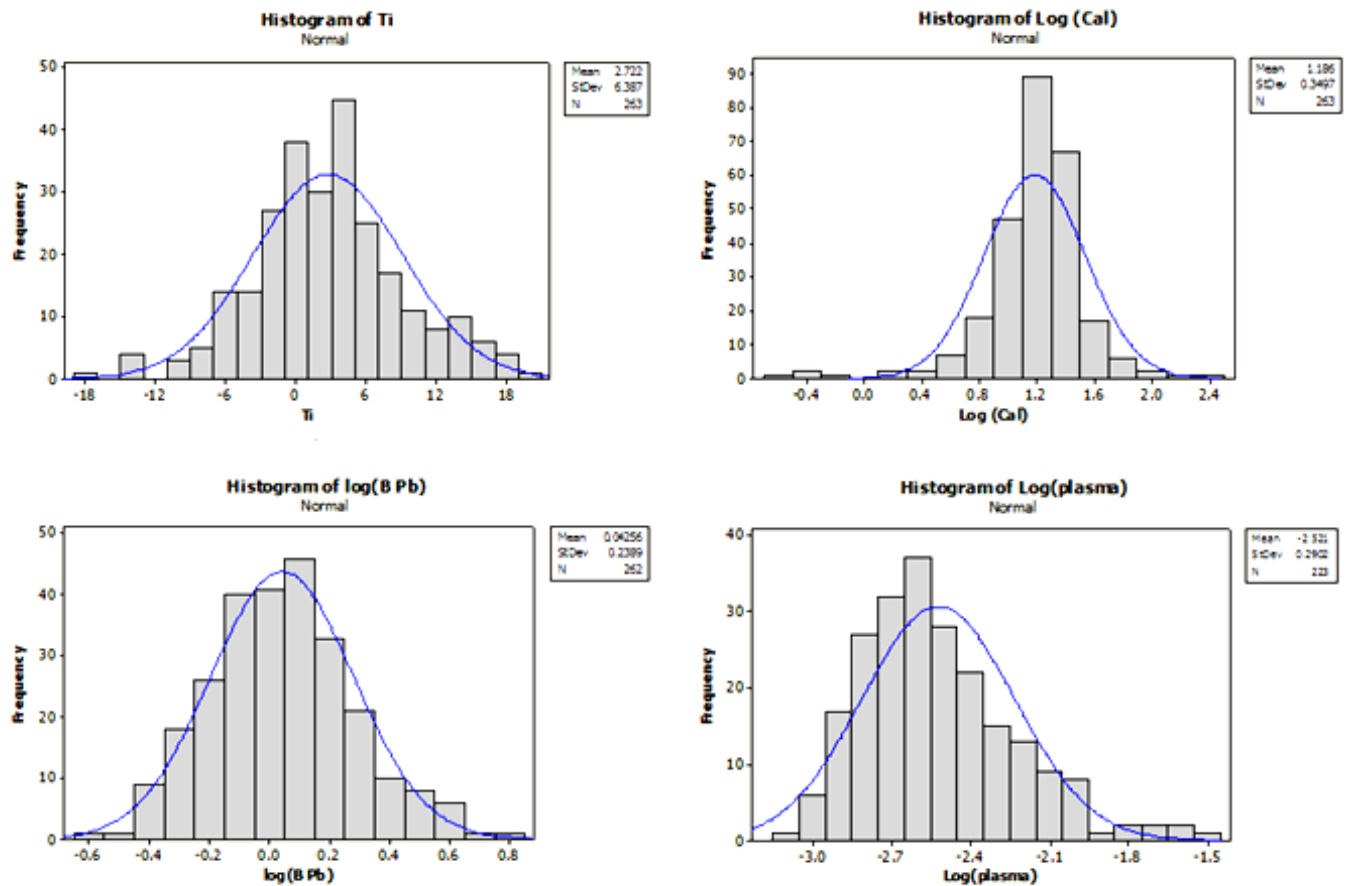
## 6.2 Plasma lead, blood lead, and bone lead concentration:

Table 1 summarizes the statistical information of body lead contents for the whole group of participants. Figure 1 shows a histogram of the frequency distribution for each of these four variables including tibia lead level (Ti), logarithmic transformation of calcaneus lead concentration (Cal), blood lead level (B Pb), and plasma lead concentration.

**Table 1: Summary statistics of bone lead levels of EG, corresponding uncertainty, and demographic variables age and BMI**

| Variable                 | N   | Median | Min    | Max    | Geometric Mean    |
|--------------------------|-----|--------|--------|--------|-------------------|
| Cal Pb <sup>2</sup>      | 263 | 5.34   | -10.70 | 287.03 | 9.40 <sup>1</sup> |
| Ti Pb <sup>2</sup>       | 263 | 2.85   | -18.93 | 19.49  | 1.59 <sup>1</sup> |
| B Pb <sup>3</sup>        | 262 | 1.07   | 0.250  | 6.03   | 1.103             |
| Plasma lead <sup>4</sup> | 224 | 4.06   | 1.88   | 34.18  | 29.22             |
| Age                      | 263 | 33.12  | 1.31   | 83.75  | 32.98             |

<sup>1</sup> For Tibia (Ti) and Calcaneus (Cal) Pb levels the arithmetic mean was reported in place of the geometric mean due to negative values. Ti lead was normally distributed therefore no transformation was required. When Cal lead was used in the analysis then the transformation  $\log(x+11)$  was used to normalize the data. Also, blood lead and plasma lead levels were transformed to  $\log(x)$  <sup>2</sup>Units in  $\mu\text{g-Pb/g-bone mineral}$  <sup>3</sup> Units in  $\mu\text{g-Pb/dl}$  <sup>4</sup>Units in  $\text{pg-Pb/ml}$



**Figure 1: This figure presents a histogram distribution of the variables. Calcaneus lead concentration has a constant of 11 added before their logarithmic transformation.**

The classification groups of age were <11, (11, 19), (20, 35), (36, 50), (51, 65),  $\geq 65$ . Analysis of variance (ANOVA) was used to test the null hypothesis: all groups of a demographic variable have similar average body lead contents. Results from univariate analyses are presented in Table 2. When pair wise comparison tests were carried out in the analysis, groups that were statistically similar have the same letter (adjacent to the confidence interval in the tables presented), and those that are statistically different have different letters (in the tables presented).

**Table 2: Univariate results for calcaneus and tibia, blood, and plasma lead level for demographic variables sex and age.**

| Variable      | Demographic Variable |             | N  | p-value <sup>1</sup> | GM (95%CI) <sup>2</sup> |                            |
|---------------|----------------------|-------------|----|----------------------|-------------------------|----------------------------|
|               |                      |             |    |                      |                         |                            |
| Calcaneus*    | Age                  | <11         | 54 | <0.0001              | D                       | 19.49 (-3.27, 20.43)       |
|               |                      | 11 ≤age <20 | 44 |                      | E                       | 4.54 (1.11, 6.04)          |
|               |                      | 20 ≤age <36 | 47 |                      | E                       | 1.42 (-0.31, 3.12)         |
|               |                      | 36 ≤age <51 | 52 |                      | E                       | 7.14 (3.01, 8.90)          |
|               |                      | 51 ≤age <65 | 46 |                      | D                       | 13.32 (3.83, 15.41)        |
|               |                      | ≥65         | 20 |                      | D                       | 10.38 (8.70, 14.46)        |
| Tibia*        | Age                  | <11         | 54 | <0.0001              | A                       | 1.58 (-0.21, 3.37)         |
|               |                      | 11 ≤age <20 | 44 |                      | A                       | 0.075 (-1.62, 1.77)        |
|               |                      | 20 ≤age <36 | 47 |                      | A                       | 0.94 (-0.49, 2.37)         |
|               |                      | 36 ≤age <51 | 52 |                      | A                       | 3.40 (1.81, 4.99)          |
|               |                      | 51 ≤age <65 | 46 |                      | B                       | 6.02 (4.13, 7.91)          |
|               |                      | ≥65         | 20 |                      | B                       | 6.49 (3.52, 9.46)          |
| Blood Lead**  | Age                  | <11         | 53 | <0.0001              | C                       | 0.936 (0.65,1.06)          |
|               |                      | 11 ≤age <20 | 44 |                      | C                       | 0.784 (0.62, 0.90)         |
|               |                      | 20 ≤age <36 | 47 |                      | C                       | 0.917 (0.82, 1.12)         |
|               |                      | 36 ≤age <51 | 52 |                      | G                       | 1.21 (0.97, 1.45)          |
|               |                      | 51 ≤age <65 | 46 |                      | H                       | 1.66 (1.34, 1.92)          |
|               |                      | ≥65         | 20 |                      | H                       | 1.73 (1.37, 2.06)          |
| Plasma Lead** | Age                  | <11         | 45 | <0.0001              | F                       | 0.00256 (0.00161,0.00332)  |
|               |                      | 11 ≤age <20 | 41 |                      | F                       | 0.00225 (0.00158, 0.00248) |
|               |                      | 20 ≤age <36 | 41 |                      | F                       | 0.00275 (0.00127, 0.00295) |
|               |                      | 36 ≤age <51 | 36 |                      | I                       | 0.00348 (0.00111, 0.00493) |
|               |                      | 51 ≤age <65 | 39 |                      | I                       | 0.00407 (0.00223, 0.00484) |
|               |                      | ≥65         | 19 |                      | I                       | 0.00420 (0.00153, 0.00637) |

<sup>1</sup> p-value for overall group effect    <sup>2</sup> Geometric Mean (GM) and corresponding 95% confidence interval (CI): the arithmetic mean has been reported for tibia and calcaneus lead; since calcaneus, blood, and plasma lead concentrations are not normalized, median has been used to report the confidence interval.    \*µg-Pb/g-bone mineral    \*\*µg-Pb/dl



Based on the results of table 2, calcaneus lead levels are the same for the ages [11, 20), [20, 36), and [36, 51). Tibia lead concentrations are not statistically different for the ages <11, [11, 20), [20, 36), and [36, 51). For blood lead concentration, the trend is similar to that for tibia lead concentration, and finally, for plasma lead concentration, the results are similar for the ages [11, 20), [20, 36), and [36, 51). For all of the compartments presented in table 2, the average lead concentration is not statistically different for the ages [51, 65) and  $\geq 65$ , where the reason can be explained due to hormonal changes for the ages 50 and older. Also the average body lead content can be assessed in different sex categories as has been presented in table 3. Significant sex differences were not observed in any of the compartments ( $p>0.05$ ).

