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Introduction and Summary. International Workshop on Lead in Bone: Implications for Dosimetry and Toxicology

by Gunnar F. Nordberg,* Kathryn R. Mahaffey,† and Bruce A. Fowler‡

Lead toxicity is a major public health problem in the United States. Recent statistics indicate that in the United States, between 3 and 4 million children ages 6 months to 5 years and approximately 400,000 infants *in utero* are exposed each year to lead in quantities associated with blood lead concentrations in excess of 15 g/dL whole blood. Blood lead concentrations chiefly reflect recent lead exposures. Lead is accumulated in the human skeleton, which is generally thought to provide an indicator of body burden of lead.

Previous information based on chemical analyses of skeletal tissues indicate that among adults, over 95% of lead is in the skeleton. For children, approximately 70% of total body lead is present in osseous tissues. Since it is rarely possible to analyze either the total skeleton, or even an entire bone, biopsy techniques have been developed. Biopsies usually are accomplished by removing a small sample of bone (for example, several hundred milligrams) from the skeleton and conducting chemical analyses for the material of interest, e.g., lead. A major question about biopsies is whether or not the sample taken is representative of the entire bone or skeleton. Techniques for measurement of lead *in vivo* have been developed to replace the invasive procedures used in bone biopsy and yet provide a measure of this body lead pool.

Data on chemical measurements of lead in the skeleton based on analyses of samples from the tibia, skull, ileum, vertebra, and rib have been used to estimate total skeletal lead for specific age and sex groups. Methods have been developed to estimate skeletal burden of lead from selected bones for persons of specified age and sex groups. During periods of rapid growth or changes in bone metabolism (e.g., chemical maturation of the skeleton), the ability to predict skeletal lead from a single bone is reduced. In general, the macrodistribution of some elements of interest within the total skeleton is not readily available.

Bone physiology is complex, and specific types of bone have different rates of growth and mineralization, as well as final density. Normal bone growth proceeds in a predictable manner in which cartilaginous matrix is converted to mineralized cartilage and layer to mature, well-calcified bone. Recent studies presented at this conference emphasized newer areas regarding the role of cation-binding proteins that are part of the organic matrix. These proteins have age-specific distributions that differ by bone type and age of the subject. For example, some of these proteins are present only among very young animals. These cationic proteins also occur in nonosseous tissues.

The process by which bone is formed and resorbed is controlled by a number of endocrine and metabolic factors. There appear to be a number of cellular targets for lead. Recent studies have specifically examined lead interference with calcium metabolism in osseous tissue with emphasis on endocrine and cellular (e.g., second messenger) alterations. There are now a number of cellular systems for studying bone resorption and effects of lead on subcellular metabolic processes.

Lead present in any particular tissue compartment represents a combination of current environmental lead exposure and internal sources of lead. Although bone is generally regarded as a major storage site for lead, under conditions of either reduced external exposure to lead or bone demineralization, bone can become an internal source of lead. Other studies presented at this conference summarized information on biokinetics of lead, noting that turnover varies greatly from bone to bone with age and sex of the subject. This mobilization of bone can greatly affect blood lead concentration. Skeletal turnover is highest among children under 10 years of age. Turnover declines to about

*Department of Environmental Hygiene, University of Umea, Umea, Sweden.

†National Institute of Environmental Health Sciences, P. O. Box 12233, Research Triangle Park, NC 27709.

‡Program in Toxicology, University of Maryland, 660 West Redwood Street, Baltimore, MD 21201.

Address reprint requests to B. A. Fowler, Program in Toxicology, University of Maryland, 660 West Redwood Street, Baltimore, MD 21201.

2 to 4% among middle-aged adults and increases among elderly females. Bone turnover is strongly influenced by factors that include nutritional status, age, and pathological conditions such as osteoporosis.