**Table 3: Univariate results for calcaneus , tibia, blood, and plasma lead concentration for demographic variables sex**

| Variable                 | Demographic Variable |        | N   | Mean (95% CI)              | p-value            |
|--------------------------|----------------------|--------|-----|----------------------------|--------------------|
| Calcaneus <sup>b</sup>   | Sex                  | Male   | 135 | 10.49 (5.63, 15.35)        | 0.787 <sup>a</sup> |
|                          |                      | Female | 128 | 8.26 (5.86, 10.66)         |                    |
| Tibia <sup>b</sup>       | Sex                  | Male   | 135 | 3.10 (2.03, 4.16)          | 0.595 <sup>a</sup> |
|                          |                      | Female | 128 | 2.33 (1.19, 3.47)          |                    |
| Blood lead <sup>c</sup>  | Sex                  | Male   | 135 | 1.28 (1.14, 1.42)          | 0.897 <sup>a</sup> |
|                          |                      | Female | 127 | 1.31 (1.58, 1.46)          |                    |
| Plasma lead <sup>c</sup> | Sex                  | Male   | 120 | 0.00378 (0.00302, 0.00454) | 0.401 <sup>a</sup> |
|                          |                      | Female | 104 | 0.00408 (0.00338, 0.00478) |                    |

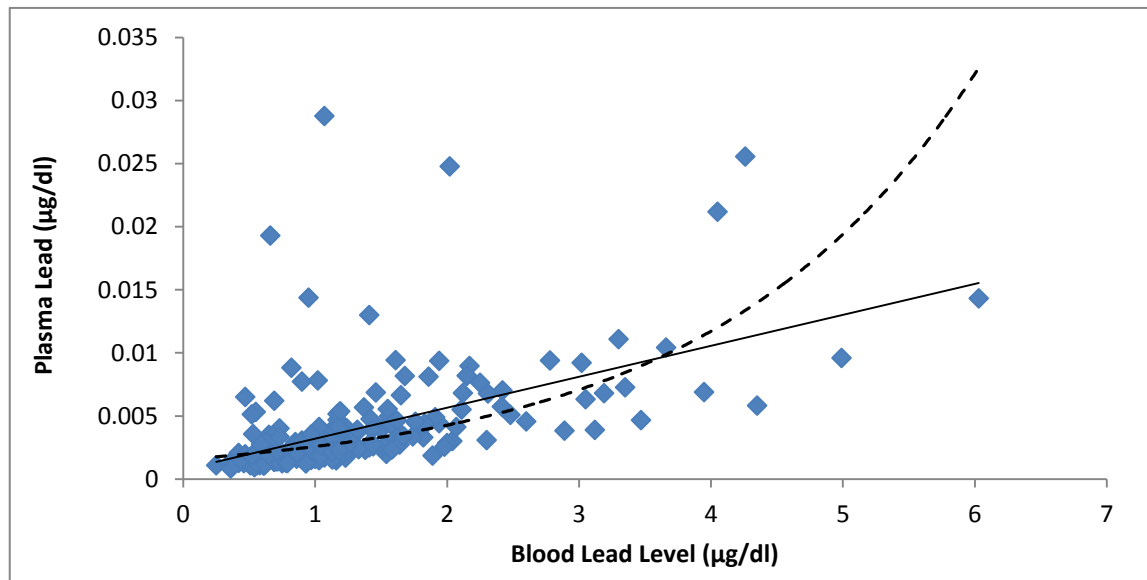
<sup>a</sup> the analysis is based on the non-parametric Kruskal Wallis      <sup>b</sup> μg-Pb/g-bone mineral      <sup>c</sup> μg-Pb/dl

### 6.3 Blood and Plasma lead levels

Blood plasma is the straw-colored/pale-yellow liquid of blood that normally holds the blood cells, and it makes up about 55% of total blood volume. Blood plasma is prepared by spinning a tube of fresh blood containing an anticoagulant (a substance that prevents clotting of blood) in a centrifuge until the blood cells fall to the bottom of the tube. Blood serum is blood plasma with the clotting factor excluded (Stedman 2006).

Marcus (1985c) revealed the nature of nonlinear relation between plasma lead and blood lead. The transfer rate of lead from the bloodstream to various body compartments is assumed to be governed by the concentration of lead in plasma. Based on Leggett's model lead kinetics are assumed to be linear as long as the concentration in erythrocytes stays below a threshold value of 60  $\mu\text{g}/\text{dl}$ , which is equivalent with the whole blood lead level of about 25  $\mu\text{g}/\text{dl}$  in adults. The Leggett model relies on a two-compartment makeup for plasma including non-diffusible and diffusible plasma. In the former, plasma proteins (notably  $\alpha$ -globulin) bind (or are bound) to the portion of lead in plasma and makes up the non-diffusible plasma lead compartment. The latter governs the transfer of lead to various tissues of interest. O'Flaherty considered the nonlinear relation between plasma lead and blood lead due to a capacity-limited binding of lead by red blood cells. A greater portion of the lead in blood will stay in the plasma at higher levels of whole blood lead concentration.

For the cohort of the general population, the association of plasma lead as a function of blood lead concentration was investigated. Figure 2 confirmed nonlinearity between the variables. Fitting the data by an exponential function improved the fitting significantly. The coefficient of determination for an exponential fit was found to be 42%; whereas, it was found to be 29% for a linear fit. Plasma lead concentrations lie in the range of 0.099-2.9% of the whole blood lead concentration, which is consistent with previous studies (DeSilva 1981; Manton and Cook 1984).



**Figure 2: Plasma lead level vs. blood lead level.**

Since plasma and blood lead concentration are not normally distributed, the logarithmic transformation of the variables should be used to assess the underlying relationship between blood lead level and plasma lead concentration as below:

$$\log (P) = (0.809 \pm 0.061) \log (B) - (2.56 \pm 0.01) \quad (R^2 = 0.444; t = 13.30; n = 224).$$

In some cases, for the purpose of comparison with the previous studies, we may not transform the variables. The Cake *et al.* (1996) study was performed on 49 active lead workers, where the ratio of serum lead to whole blood lead in their study varied from 0.8% to 2.5%. The authors found the relationship between blood lead level (B) and serum lead (S) was expressed by a linear function as:

$$S = (0.0206 \pm 0.0021) B - (1.58 \pm 0.78) \quad (R^2 = 0.670; t = 9.82).$$

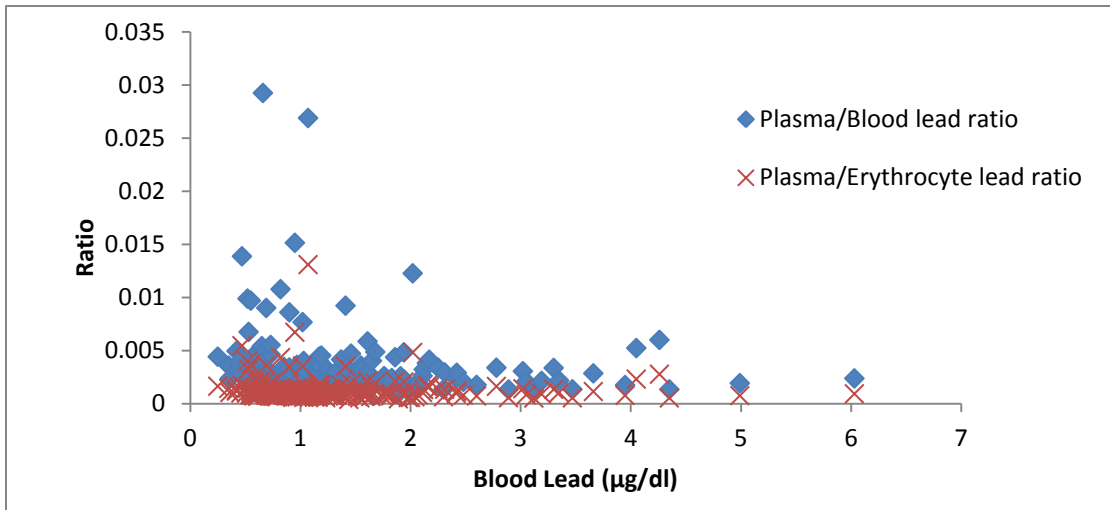
For the current study, the linear regression equation of plasma lead (P) and blood lead was found to be:

$$P = (0.00246 \pm 0.00025) B + (0.000721 \pm 0.000402) \quad (R^2 = 0.291; t = 9.54; n = 224).$$

The Rosen *et al.* (1974) study showed that plasma concentrations of lead remain constant over a wide range of haematocrit and whole blood lead concentration, where the concentration of lead in plasma was found to be 3µg/dl, which is about 100 times higher than the highest plasma lead level in the current study. Cavalleri *et al.* (1978) reported that plasma lead increased linearly and significantly as whole blood lead level increases with a constant at about 3%. Desilva (1981) reported that the plasma lead concentration increased with the erythrocyte lead concentration with a constant of about 0.74%. Desilva compared the plasma lead concentration (P) with the erythrocyte lead concentration (E) which was calculated according to the formula below:

$$E = \frac{100B}{Ht} - \frac{P(100 - Ht)}{Ht}$$

Where, B and Ht stand for blood lead concentration and haematocrit respectively. Haematocrit is the volume percentage of red blood cells in blood (Medical Dictionary). For the purposes of direct comparison with Cavalleri and Desilva work, we presented some of the results in terms of these comparisons. Figure 3 shows the ratio of plasma lead and blood lead level (erythrocyte lead concentration). Haematocrit results are available for 220 participants; therefore, plasma results were removed for participants whose haematocrit results were not available.



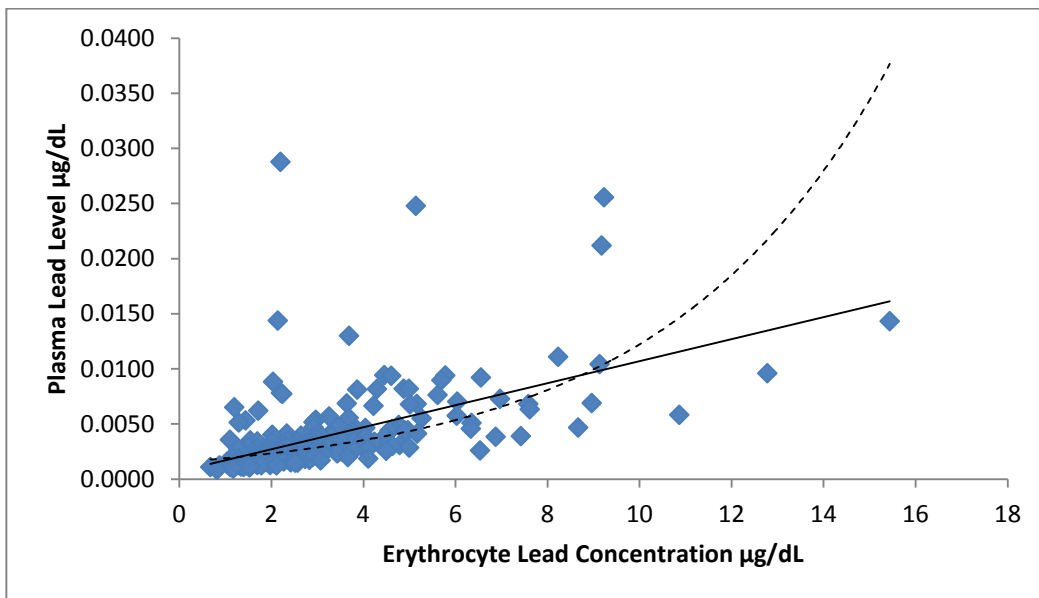
**Figure 3: The ratio of plasma lead concentration and blood lead level (Erythrocyte lead) versus blood lead values. The average of P/E and P/B were found to be 0.13% and 0.32% respectively.**

Figure 4 shows a scatter-gram of the plasma lead versus erythrocyte linear concentration. The underlying relationship between the parameters is expressed by the following equation as:

$$P = (0.000998 \pm 0.000103) E + (0.000708 \pm 0.000391) \quad (R^2 = 0.301; P < 0.0001).$$

In above equation, the regression coefficient is highly significant. However, the intercept is not significantly different from zero. The equation that was reported by Desilva is as:  $P = 0.009 E - 0.13$ , where the intercept as reported was not significantly different from zero. One of the reasons of having discrepancy between the slope of the regression of the current work and Desilva work can be due to the accuracy of the plasma results obtained. Since the concentration of blood lead levels are significantly lower than previous works, which can be seen in figure 4, the concentration of lead in plasma could be much closer

to the minimum detection limit. On the other hand, in Desilva's work, the plasma lead concentration could be overestimated due to moving some lead concentrations from red blood cells to plasma during the separation processing. Also, based on the information presented by figure 4, having a nonlinear fit, such as an exponential fit, will improve the  $R^2$  value by 13%; therefore, the nonlinearity between plasma lead concentration and erythrocyte still stays valid.