Various techniques exist for measurement of tissue lead concentrations. However, nuclear techniques have been developed that are applicable to *in situ, in vivo* analysis of metals. There was extensive discussion of fundamental design issues with regard to geometry and radiation used in X-ray fluorescence with emphasis on efficiency of excitation, background scatter, and penetration depth. The currently available methodologies for *in situ, in vivo* analysis of bone lead concentrations are based on L X-ray using an iodine-125 source and K X-ray using either cadmium-109 or cobalt-57 sources. L X-ray and K X-ray techniques analyze only extremely superficial bones very near the skin, such as tibia or calcaneus. Even among these bones, the L X-ray is limited to extremely superficial layers, measuring at most the top 1 or 2 mm of bone depending on the layer of skin covering. The K X-ray penetrates to a much greater depth, approximately 20 to 40 mm of bone.

The distribution of lead between the most superficial and deeper part of the cortex will be of immense importance in interpreting the results of bone the L and K X-ray fluorescence analyses. Data presented by several investigators demonstrated variations in concentrations of lead in specific bone regions. The L X-ray and K X-ray are very likely detecting different pools of lead within bone. For example, the L X-ray appears to identify a peak of lead just under the top millimeter of the periosteum. This peak appears to correspond to recently deposited lead and correlate well with the ethylene diamine tetraacetate (EDTA)-chelatable lead pool. By contrast, the K X-ray method provides biopsy of a much larger sample of bone lead. Whether or not these methods sample pools of lead that have different metabolic significance remains a research issue.

Conference participants shared their experiences and expertise to assess research needs for further development of X-ray fluorescence methodology. The K X-ray method expresses lead on the basis of bone mineral concentration (e.g., μg lead/g bone mineral or μg lead/g calcium). The L X-ray system, although it samples only a much smaller depth, expresses lead relative to an area rather than on a bone mineral basis. Both methods offer advantages depending on the research question. Under some circumstances, it may be useful to measure lead based on surface area (e.g., rapidly changing conditions of external lead exposure), while under other conditions expressing lead content only on the basis of bone mineral (e.g., osteoporosis) would provide more useful information.

The extent to which the skin and bone marrow in children are exposed to radiation from these methods has been assessed. The radiation dose was generally considered to be less than whole body exposures to less than a day of natural earth radiation or, stated another way, a fraction of the radiation from a dental X-ray. It should be noted that these are difficult comparisons because the radiation estimates are to relatively broad body surface areas, whereas this dose is focused on a small area. Overall, however, the health physicists regarded the quantities of radiation used by these techniques as having very minor health significance.

Bone lead concentration and its measurement quality are determined, in part, by measurement variation, exposure variation, and individual metabolic conditions. There are a number of different approaches that can be used to evaluate techniques for measurement of lead in bone. The predominant issue at this stage of research development is the extent to which these measurements reflect overall bone lead pool. Bone lead is a good indicator of long-term lead absorption. Cortical bone has an average half-time for lead of some 10 to 20 years, while trabecular bone has approximately a 5-year half-time.

Most of the experience in measurement of bone lead *in vivo* among adults has been conducted with the K X-ray instrumentation. Efforts to evaluate the contribution of skeletal lead to blood lead have been carried out by several groups of investigators among active and retired lead workers. Among retired workers, a substantial proportion of lead in blood (e.g., two-thirds) was related to previously accumulated skeletal stores of lead. Based on chemical analyses of lead concentrations from vertebral biopsy samples of bone, a good relation was observed between blood lead and vertebral lead for both active and retired workers. Comparison of such values with lead excretion by provocative EDTA chelation tests indicated a lower correlation. However, the lead pool removed by provocative EDTA chelation may be representative of a biologically more active lead pool.

The L X-ray system has been used to measure bone lead among children. Instrumentation developed using the L X-ray system measures lead in the most superficial portion of the bone. The technique has been used for investigations of children who required treatment for lead toxicity. It is worthy of substantial attention that after recommended medical treatment, following guidelines from professional associations and the Centers for Disease Control, that these children had bone concentrations generally in excess of adults having typical and occupational exposures to lead. Lead produces chronic adverse effects on health that makes these highly elevated bone lead concentrations in such young children particularly dangerous.