**Figure 4: Relationship between plasma lead concentration and erythrocyte lead level**

The O'Flaherty's model expresses the relationship between concentration of lead in whole blood (B) and concentration in plasma (P) as follows:

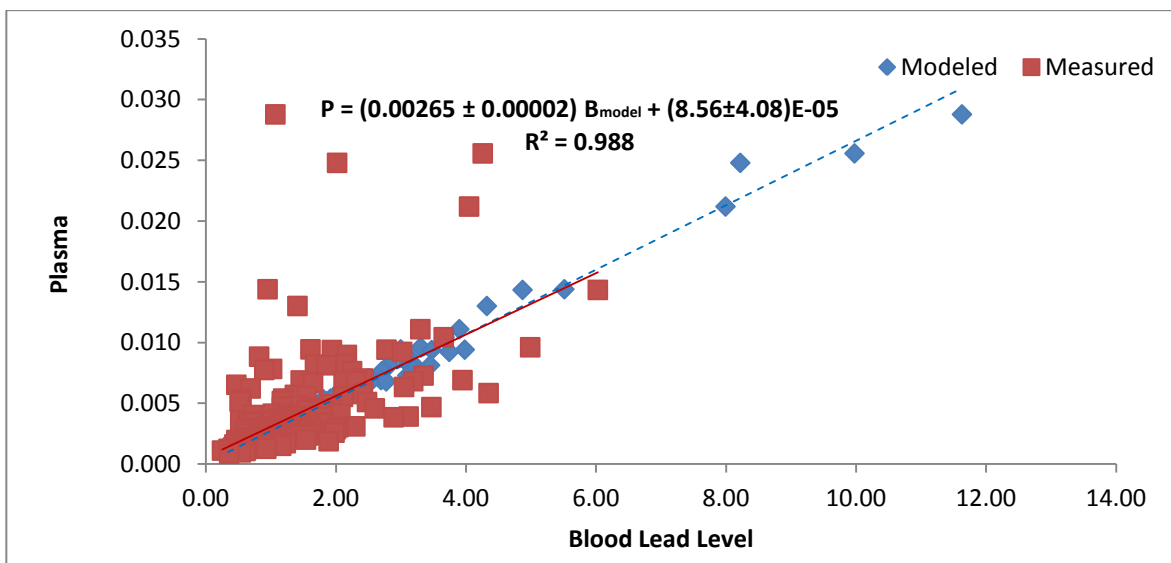
$$B = (1 - Ht) \times P + (Ht \times P) \times (G + BIND / (KBIND + P)) \quad (1).$$

Where in the above formula, G is the ratio of unbound red cell lead to plasma lead; BIND is the maximum binding capacity for lead in erythrocytes (mg Pb / l of red blood cell),

and KBIND is half-saturation binding constant (mg Pb/l cell). The default value of G, BIND, and KBIND in the O’Flaherty model have been defined to be 1.2, 2.7 mg/l, and 0.0075 mg/l respectively. The above mentioned model parameters may need to be changed from their default values based on new experimental information (O’Flaherty 1993). In equation 1, by defining G, BIND, and KBIND values to be 1.2, 3.6 (mg/l), and 0.004 (mg/l) respectively, and replacing haematocrit and plasma concentration for each participant, blood lead level was modeled ( $B_{\text{model}}$ ) and the result was compared with the measured values ( $B_{\text{measure}}$ ). The equation below shows a good agreement between the measured and calculated blood lead level.

$$B_{\text{model}} = (0.930 \pm 0.093) B_{\text{measure}} + (0.211 \pm 0.145) \quad (R^2 = 0.315; P < 0.0001)$$

The relationship of plasma lead concentration and modeled (measured) blood lead concentration is shown in figure 5.



**Figure 5: Plasma lead concentration as a function of measured and modeled blood lead concentration using the revised values of BIND, KBIND, and G.**



The slope of the linear regression equation of plasma lead concentration and modeled blood lead level is not significantly different from that of plasma lead concentration and measured blood lead level ( $0.00252 \pm 0.00025$  versus  $0.00265 \pm 0.00002$ ). Also, the result of O'Flaherty's model suggests the linearity in relationship of blood lead and plasma lead concentration. Fleming et al. (1999), reduced the red cell coefficients (BIND= 2.6 and KBIND = 0.0060), while the authors applied the model to smelter employees who were heavily exposed to lead. Reducing the coefficients will result in a proportionately higher uptake of lead to bone as the slope of plasma lead concentration versus blood lead level increases.

#### **6.4 Plasma lead and Bone lead concentration relationship**

Cake *et al.* (1996) found a positive correlation between serum and tibia lead ( $n=49$ ;  $r = 0.51$ ;  $t = 4.06$ ) and calcaneus lead ( $n=49$ ;  $r = 0.65$ ;  $t = 5.83$ ). Also, the authors found a significant correlation between blood lead level and calcaneus lead concentration ( $r = 0.57$ ;  $t = 4.72$ ), and blood lead level and tibia lead concentration ( $r = 0.39$ ;  $t = 2.94$ ). In the present study<sup>1</sup>, plasma lead concentration marginally correlated with tibia lead levels ( $r^2 = 0.0240$ ;  $t = 2.33$ ;  $P < 0.05$ ); whereas, no correlation was found between the plasma (P) and calcaneus lead concentration (Cal). Since in this study, we are dealing with a wide age range of participants (1.2 to 83 years old), the association of plasma lead concentration can be tested as a function of predictors age and tibia lead concentration (Ti), where the following equation can express the underlying relationship.

---

<sup>1</sup> In the regression equations we used logarithmic transformation of blood lead level ( $B_i$ ) and plasma lead concentration ( $P_i$ ). Calcaneus lead levels were transformed to  $\log(x+11)$  ( $Cal_i$ ). Tibia lead levels were not transformed.

$$P_t = (0.00218 \pm 0.00303) Ti + (0.00422 \pm 0.00093) Age - (2.66 \pm 0.03) \quad (n=223; R^2 = 0.107; P < 0.0001).$$

In above equation, the coefficient of Ti is not significantly different from zero (P = 0.474); whereas, that of age is significantly different from zero (P < 0.0001). Similarly, the underlying association of plasma lead concentration as a function of calcaneus lead and age can be expressed as:

$$P = (2.14 \pm 1.06) \times 10^{-5} Cal + (5.39 \pm 1.16) \times 10^{-5} Age + (0.00192 \pm 0.00047) \quad (n=223; r^2 = 0.102; P < 0.0001).$$

Where, both calcaneus lead coefficient (P < 0.05) and age coefficient (P < 0.0001) are different from zero. For males (n = 119), plasma lead correlated neither with tibia lead (P = 0.0740) nor with calcaneus lead (P = 0.101) significantly. However, plasma lead concentration marginally correlates with age and tibia lead as follows:

$$P_t = (0.00339 \pm 0.00424) Ti + (0.00360 \pm 0.00126) Age - (2.66 \pm 0.04) \quad (n=119; r^2 = 0.90; P = 0.0040).$$

Similarly, for calcaneus lead we have:

$$P_t = (0.121 \pm 0.076) Cal_t + (0.00387 \pm 0.00118) Age - (2.81 \pm 0.10) \quad (n=119; r^2 = 0.105; P = 0.0020).$$

Equally, for females the equations are as follows:

$$P_t = (0.00149 \pm 0.00447) Ti + (0.00473 \pm 0.00141) Age - (2.66 \pm 0.05) \quad (n=103; r^2 = 0.125; P < 0.001).$$

$$P_t = (0.0986 \pm 0.0825) Cal_t + (0.00485 \pm 0.00130) Age - (2.78 \pm 0.11) \quad (n=103; r^2 = 0.136; P < 0.001).$$

## 6.5 Blood lead and Bone lead concentration relationship

For the whole group with available blood lead level and bone lead concentration, tibia lead concentration (Ti) and calcaneus lead (Cal) correlates significantly with blood lead level (B) as the relationship can be expressed by the following equation.

$$B = (0.0252 \pm 0.0080) Ti + (1.23 \pm 0.06) \quad (n = 262; r^2 = 0.0368; P = 0.00181).$$

$$B = (0.0125 \pm 0.0022) Cal + (1.18 \pm 0.05) \quad (n = 262; r^2 = 0.108; P < 0.0001).$$

More than 90% to 95% (70-80% in children) of the total lead body burden accumulates in bone (Barry and Mossman 1970; Schroeder and Tipton 1968). Lead is mobilized more than usual from bone to blood during periods of pregnancy, lactation, menopause, aging, kidney disease, hyperthyroidism, chronic disease, and physiologic stress (Manton 1985; Hu et al. 1991). The endogenous exposure coming from tibia and calcaneus bones, can be estimated by looking at the linear regression equations of blood lead levels and a function of tibia and calcaneus lead concentration. Brito *et al.* (2002) assessed the endogenous release of lead from bone to blood in 204 occupationally exposed subjects at a smelter. The authors suggested that using structural analysis predicts release of lead from bone to blood higher than those predicted by the generally used linear regression equations. Since for the whole group of participants the relationship between the blood lead level and bone lead concentration is stronger, we use the equations to predict the endogenous release of lead from bone to blood. Since there are variances in predictors and responses, factor analysis was used to find the true slope. In figure 6, if  $m$  presents the slope of tibia lead

vs. blood lead and  $m'$  presents the slope of blood lead vs. tibia lead, then the true slope ( $m_T$ ) of blood lead level vs. tibia lead concentration is calculated as (Quenouille 1952):

$$m_T = \frac{\sigma_B^2 \frac{m}{\sigma_{Ti}^2} + \sigma_{Ti}^2 \frac{(m')^{-1}}{\sigma_B^2}}{\frac{1}{\sigma_{Ti}^2} + \frac{1}{\sigma_B^2}} = \frac{\sigma_B^2 m + \sigma_{Ti}^2 (m')^{-1}}{\sigma_{Ti}^2 + \sigma_B^2}$$

The variances of blood lead level (transformed), tibia lead, calcaneus lead concentration (transformed) were found to be 0.709  $\mu\text{g/dl}$ , 40.79  $\mu\text{g/g}$ , and 508.76  $\mu\text{g/g}$  respectively. Therefore, the true slope of blood lead level versus tibia lead concentration can be found as:

$$m_T = \frac{0.709 \times 0.0252 + 40.79 \times (1.46)^{-1}}{40.79 + 0.709} = 0.674$$

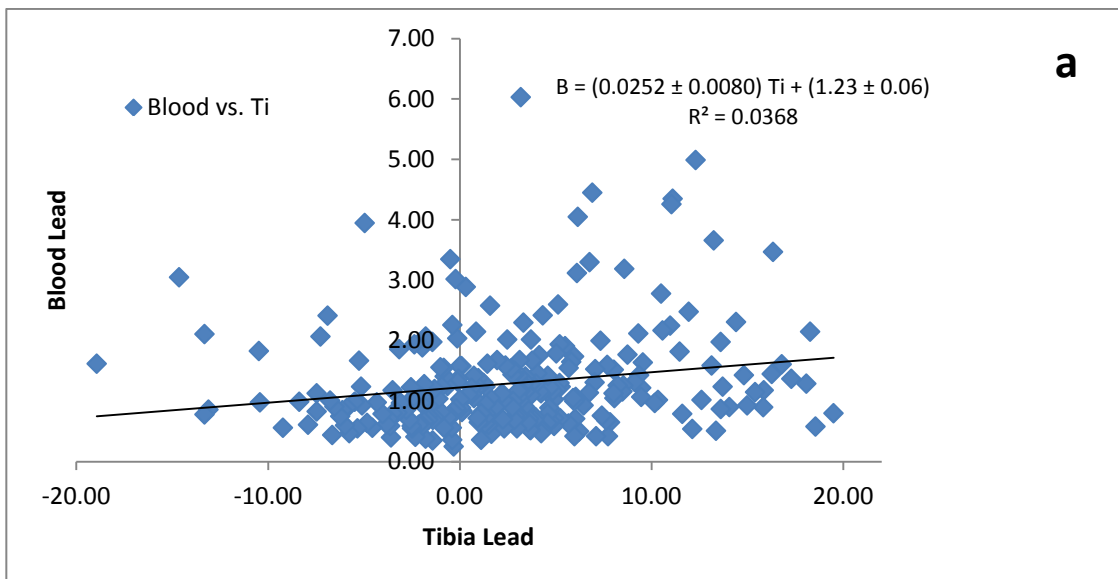
Similarly, for calcaneus lead and blood lead concentration we have:

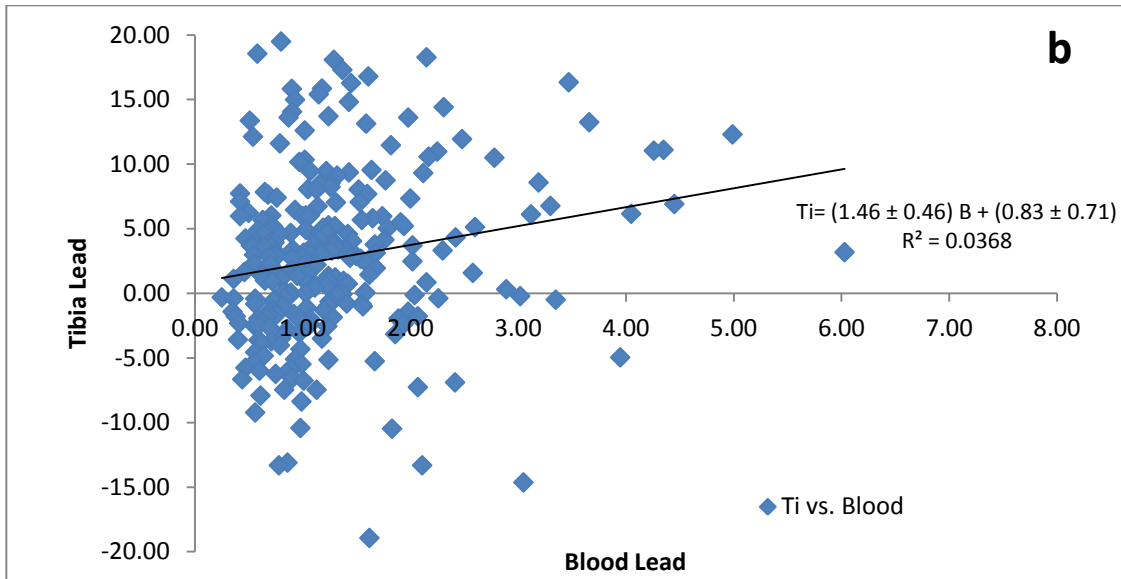
$$\text{Cal} = (8.60 \pm 1.53) \text{ B} - (2.03 \pm 2.37)$$

By using the linear regression equations of the blood lead level as a function of calcaneus and calcaneus lead as a function of blood lead level, the true line slope of blood lead level as a function of calcaneus lead using the structural model can be expressed as follows:

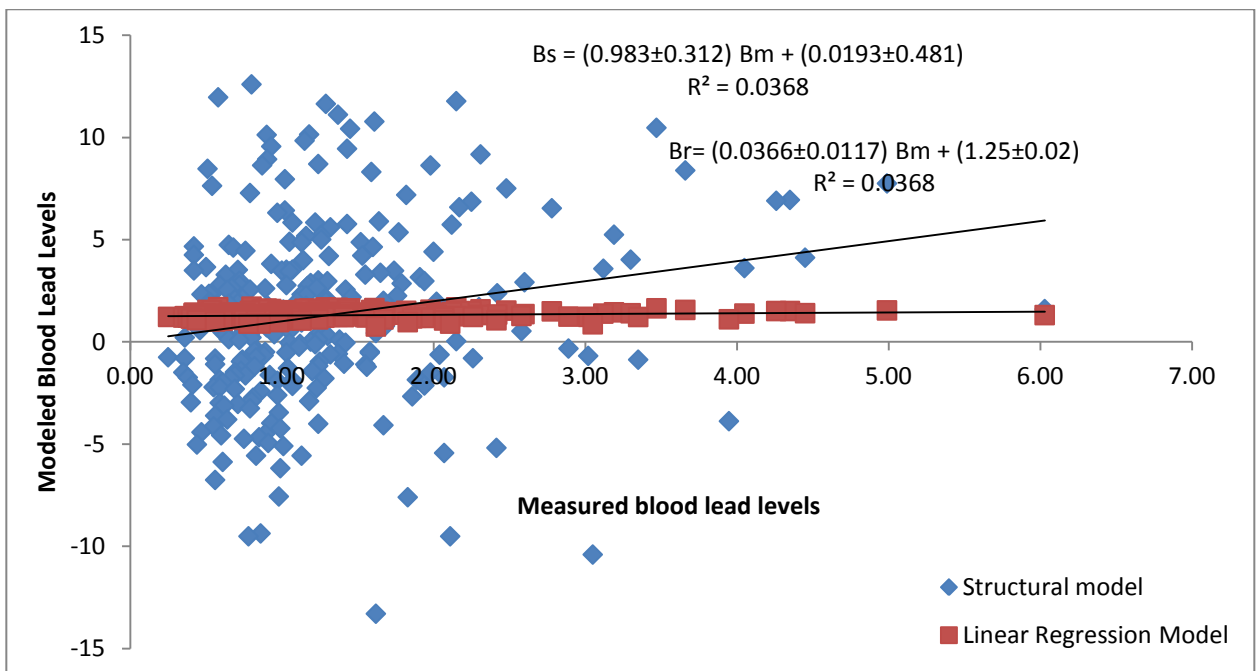
$$m_T = \frac{0.709 \times 0.0125 + 508.76 \times (8.6)^{-1}}{508.76 + 0.709} = 0.116$$

The resulted calculated blood lead levels by using the structural model and regression model has been shown in figures 7-8, while using the structural model, the slope linear regression equation of the modeled blood lead level versus the measured blood lead level is close to one. The average lead concentration in calcaneus was found to be 3.4 times lead concentration in tibia. Therefore, about 63% ( $0.674 / (0.674 + 3.4 \times 0.116)$ ) of the endogenous component of blood lead level comes from tibia lead and 37% of that comes from calcaneus bone lead.

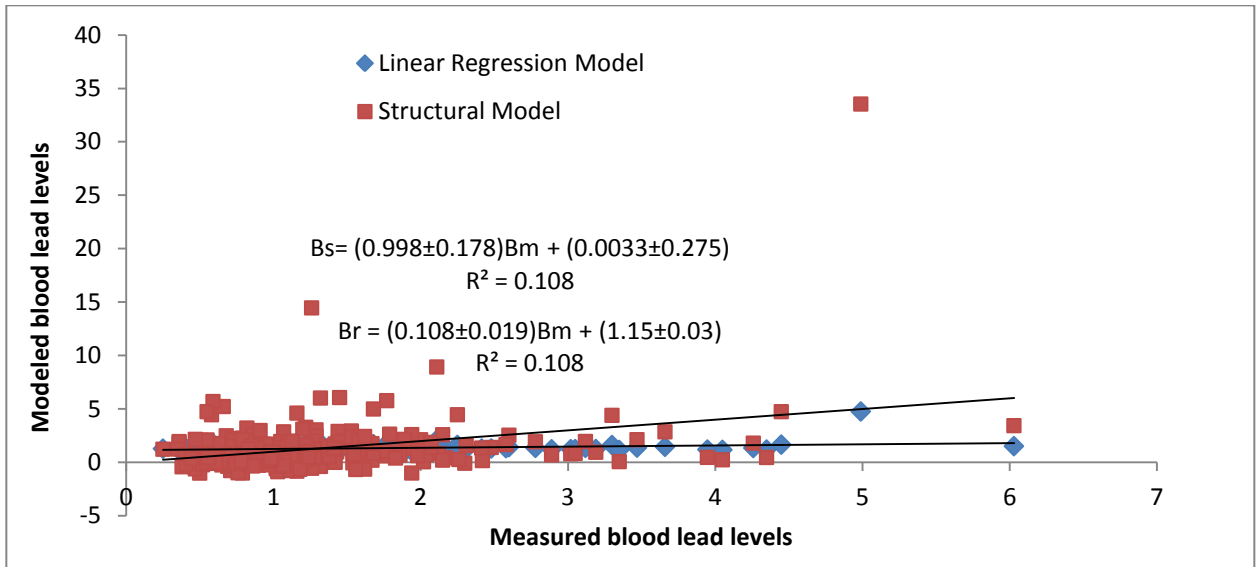




**Figure 6:** This figure shows a linear regression equation of (a) blood lead ( $\mu\text{g}/\text{dl}$ ) versus tibia lead ( $\mu\text{g}/\text{g}$ ) and (b) tibia lead as a function of blood lead concentration.



**Figure 7:** This figure shows a direct comparison of the linear regression model ( $B_r$ ) and the structural model ( $B_s$ ) with measured blood lead levels ( $B_m$ ) by using tibia lead values.



**Figure 8:** This figure shows a direct comparison of the linear regression model (Br) and the structural model (Bs) with measured blood lead levels (Bm) by using calcaneus lead values.

## Chapter 7

### Age and sex influence on bone and blood lead level in a cohort of the general population living in Toronto

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#### Abstract:

Among 263 non-occupationally exposed healthy subjects, ranging from approximately one to eighty years old, we show how quadratic equations can best describe the underlying relationship between blood / bone concentration and age in both male and female subgroups. Restricting the analysis to 15 years and older, we found that lead content increased linearly with age in both sexes. In this study, the number of previous pregnancies and menopausal status had significant influence neither on bone nor blood lead concentration and age profile. Similarly, sex was not found to be associated with bone/blood lead level and age regression models.

#### 1. Introduction

Low-level lead exposure has been reported to induce a range of adverse health effects, among which are intellectual deficits (especially in children) (Lanphear *et al* 2005) and cardiovascular disease (Navas-Acien *et al.* 2007). Approximately 90% to 95% of the total lead body burden is in bone (Barry 1975). Leggett (1993) and O’Flaherty (1991) have developed two widely used models of lead (Pb) metabolism; where in both models bone has been identified as the tissue compartment in which the overwhelming proportion of the mass of Pb is stored in the body. Study of the bone lead concentration not only provides us with an index of cumulative exposure, but also represents a source of endogenous lead that can be redistributed to sensitive target tissues, a process that can be enhanced during physiological and pathological states like pregnancy, lactation, and menopause (Kosnett *et al.* 1994).