Using the X-ray methodology, the actual depth of bone mineral measured is highly dependent upon the thickness of soft tissue (e.g., skin) covering the bone. This method measures the most superficial layer of bone mineral, immediately under the periosteum. The radiation dose to the skin is approximately 1/20th that of a chest X-ray. The current detection limit of the L X-ray method has a detection limit of 7 μg lead/g of bone with 9.2% *in vivo* reproducibility of these methods. One of the major difficulties with application of the L X-ray methodology is that the general population of children

has bone lead concentrations lower than 7 μg lead/g of bone. A figure frequently cited for children is 5 μg lead/g of bone. Improvement in the L X-ray methodology could be achieved through columination of the electron beam to reduce background scatter; improved beam polarization; more sensitive detectors; and alternative methods for statistical analysis of the signal. Both the L X-ray and K X-ray quantitative limits could be improved by longer counting time or changing the configuration of the detectors to capture energy emitted from the bone. Longer counting times for *in vivo* measurements may create problems because the lead is expressed on the basis of bone mineral. The K X-ray has analytical sensitivity to approximately ±5 μg lead/g bone mineral.

The overall view of the conference participants was that the L X-ray and K X-ray measure different fractions of bone lead, although the K and L X-rays may be compared on technical issues, such as quantitative limits. It appears that L X-ray quantifies lead nearest the periosteum, which may be associated closely with lead accessible by provocative EDTA chelation (i.e., a diagnostic technique) reflecting relatively more recent lead exposure than that identified in deeper bone layers by the K X-ray techniques. The K X-ray methodology samples a much larger area of bone and appears to be more reflective of chronic, cumulative exposure to lead. The radiation dose was generally not regarded as substantial issue for either of these techniques.

Advances in being able to measure bone lead *in vivo* are advantageous in diagnosis of lead toxicity. Another use of *in vivo* measurement of bone lead is to develop improved methods to be used in screening or epidemiological studies that assess potential association of body lead burden with adverse health effects. Current research on lead and health indicate subtle but significant neurobehavioral and cognitive dysfunction at lead exposures present in approximately 40 to 50% of the general population of young children. These critical effects are considered to be associated with lead in nonosseous tissues, e.g., the central nervous system. To date, lead exposures associated with these effects have been referenced to blood lead concentrations and, in some instances, to dentine lead concentrations and the indices of internal exposure.

Bone and dentine lead are indicators of an integrated soft tissue dose of lead over time. Bone lead concentrations have been associated with blood lead concentrations, however, typically at higher blood lead quantities, e.g., > 20 μg lead/dL whole blood. Numerous strategies exist to improve the count rate (i.e., capture of fluorescence emitted from the bone or capture of emitted radiation from the bone) without increasing radiation of the bone or increasing counting time.

A number of physiological and pathological conditions can alter the distribution of lead between bone and nonosseous tissues. These include age, nutritional and endocrine status of the subject, pregnancy, lactation, osteoporosis, and renal disease. Children have a far higher rate of skeletal turnover and remodeling of bone, resulting in a potentially larger pool of biologically active lead. Pregnancy and lactation represent severe calcium stresses on the adult woman that are typically accompanied by mobilization of skeletal mineral. Lead is mobilized along with bone mineral and transferred across the placenta or is secreted in milk during lactation.

A critical factor in designing effective methods for measurement of bone lead concentrations is understanding the types of research questions that such measurements can be used to answer. In our enthusiasm to be able to measure bone lead *in vivo* and *in situ*, it is critical not to lose sight of the fundamental recognition that development of biological indicators of the physiological active pool of lead remains an essential consideration. To date most research on adverse effects of lead have been referenced to blood lead concentration, which does not estimate long-term lead exposure. Lead accumulation in bone can become an internal source of lead that may be particularly important in development of chronic disease. Both L X-ray and K X-ray instrumentation potentially provide important contributions to understanding the association between external lead dose, internal lead dose, and human health effects. Further research is also clearly needed on the cellular and subcellular deposition of lead in bone, since such data may provide useful interpretation of information about the biochemical active lead pool in bone.