The first *in vivo* lead measurement using x-ray fluorescence, used <sup>57</sup>Co as the radioactive source with a 90° scattering angle (Ahlgren *et al.* 1976). Using <sup>109</sup>Cd instead of <sup>57</sup>Co improved the precision significantly since <sup>109</sup>Cd emits 88.03 keV gamma rays which are only 34 eV above the lead K absorption edge energy. The person or calibration standard is typically irradiated for twenty to thirty minutes, and the generated photons are collected as a number of counts by the germanium detector located behind the source in the backscatter geometry with an average 153° scattering angle. Choosing backscatter



geometry for  $^{109}\text{Cd}$  is to minimise overlap between Compton scatter and lead  $K_{\alpha}$  x-rays (at 72.804 keV and 74.969 keV) (Todd *et al.* 1992). Developing the four-detector system in clover leaf geometry (Nie *et al.* 2006) further improved the precision in determining bone lead contents compared to a conventional (one detector) system. The four detector clover-leaf geometry detector system has enabled us to measure bone lead content with the minimum detection limit (MDL) of  $3.2 \mu\text{g Pb (g bone mineral)}^{-1}$  and  $4.8 \mu\text{g Pb (g bone mineral)}^{-1}$  for tibia and calcaneus respectively (Behinaein *et al.* 2011). Hence, by using the four-detector system to assess bone lead concentration, low-level environmental lead exposure studies relating to public health are possible.

Age and sex have been shown to be variables correlated with bone lead concentration. It has been reported that bone lead level increases with age (Hu *et al.* 1991, Somerville *et al.* 1988, Kosnett *et al.* 1994). Kosnett *et al.* (1994) reported that sex influences bone lead concentration after the age sixty. Also, Drasch *et al.* (1987) concluded that lead level in males' bone were higher than those in females' bone. Similarly, Gamblin *et al.* (1994) reported that the correlation between tibia lead and age was stronger in men than women.

## **2. Materials and Methods:**

### **2.1. Environmentally exposed group of participants:**

A total of 263 of individuals participated in this pilot study of bone and blood lead measurements supported by Health Canada's Chemical Management Plan. The study was conducted in collaboration with McMaster University and St. Joseph's Health Centre located in Toronto. Participants were measured for bone lead (calcaneus and tibia) concentration and whole blood and plasma lead levels. Among participants 17 (6.5%) of them were 1 to 5 years old; 37 (14%) of them 6 to 10; 44 (17%) of them 11 to 19; 47 (18%) of them 20 to 35; 36 (14%) of them 36 to 50; 46 (17.5%) of them 51 to 64, and finally 19 (7%) of them belonged to age range of 65 to 85 years old. Calcaneus and tibia lead concentration were measured using a  $^{109}\text{Cd}$  source in a backscattering geometry. The source was positioned in a four-detector geometry system with 16 mm diameter hyper pure germanium crystals owned by McMaster University; each measurement lasted about 22 min. During the time of measurement, participants were asked to complete a questionnaire. Adults were asked socioeconomic questions such as level of education and household income; age of their house, renovation of house; use of traditional medicines and foodstuffs. Women were asked about use of oral contraceptive, menopausal status and number of pregnancies. For children over age 7, the questionnaire was similar to adults, except the questions that were non-relevant to children were excluded, finally for children under the age of 7, parents were asked to identify the possible sources of lead exposure such as putting soil or paint chips in their mouth (pica).

## 2.2.Outliers

A schematic *box-and-whisker plot* (box-plot) was used to indentify outliers. Minitab uses the 3IQR rule, which includes 50% of data (the differences between the third and first quartile), to detect outliers. The following subjects were considered as outliers and were excluded from the data set. One had tibia and calcaneus lead concentrations of -  $13.31 \pm 18.04$  ppm and  $74.93 \pm 15.44$  ppm respectively (t=1.3 years old). Two other subjects had calcaneus lead levels of  $88.45 \pm 7.39$  (t=4.35 years old) and  $287.03 \pm 66.94$  (t=1.2 years old). The three measurements were removed due to the imprecision of the measurements, because of the young age of these participants, which meant that they moved a great deal and it was not possible to position them satisfactorily.

## 2.3.The K X-Ray Fluorescence Technique

The  $^{109}\text{Cd}$  K XRF bone lead measurement system detector was transported from McMaster University in Hamilton to Toronto by the research team and set up in a room in St Joseph's Healthcare Centre. The source activity at the time of the start of measurements was calculated to be 1.27GBq. The end of the measurements was about 18 months later; by that time the source activity had declined to 0.57GBq. To find the lead concentration in participants, related spectrum files were analyzed for the coherent,  $\text{PbK}_\alpha$ , and  $\text{PbK}_\beta$  spectral peak areas by mathematically fitting the spectrum. The coherent scatter signal comes mainly from the calcium in bone. The unit of measurement is in micrograms lead/gram bone mineral (or ppm) as the Pb x-ray signal is normalized to the coherent peak. The results of (alpha/coherent) and (beta/coherent) for all four detector systems were evaluated against their corresponding calibration lines. The outcome was four sets each having two estimates of lead concentration resulting from alpha and beta routines for all four detector systems (clover –leaf geometry). Results were combined by using an inverse –variance weighted mean to find one value of lead concentration (+/- uncertainty) for each of the participants. The inverse weighted mean is calculated by using the formula below, where index (i) addresses the detector number (i.e. 1, 2, 3, and 4);  $\text{Pb}_i$  presents the inverse weighted mean of the calculated lead ( $\text{Pb}_\alpha (\pm \sigma_{\text{Pb}\alpha} )$ ) and ( $\text{Pb}_\beta (\pm \sigma_{\text{Pb}\beta} )$ ) based on  $\text{PbK}_\alpha$  and  $\text{PbK}_\beta$  lead x-rays respectively, and  $\text{Pb}_\mu$  is the lead inverse weighted mean of the eight possible estimates.

$$\text{Pb}_\mu = \frac{\sum_i \frac{\text{Pb}_i}{\sigma_{\text{Pb}i}^2}}{\sum_i \frac{1}{\sigma_{\text{Pb}i}^2}} \quad \sigma_{\text{Pb}\mu}^2 = \left( \sum_i \frac{1}{\sigma_{\text{Pb}i}^2} \right)^{-1}$$

## 2.4. Dummy variables in linear regression equations

An indicator or dummy variable is used in a regression equation to identify different categories of a nominal variable. An example of a dummy variable includes the following (Kleinbaum *et al.* 2007):

$$Z = \begin{cases} 1 & \text{if subject is male} \\ 0 & \text{if subject is female} \end{cases}$$

The term dummy reflects the fact that the values like 0 and 1 are not meaningful measurements but indicate the categories of interest. In this paper, dummy variables were used in variety of categories including males and females, to indicate the number of pregnancies in females and to differentiate females having or not having breastfed children. There are two approaches to setting up the regression equations. In the first approach, the male and female are treated separately by fitting the two separate regression equations, and then the appropriate t test is conducted. As an example, the following equations stand for systolic blood pressure (Y) as a function of age (t) for males (M) and females (F):

$$Y_M = \beta_{0M} + \beta_{1M}t + E_M$$

and

$$Y_F = \beta_{0F} + \beta_{1F}t + E_F$$

With the appropriate null hypothesis for comparing the slopes, we have:

$$H_0 = \beta_{1M} = \beta_{1F}$$

When the null hypothesis  $H_0 = \beta_{1M} = \beta_{1F}$  is true, the two regression lines simplify to  $Y_M = \beta_{0M} + \beta_1 t + E_M$  for males and  $Y_F = \beta_{0F} + \beta_1 t + E_F$  for females, where  $\beta_1$  in the equations is common slope when  $\beta_{1M} = \beta_{1F}$  (parallelism), and  $E_F$  ( $E_M$ ) stand for the error component, which is the difference between the observed and true average response of

$$Y_M \text{ (} Y_F \text{) at } t. \hat{\beta}_1 = \frac{(n_M - 1)S_{X_M}^2 \hat{\beta}_{1M} + (n_F - 1)S_{X_F}^2 \hat{\beta}_{1F}}{(n_M - 1)S_{X_M}^2 + (n_F - 1)S_{X_F}^2}$$

Any of the following three alternative hypotheses can be used:

$$H_A = \begin{cases} \beta_{1M} > \beta_{1F} & \text{(one sided)} \\ \beta_{1M} < \beta_{1F} & \text{(one sided)} \\ \beta_{1M} \neq \beta_{1F} & \text{(two sided)} \end{cases}$$

The test statistic for evaluating parallelism (i.e.  $\beta_{1M}=\beta_{1F}$ ) is calculated by:

$$T = \frac{\hat{\beta}_{1M} - \hat{\beta}_{1F}}{S_{(\hat{\beta}_{1M} - \hat{\beta}_{1F})}} \quad (1)$$

Where,

$\hat{\beta}_{1M}$  : Least-squares estimate of the slope, using  $n_M$  observations on males.

$\hat{\beta}_{1F}$  : Least-squares estimate of the slope, using  $n_F$  observations on females.  $S_{(\hat{\beta}_{1M} - \hat{\beta}_{1F})}$  : Estimate of the standard error of the estimated difference between slopes ( $\hat{\beta}_{1M} - \hat{\beta}_{1F}$ ).

$$S_{(\hat{\beta}_{1M} - \hat{\beta}_{1F})}^2 = S_{P,Y|t}^2 \left[ \frac{1}{(n_M - 1)S_{t_M}^2} + \frac{1}{(n_F - 1)S_{t_F}^2} \right]$$

$$S_{P,Y|t}^2 = \frac{(n_M - 2)S_{Y|t_M}^2 + (n_F - 2)S_{Y|t_F}^2}{n_M + n_F - 4}$$

$S_{Y|t_M}^2$  = Residual mean-square error for the males.

$S_{Y|t_F}^2$  = Residual mean-square error for the females.

$S_{t_M}^2$  = Variance of the age for the males.

$S_{t_F}^2$  = Variance of the age for the females.

Then, the critical regions for different hypotheses and significance level  $\alpha$  is defined as:

$$\begin{cases} T \geq t_{n_M + n_F - 4, 1 - \alpha} & \beta_{1M} > \beta_{1F} \\ T \leq t_{n_M + n_F - 4, 1 - \alpha} & \beta_{1M} < \beta_{1F} \\ |T| > t_{n_M + n_F - 4, 1 - \alpha / 2} & \beta_{1M} \neq \beta_{1F} \end{cases}$$

In the next step, we should determine whether both straight lines have the same intercept.

The null hypothesis in this case is given by:  $H_0: \beta_{0M}=\beta_{0F}$

If the null hypothesis is true, then the common intercept,  $\beta_0$  is defined by the following equation:

$$\hat{\beta}_0 = \frac{n_M \hat{\beta}_{0M} + n_F \hat{\beta}_{0F}}{n_M + n_F} \quad (2)$$

$$T = \frac{\hat{\beta}_{0M} - \hat{\beta}_{0F}}{S_{(\hat{\beta}_{0M} - \hat{\beta}_{0F})}} \quad (3)$$

$$S_{(\beta_{0M} - \beta_{0F})}^2 = S_{P,Y|t}^2 \left[ \frac{1}{n_M} + \frac{1}{n_F} + \frac{\bar{t}_M^2}{(n_M - 1)S_{t_M}^2} + \frac{\bar{t}_F^2}{(n_F - 1)S_{t_F}^2} \right]$$

The test statistic in this case is given by:

$$\begin{cases} T \geq t_{n_M+n_F-4, 1-\alpha} & \beta_{0M} > \beta_{0F} \\ T \leq t_{n_M+n_F-4, 1-\alpha} & \beta_{0M} < \beta_{0F} \\ |T| > t_{n_M+n_F-4, 1-\alpha/2} & \beta_{0M} \neq \beta_{0F} \end{cases}$$

1. In the second approach, the dummy variable Z is defined to be 1 and 0 if the subject is male and female respectively. For the combined data, the single multiple regression models ( $Y = \beta_0 + \beta_1 t + \beta_2 Z + \beta_3 t Z + E$ ) yields the following two models for the two values of Z:

$$Z: \quad \text{If } Z=0: Y_M = \beta_0 + \beta_1 t + E$$

$$\text{If } Z=1: Y_F = (\beta_0 + \beta_2) + (\beta_1 + \beta_3) t + E$$

The value of F statistic (eq. 3) is used to test the parallelism:

$$F = \frac{[\text{Residual SS}(\text{reduced model}) - \text{Residual SS}(\text{full model})]/v}{\text{Residual MS}(\text{full model})} \quad (4)$$

- 2.

Where v in our cases is equal two, since the null hypothesis of coincidence specifies that the difference in slopes (e.g. for males and females) and that in intercepts is zero. In the other word, v

is the number of linearly independent parametric functions specified to be zero under the null hypothesis.

### 3. Analysis and Results

#### 3.1. Age influence on bone and blood lead level of environmentally exposed group of participants

Age and sex are presumed to be two variables likely to be correlated with bone lead concentration. Figure 1(a) represents bone lead concentration as a function of age for the whole group of participants. The underlying relationship between tibia lead concentration (Ti) and age (t) is expressed by the following equations:

$$Ti = (0.0935 \pm 0.0181) t - (0.303 \pm 0.703) \quad (n=262; r=0.315; P \leq 0.0001).$$

$$Ti = (0.00267 \pm 0.00096) t^2 - (0.0974 \pm 0.0708) t + (1.95 \pm 1.07) \quad (n=262; r=0.346; F=17.62^2).$$

It is seen that there is a significant linear correlation between tibia and age, but that a second order polynomial relationship with age explains significantly more of the spread in tibia lead ( $P < 0.0001$ ).

A brief inspection of Figure 1 shows increased spread in the plot of calcaneus lead (Cal) versus age (t) at both young and old ages. So, it is not surprising that calcaneus lead did not show a linear correlation with age ( $n=260; r=0.0609; P=0.328$ ). However, there is a significant relationship between calcaneus lead and a second order polynomial in age, such that:

$$Cal = (0.00793 \pm 0.0017) t^2 - (0.536 \pm 0.129) t + (13.06 \pm 1.96) \quad (n=260; r=0.279; F=10.84; P \leq 0.0001)$$

Blood lead levels (BLL) have a significant linear correlation with age (figure 2). Again, more of the variation in blood lead is explained by a second order polynomial than by the linear relationship. The underlying relationships between two variables can be expressed as:

$$BLL = (0.0169 \pm 0.0023) t + (0.736 \pm 0.089) \quad (n=262; r=0.416; P \leq 0.0001).$$

$$BLL = (0.0003 \pm 0.0001) t^2 - (0.0042 \pm 0.0090) t + (0.98 \pm 0.14) \quad (n=262; r=0.438; F=30.65; P < 0.0001).$$

By classifying the group of participants into two main categories, males and females, the association of the potential predictor variables, age and sex, on bone and blood lead level is investigated further (figure 3). The related regression equations are:

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<sup>2</sup> The partial F statistic is the ratio of the extra sum of squares due to adding the quadratic term to the residual of mean squares for the second order model.

Males:  $Ti = (0.0839 \pm 0.0252) t + (0.551 \pm 0.923)$  (n=135; r=0.278; P≤0.001)

Females:  $Ti = (0.112 \pm 0.026) t - (1.57 \pm 1.08)$  (n=127; r=0.376; P≤0.0001)

Based on the related statistical test (dummy variable, see section 2.4. above), the t test for evaluating parallelism was not significant (t = 0.99). Also, there is insufficient evidence to reject the null hypothesis of having common intercepts (t = 1.76). In both male and female subgroups, age did not correlate linearly with calcaneus (males: r=0.0324; P=0.711; females: r=0.00136; P=0.988). However, in males, the two variables correlate with each other through a quadratic function significantly, and in females, the correlation of two variables is marginally rejected at the 95% confidence level (figure 4).

Males:  $Cal = (0.0116 \pm 0.0027) t^2 - (0.826 \pm 0.196) t + (17.0 \pm 2.7)$  (n=133; r=0.350; P<0.0001; F=9.06>)

Females:  $Cal = (0.00419 \pm 0.00223) t^2 - (0.218 \pm 0.172) t + (7.82 \pm 2.86)$  (n=127; r=0.250; P=0.0626; F=4.1)

The approach to test the hypothesis of having a coincident regression line for both males and females is a little more complex with the polynomial function than with the linear relationship as explored above for tibia lead versus age. In this case, one has to use a single multiple regression model that contains one dummy variable (Z) to differentiate males and females in the following equation.

$$Cal = (0.0116 \pm 0.0026) t^2 - (0.826 \pm 0.185) t - (0.00741 \pm 0.00352) t^2 Z + (0.608 \pm 0.262) t Z - (9.18 \pm 4.01) Z + (17.03 \pm 2.57) \quad (n = 260; r = 0.314; F = 5.53)$$

The equation above yields the following separate quadratic equations:

By assuming Z=0 for males:  $Cal = (0.0116 \pm 0.0027) t^2 - (0.826 \pm 0.196) t + (17.0 \pm 2.7)$

By assuming Z=1 for females:  $Cal = (0.00419 \pm 0.00223) t^2 - (0.218 \pm 0.172) t + (7.82 \pm 2.86)$

By using information given in table 1 and equation 4 in section 2.4 above, the F statistic is calculated to be:

$$F = \frac{(3129.2 - 2484.4) / 2}{113.2} = 2.84$$

Based on the calculated F value, two lines are not coincident (P = 0.0162).

**Table 1: AVOVA table for the calcaneus lead-age regression equation**

| Source                            | d.f. | SS      | MS     | F     |
|-----------------------------------|------|---------|--------|-------|
| Regression ( $t^2$ )              | 2    | 2479.6  | 1239.8 | 10.84 |
| Residual                          | 256  | 29282.6 | 114.4  |       |
| Regression ( $t^2, Z$ )           | 3    | 2484.4  | 828.15 | 7.21  |
| Residual                          | 255  | 29277.7 | 114.8  |       |
| Regression ( $t^2, Z, tZ$ )       | 4    | 2625.8  | 656.5  | 5.72  |
| Residual                          | 254  | 29136.4 | 114.7  |       |
| Regression ( $t^2, Z, tZ, t^2Z$ ) | 5    | 3129.2  | 625.8  | 5.53  |
| Residual                          | 253  | 28633   | 113.2  |       |

Similarly, the linear relationship between blood lead level and age is expressed as:

$$\begin{aligned} \text{Males: } \text{BLL} &= (0.0163 \pm 0.0031) t + (0.791 \pm 0.115) & (n=135; r=0.409; P \leq 0.0001) \\ \text{Females: } \text{BLL} &= (0.0182 \pm 0.0034) t + (0.654 \pm 0.141) & (n=127; r=0.428; P \leq 0.0001) \end{aligned}$$

The t test of parallelism ( $t = 0.40$ ) was not significant. Thus, the lines for males and females have slopes which are consistent. Similarly, for the intercepts, for a two-sided test with  $\alpha = 0.05$ ,  $|t|$  was found to be 0.745 which accepts the null hypothesis of having a common intercept.

As a 'pilot,' the study had this examination of children's bone-Pb as a goal - to see if it would be feasible to do in the future. However, we observed that that in adults older than 15 as age increases, uncertainties in bone lead levels increases. Moreover, since adolescence happens at an average age of 15 in the next step of the study, the ages younger than 15 were excluded and the underlying relationships between bone lead concentration and age were investigated. The association of tibia lead and age were examined as:

$$\begin{aligned} \text{Males: } \text{Ti} &= (0.125 \pm 0.037) t - (1.41 \pm 1.66) & (n=86; r=0.349; P=0.000996) \\ \text{Females: } \text{Ti} &= (0.097 \pm 0.043) t - (1.03 \pm 2.04) & (n=95; r=0.225; P=0.0284) \end{aligned}$$

The t test of parallelism was not significant ( $t = 0.495$ ), and there is insufficient evidence to reject the null hypothesis of having common intercepts ( $t = 0.142$ ). Therefore, the underlying relationship of tibia lead concentration as a function of age for both males and females is expressed as:

$$\text{Ti} = (0.109 \pm 0.028) t - (1.17 \pm 1.32) \quad (n=181; r=0.275; P=0.000181)$$

Similarly, calcaneus lead and age relationships can be expressed as:

$$\begin{aligned} \text{Males: } \text{Cal} &= (0.197 \pm 0.061) t - (2.38 \pm 2.75) & (n=85; r=0.336; P=0.0017) \\ \text{Females } \text{Cal} &= (0.152 \pm 0.067) t + (1.19 \pm 3.15) & (n=95; r=0.230; P=0.0252) \end{aligned}$$



Based on t test, two lines are coincident; hence, the equation below is used to express the linear association of calcaneus lead and age:

$$\text{Cal} = (0.178 \pm 0.045) t - (0.714 \pm 2.089) \quad (n=180; r=0.283; P=0.000118)$$

Finally, for blood lead level and age relationships are given as:

$$\text{Males: BLL} = (0.0226 \pm 0.0052) t + (0.493 \pm 0.236) \quad (n=86; r=0.429; P \leq 0.0001)$$

$$\text{Females BLL} = (0.0133 \pm 0.0059) t + (0.888 \pm 0.279) \quad (n=95; r=0.228; P=0.0264)$$

Based on t test, the differences in linear regression equations of blood lead level and age are not significant, and the overall equation to express the relationships is:

$$\text{BLL} = (0.0179 \pm 0.0039) t + (0.689 \pm 0.182) \quad (n=181; r=0.321; P \leq 0.0001)$$

### 3.2. Analysis of Pb bone and blood in relation to female reproductive status

It has been shown that lead can be mobilised out of bone during pregnancy and lactation. So, one might expect that the bone lead of an adult female would be negatively related to number of pregnancies. Table 2 represents the bone lead level of females of ages 18 to 63.9 who had experienced between zero and eight pregnancy during their life time. An indicator Z was set from 0 to 8 to present the number of pregnancies. The equations below express the association of age and Z with tibia and calcaneus lead concentration:

$$\text{Ti} = (0.169 \pm 0.0647) t + (0.987 \pm 1.486) t Z - (0.0153 \pm 0.0278) Z - (4.52 \pm 2.89) \quad (n=89; r=0.353)$$

$$\text{Cal} = (0.264 \pm 0.103) t + (1.54 \pm 2.37) t Z - (0.0245 \pm 0.0444) Z - (5.19 \pm 4.62) \quad (n=89; r=0.342)$$

Using ANOVA information tabulated in table 3, F statistic for both tibia lead/age and calcaneus lead/age was calculated to be:

$$F(Z|t)_{Ti} = \frac{(479.41 - 458.6) / 2}{39.75} = 0.262$$

$$F(Z|t)_{cal} = 0.238$$

Comparing this F with  $F_{3, 85, 0.999} = 5.95$ , the null hypothesis of having coincidence lines with  $P < 0.001$  is accepted. Therefore, based on the results presented here, pregnancy does not have any effect in bone lead concentration and age relationship. Hence, the resultant equations are:

$$\text{Ti} = (0.157 \pm 0.046) t - (3.59 \pm 2.21) \quad (n=89; r=0.345; P=0.000938)$$

$$\text{Cal} = (0.242 \pm 0.0731) t - (3.69 \pm 3.53) \quad (n=89; r=0.334; P=0.00136)$$

**Table 2: Average tibia and calcaneus lead concentration in females with and without the history of pregnancy**

| # pregnancies | N  | Ti<br>(Avg ± StDev) | Cal<br>(Avg ± StDev) | Age Range | Correlation (r) of age to Ti | Correlation (r) of age to Cal |
|---------------|----|---------------------|----------------------|-----------|------------------------------|-------------------------------|
| 0             | 28 | 2.31±4.43           | 6.36±10.88           | 18-68     | 0.370 (P=0.0528)             | 0.613 (P=0.00053)             |
| 1             | 13 | 4.48±9.46           | 4.00±4.40            | 18.3-83   | 0.715 (P=0.006)              | 0.423 (P=0.149)               |
| 2             | 20 | 3.73±5.62           | 8.13±10.05           | 32.7-66.3 | 0.168 (P=0.479)              | 0.073 (P=0.761)               |
| 3             | 15 | 2.41±8.90           | 9.62±11.8            | 34.5-65.3 | 0.251 (P=0.368)              | 0.0058 (P=0.984)              |
| 4             | 7  | 6.57±5.17           | 8.05±7.03            | 39.7-75.4 | 0.173 (P=0.711)              | 0.235 (P=0.612)               |
| 5             | 5  | 6.02±5.48           | 9.60±20.17           | 38.8-70.1 | 0.626 (P=0.258)              | 0.623 (P=0.262)               |
| 8             | 1  | 13.24               | 22.64                | 63.9      |                              |                               |

**Table3: ANOVA information for tibia and calcaneus lead as a function of age for females with or without having any pregnancy during their life time.**

| Source               | d.f. | Ti     |        | Cal    |        |
|----------------------|------|--------|--------|--------|--------|
|                      |      | SS     | MS     | SS     | MS     |
| Regression (t)       | 1    | 458.6  | 458.6  | 1092.1 | 1092.1 |
| Residual             | 87   | 3399.6 | 39.08  | 8669.8 | 99.7   |
| Regression (t,Z)     | 2    | 467.5  | 233.8  | 1109.5 | 554.8  |
| Residual             | 86   | 3390.7 | 39.4   | 8652.4 | 100.6  |
| Regression (t, Z,tZ) | 3    | 479.49 | 159.83 | 1140.3 | 380.1  |
| Residual             | 85   | 3378.8 | 39.75  | 8621.6 | 101.4  |

33% of all females measured were menstruating at the time of the study; their ages ranged from 11.7 to 39.2 (table 4). To study the influence of menopause on bone lead levels, an indicator Z was set to zero and one to differentiate menopausal and menstrual status respectively.

$$Ti = (0.171 \pm 0.111) t - (0.038 \pm 0.17) tZ + (2.13 \pm 7.13) Z - (4.98 \pm 6.79) \quad (n=101; r=0.369)$$

$$Cal = - (0.133 \pm 0.166) t + (0.299 \pm 0.190) tZ - (21.53 \pm 10.67) Z + (20.5 \pm 10.2) \quad (n=101; r=0.418)$$

In this case, the respective F statistic for tibia and calcaneus lead was computed to be 0.0452 (P = 0.987) and 2.61 (P = 0.0558). Hence, the null hypothesis of having coincidence lines is accepted. Therefore, in this study, menopause did not have any effect in bone lead concentration and age regression model.

**Table 4: Human body lead levels in menopausal, premenopausal, and menstrual females**

| Status              | N  | Ti<br>(Avg±StDev) | Cal<br>(Avg±StDev) | BL<br>(Avg±StDev) | Age Range | Correlation (r) of<br>age to Ti | Correlation (r) of<br>age to Cal |
|---------------------|----|-------------------|--------------------|-------------------|-----------|---------------------------------|----------------------------------|
| Menopause           | 33 | 5.36±7.82         | 12.51±11.08        | 1.83±0.95         | 39.2-83   | 0.217 (P=0.224)                 | 0.123 (P=0.494)                  |
| Peri-<br>menopausal | 23 | 4.64±6.03         | 7.64±11.02         | 1.47±1.12         | 40.4-54.7 | 0.551 (P=0.006)                 | 0.119 (P=0.589)                  |
| Menstruation        | 45 | -0.01±4.59        | 2.86±6.33          | 0.92±0.49         | 11.7-39.7 | 0.149 (P=0.328)                 | 0.206(P=0.175)                   |

#### 4. Discussion:

For the whole group of participants, the quadratic equation of tibia lead (n=262) and calcaneus lead (n=259) and age implies that the rate of changes in tibia and calcaneus lead with respect to time is equal to:

$$\frac{\partial Ti}{\partial t} = (0.00534 \pm 0.00192)t - (0.0974 \pm 0.0708) \quad (5)$$

$$\frac{\partial Cal}{\partial t} = (0.0159 \pm 0.0034)t - (0.536 \pm 0.129)$$

According to the above equations, tibia and calcaneus lead are reaching their minimum values at about ages  $18.2 \pm 14.8$  and  $33.7 \pm 10.9$  respectively. Figure 5 represents the slope of calcaneus and tibia polynomial equations as a function of age (eq. 5) which is an indication of behavioural differences of lead in tibia and calcaneus. The rate of changes of lead in calcaneus (slope) as a function of time is faster than that in tibia; the rate of change of lead in two bone sites reach the balance at about age 42, and afterward, as age increases the differences in slopes becomes more significant. Furthermore, by figure 1, one may conclude that calcaneus lead concentration has its highest level at very young ages (2.6-11 years old), and oppositely, tibia lead level is higher in older population.

The slope of the bone lead concentration and blood lead level as a function of age is not constant over the entire age range. Morgan *et al.* (1990) studied the tibia lead concentration as a function of age in 59 subjects aged 21 to 80 years old and found a quadratic relationship between the parameters as: tibia lead (ppm) =  $-6.24 + 0.0047 \times \text{age}^2$  ( $r=0.65$ ). To have the fair comparison with Morgan's equation, the underlying relationships for the ages 21 to 80 by excluding the linear part of the regression model resulted in an equation as follow:

$$Ti = (0.0015 \pm 0.0004) t^2 + (0.150 \pm 0.933) \quad (n=163; r=0.325; P<0.0001)$$

Moreover, for females of age 15 and older (n=95), the correlation factors (r) for tibia and calcaneus was found to be 22.5% and 23% with the respective slope values of  $0.097 \pm 0.043$  and  $0.152 \pm 0.067$ , where the respective correlation factors for females of age 18 and older (n=89) were found to be 34.5% and 33.4% with the respective slope values of  $0.157 \pm 0.046$  and  $0.242 \pm 0.073$ . Based on the results, one may conclude that linearity in

bone lead contents and age become stronger as age increases. Similar to our study, Kosnett et al. (1994) reported that the slope of the relationship between age and bone lead concentration is not constant over the entire age range. The authors found that after the age of 20 years, tibia lead values inflect upward at an average slope of 0.38 ppm/y. Table 5 summarizes a comparison of tibia bone lead concentrations and the regression analysis of tibia lead content and age from some environmental in vivo XRF studies. In each case, age ranges were selected from the present study to match the earlier work. Results indicate that for significant computed  $|t|$  values, tibia lead concentrations are lower than those observed in the previous studies.

Based on the information provided in table 4, the differences between the bone lead concentrations in menopausal and peri-menopausal group of participants are not significant ( $|t| = 0.39$  for tibia lead and  $|t| = 1.62$  for calcaneus lead). However, in menopausal women because of an increase in bone remodeling (Heaney *et al.* 1978), one may expect the lower bone lead concentrations in menopausal groups. The reason for disagreement between the results presented here and post-mortem studies might be because of having the participants exposed to lead via different sources. In other words, in our study, participants are neither exposed to lead occupationally nor exposed to the common source of lead in their routine life (e.g. using lead pots for cooking). Roy *et al.* (1997) found the slopes of the regression lines of tibia lead vs. age for females (n=90) and males (n=59), ages from 6 to 80,  $0.277 \pm 0.038$  and  $0.285 \pm 0.041$  respectively. Also, the authors reported on no statistically significant difference between the age dependence of tibia lead in males and females. Gamblin *et al.* (1994) reported the slope of tibia lead as a function of age for males (n=38 ages 23-70) and females (n=73 ages 19-81) to be  $0.44 \pm 0.05$  and  $0.26 \pm 0.05$  respectively. In the study presented here, we reported the respective slopes for females and males to be  $0.120 \pm 0.026$  and  $0.0839 \pm 0.0252$ . To have a fair comparison with Gamblin *et al.* results, we consider the same age range that they reported and the slopes for males and females tibia lead as a function of age was found to be  $0.167 \pm 0.047$  (n=73,  $r=0.390$ ,  $P=0.000592$ ) and  $0.109 \pm 0.032$  (n=105,  $r=0.314$ ,  $P=0.0011$ ) respectively. The change in the slope of (bone Pb vs. age) vs. time is a decline in input rates as background lead level has been decreased. In all of the cases, the correlation between the bone lead is stronger in males than in females that can be an indication of nonlinearity in relationships for females since during the menopause due to the bone resorption process, bone acts as a secondary source of lead exposure, and therefore, blood lead level increases, and bone lead concentration declines (Gamblin *et al.* 1994). Kosnett et al. (1994) reported that sex differences in regression models of bone lead values and age were insignificant up until the sixth decade, where the conclusion was not consistent for the current study as no significant correlation was found between bone lead concentration and age in females and males of age 60 and older.

**Table 5: Tibia lead level and Regression Models for Bone Lead Concentration in Several Environmental Cohorts**

| Previous Studies |      |     |                                     |  |                    |                                      | Present Study |  |                      |                                     |  |
|------------------|------|-----|-------------------------------------|--|--------------------|--------------------------------------|---------------|--|----------------------|-------------------------------------|--|
| Study            | Year | n   | Age Range                           | Min/<br>Max<br>Ti<br>$\mu\text{g Pb g}^{-1}$ | Mean $\pm$<br>SD   | Regression<br>Model                  | n             | Min/Max<br>Ti<br>$\mu\text{g Pb g}^{-1}$ | Mean $\pm$<br>SD     | [T]<br>values<br>for mean<br>values | Regression Model   |
| Hoppin           | 1995 | 23  | 18-21                               | -1.5 / 8.2                                   | 3.0 $\pm$<br>2.3   | Ti=1.5t-<br>25.8<br>( $r^2=0.3033$ ) | 8             | -9.22 /<br>8.58                          | 2.28 $\pm$<br>5.73   | 0.34                                | No Correlation   |
| Hoppin           | 1997 | 168 | 13-19                               | -7.15 /<br>14.23                             | 4.0 $\pm$<br>4.4   | No<br>Correlation                    | 31            | -10.42 /<br>13.36                        | -0.095 $\pm$<br>5.61 | 3.85                                | No Correlation   |
| Kosnett          | 1994 | 100 | 11-78                               | -12 / 69                                     | 12.7 $\pm$<br>14.6 | ( $r=0.69$ ;<br>P<0.0001)            | 21<br>5       | -18.93 /<br>18.27                        | 2.81 $\pm$ 6.29      | 6.50                                | ( $r=0.376$ ;<br>P<0.0001)   |
| Morgan           | 1989 | 59  | 21-80                               | -10/ 42                                      | 7.2 $\pm$<br>10.8  | $r=0.65$<br>non-linear<br>model      | 16<br>3       | -18.93 /<br>18.27                        | 3.68 $\pm$ 6.25      | 2.36                                | $r=0.325$ ;<br>P<0.0001  |
| Ahmed            | 2005 | 15  | 19.3-34.1<br>Vermont<br>Study       | -6.5 /<br>6.8                                | 0.7 $\pm$<br>3.1   | Not tested                           | 41            | -14.63 /<br>12.61                        | 0.85 $\pm$ 5.27      | 0.13                                | Ti= $(-0.403 \pm 0.184)$<br>$+ (12.09 \pm 5.19)$<br>( $r=0.365$ ;<br>P=0.0344) |
| Ahmed            | 2005 | 19  | 18.2-30.5<br>New Brunswick<br>Study | -2.1/ 5.7                                    | 0.5 $\pm$<br>1.7   | Not tested                           | 33            | -9.22 /<br>12.61                         | 1.75 $\pm$ 4.83      | 1.34                                | No correlation   |

## **5. Conclusion**

In this data set, both bone and blood lead were lowest in young adults. The lead levels tended to rise steadily with age beyond the age of 40 or so. Perhaps surprisingly, higher bone and blood lead levels were also observed amongst the pre-pubertal participants. Because of these different trends over different age ranges, the overall relationship of bone or blood lead with age was better described by a quadratic, rather than a linear function. If only those subjects older than 15 years were considered, linear relationships were adequate. Amongst these data, differences attributable to the sex of the participant were not sufficiently strong to achieve statistical significance. Also, expected variation of bone and blood lead amongst the women with menstrual status, parity or breast feeding was not apparent amongst these data.

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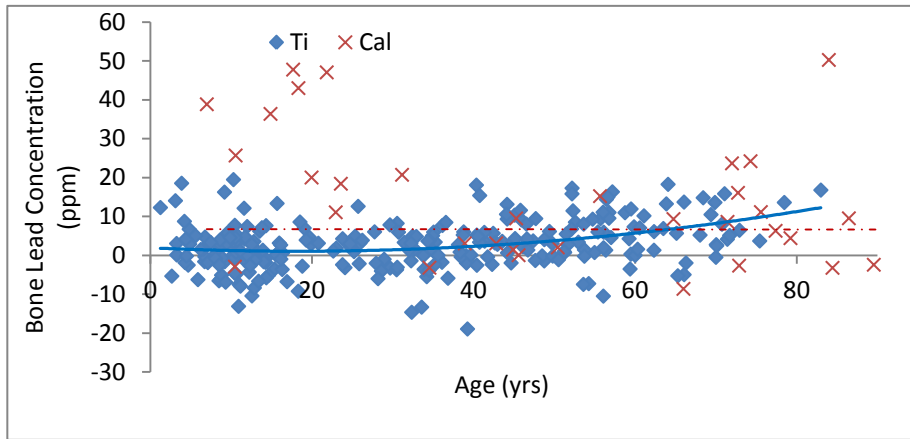


Figure 1: Bone lead concentration as a function of age for the environmentally exposed group of participants

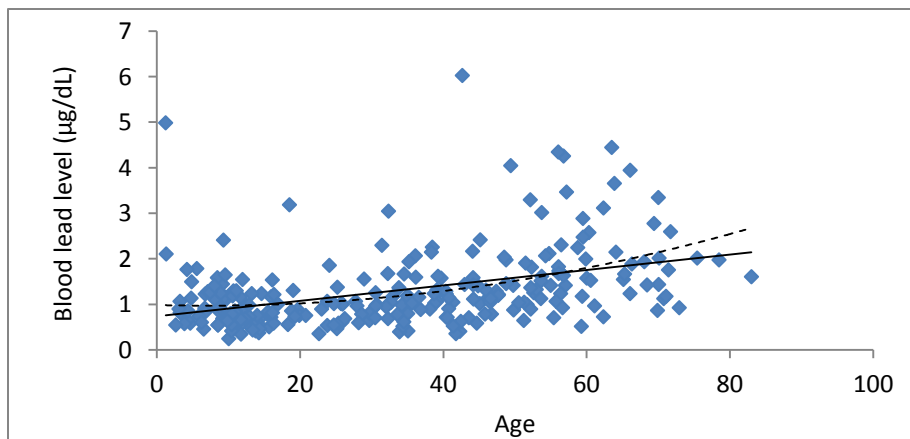


Figure 2: Blood lead levels as a function of age

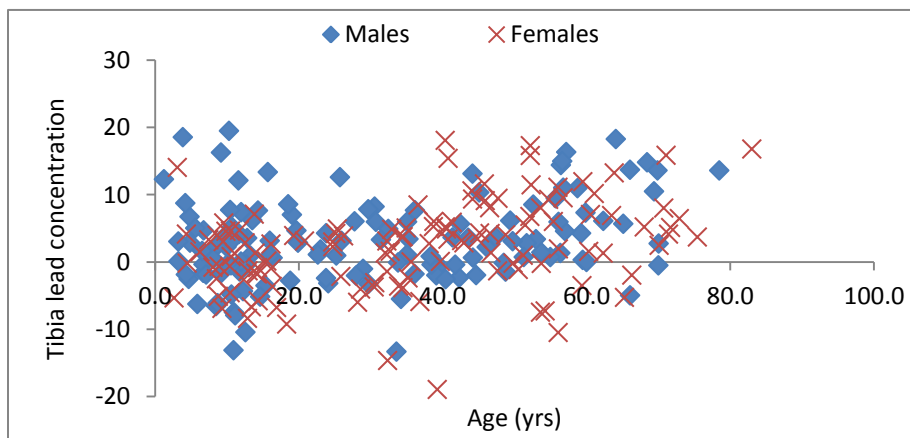
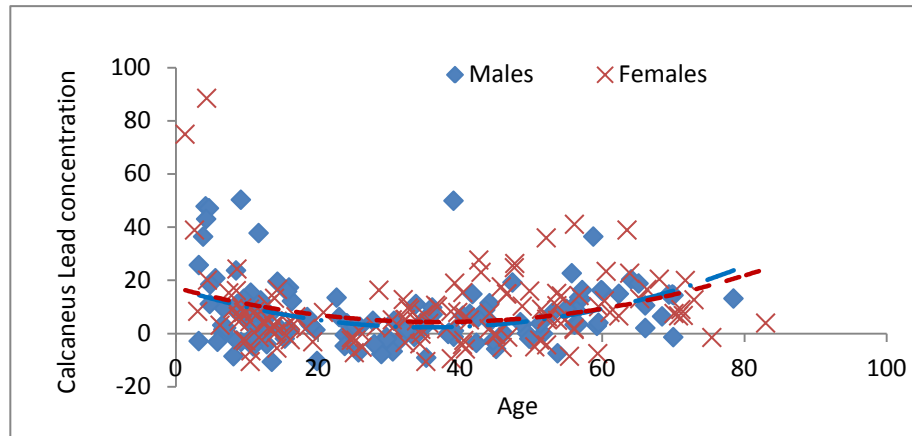
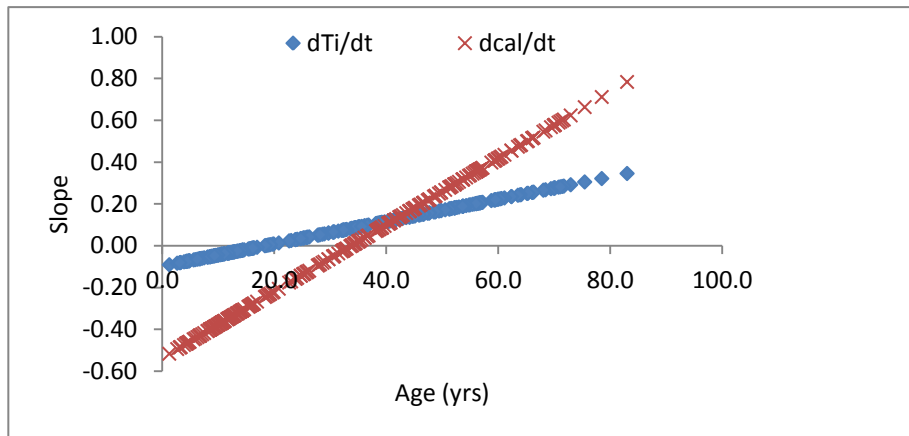


Figure 3: Tibia lead concentration as a function of age for males and females



**Figure 4: Calcaneus lead concentration as a function of age for males and females**



**Figure 5: The figure presents the slope of tibia and calcaneus lead concentration as a function of age**



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## Chapter 8

### Conclusion and Discussion

In this thesis, for the first time, we confirmed a nonlinear relationship between bone lead concentration and CBLI. The transfer rate of lead from blood becomes more efficient for the higher levels of exposure. In most of the previous works, the relationship between tibia and calcaneus lead concentration have been reported to be linear as, Cake *et al.* (1996), Fleming *et al.* (1997) and Somerville *et al.* (1989) have reported a strong correlation between lead concentration in calcaneus and tibia with the slope values of  $1.17 \pm 0.16$  (n = 49),  $1.7 \pm 0.04$  (n = 367), and 2.03 (n = 120) of an occupationally exposed group of participants respectively. The differences in slopes of various studies can be an indication of having non-linear relationship between tibia and calcaneus lead levels. For the first time, we examined the underlying relationships between lead concentration in two bone sites by a polynomial function, which means the changes of lead in one bone site depends on the concentration of lead in another bone site. We have not observed a strong correlation in tibia and calcaneus lead of the general population study.

One of the challenging goals at lead studies is to detect lead at very low levels of concentration. In the study of the general population, the factors that influence uncertainties in lead contents in tibia (cortical bone) and calcaneus (trabecular bone) were discussed based on sex, age, and BMI. We observed significant differences for both Cal and Ti uncertainty measures among the age groups, where the uncertainties were highest

in the lowest age group (<11). Also, we found that the product of source activity and measurement time will influence the precision of measurements significantly.

The transfer rate of lead from bone to blood was reported for the first time to be not a constant value, but a function of body lead contents, age, and employment time. The transfer rate of lead from cortical bone to blood can be solely expressed as a function of CBLI. The model was successfully developed for smelter employees. For future work, the model can be expanded for the general population.

Both bone and blood leads were lowest in young adults. The lead levels tended to rise steadily with age beyond the age of 40 or so. Because of these different trends over different age ranges, the overall relationship of bone or blood lead with age was better described by a quadratic, rather than a linear function.

Regarding the etiologic contribution of lead to increase the risk of hypertension, questions are still unanswered since the few longitudinal studies have examined the association of blood lead on the risk of hypertension (Cheng *et al* 2001, Glenn *et al* 2003). On the other hand, the studies were not unanimous in identifying lead as a risk factor. Selecting appropriate lead biomarkers may be essential to answering the questions. For smelter employees, having blood lead levels and blood pressures available in many time points and bone lead levels in three time points can provide us with a solid longitudinal study to find out whether the association of blood lead or bone lead with increased blood pressure is acute or chronic in nature. Furthermore, the influence of employment time and strike in different subgroups (G1, G2, G3, and G4) on the relationship of body lead contents and blood pressure can be assessed, where the

relationship can be identified to be nonlinear depending on the level of lead exposure, age, and employment time. Moreover, since CBLI can be a good estimate of total exposure, we are able to examine the relationship of CBLI and blood pressure for the first time.

Furthermore, lead screening during pregnancy and lactation is essential for immigrants who have been exposed to high levels of air pollutants in their original countries. Adverse effects of lead exposure are being identified at ever lower levels of exposure in both children and adults and can cause infants' and children's intellectual deficits. The adverse effects of prenatal lead exposure on maternal health and infant outcomes across a wide range of maternal blood lead levels have been well documented (Bellinger 1991, 1995). Therefore, women who have been exposed to high levels of air pollutants, despite having low/moderate blood lead level, may have high bone lead concentration which can be released back to their blood stream during their pregnancy and lactation and have adverse effects on their fetuses and infants' health.

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