Effects of Lead on Target Organ Systems

The second phase of the workshop examined the known and potential consequences of lead mobilization from bone on various target organ systems. There are published clinical studies that indicate that mobilization of lead from the maternal skeleton during pregnancy is associated with significant exposure to both the mother and fetus. Clinical case studies suggest that in some instances this mobilization may be of sufficient magnitude to acutely intoxicate both mother and infant. At lower dose levels, skeletal mobilization of lead may produce decreases in stature and behavioral and cognitive function deficits in infants. Measurements of maternal skeletal burdens of lead thus have great potential for assessing risk in this critical human population.

A similar set of concerns was expressed for geriatric populations where demineralization of the skeleton is also known to occur as part of the aging process. In particular, post-menopausal women undergoing osteoporotic changes are of special concern. Mobilization of lifetime lead stores during the later stages of life with redistribution to soft tissues such as the brain and kidney would have widespread societal and public health implications.

The influence of nutrition factors such as dietary intake of calcium and other essential minerals in both lead absorption from the gastrointestinal tract and

mobilization from skeleton was also extensively examined. It was concluded that such dietary factors are of clear importance in mediating the availability of lead *in vivo*. The influence of diet at various life stages is an area of great potential significance in preventing or attenuating internal lead exposure.

Release of lead from bone stores as a function and cause of renal disease was also examined in detail. Renal osteodystrophy is a common complication of chronic kidney disease. Lead incorporation into bone and structure of metaphysis bone have not been found to be altered in lead-intoxicated rats, but further studies in humans are needed. Statistical studies of lead, blood pressure, and cardiovascular disease showed a small but attributable risk associated with lead. Various analyses suggested that a change in the population mean blood lead from 25 to 10 μg/dL would reduce myocardial infarctions by approximately 30,000/year and the incidence of all cardiovascular disease in the United States by several million.

The well-known elevated incidence of renal disease in lead-exposed workers was examined in light of recent mechanistic studies concerning the effects of lead in the kidney. The known and possible roles of high-affinity renal lead-binding proteins (PbBP) which appear to play a central role in mediating the effects of lead in renal proximal tubule cells of rats at low dose levels was extensively discussed. The renal PbBP in rats was found to be a specific cleavage product of α_2-microglobulin, which facilitated the nuclear uptake of lead and bound to renal chromatin. This molecule, which is a member of the retinol-binding protein family, was immunologically distinct from a chemically similar protein in rat brain and was highly localized in only certain nephrons and proximal tubule segments. It was hypothesized that there are analogous lead receptor molecules in humans that act to determine individual susceptibility to lead toxicity on the basis of genetic expression. Such a finding would have a far-reaching impact on delineation of highly sensitive human populations at special risk for lead toxicity.

Strategies using new techniques for epidemiological studies in known human populations at risk for lead toxicity were discussed on the final day of the workshop. The special problems associated with elevated lead exposure in pediatric populations received special attention. Decrements in IQ at both ends of the intelligence spectrum and delinquent behaviors associated with lead may have important societal consequences.

There was a review of the potential utility of bone lead measurements in exposed worker populations and the need for more thorough evaluations of lead effects on target organs such as the kidney, immune, and reproductive organ systems. The high incidence of renal failure (2–6 times) among lead-exposed workers is of particular concern since over 50% of end-stage renal disease in the United States is of unknown etiology. The critical need for the development of new and more sensitive biological indicators of lead-exposure, effect, and susceptibility in workers and the general populations was also discussed. Examples of such indicators might include refinements (e.g., molecular biology) of known approaches involving lead-induced disturbances of the heme biosynthetic pathway or development of radioimmunoassays for specific excreted proteins such as the renal PbBP that appear to be involved in mediating the low-level effects of lead in target cell populations of the nephron. There appeared to be general agreement among the workshop participants that development of such indicators coupled with the better estimates of internal lead stores provided by X-ray fluorescence analyses of bone would provide the more rigorous and sensitive analytical tools which will be required to making risk assessments at low-level exposures.

Summary Statements

The L X-ray and K X-ray appear to measure different pools of lead in bone. It is not yet clear which of these pools (or if both of these pools) is most clearly associated with recent lead exposures, with other parameters of lead exposure (e.g., blood lead, EDTA chelatable lead, or hemopoietic indicators of lead exposure), and most importantly with disease outcome.

Distribution of lead between bone and nonosseous tissues depends on factors including age, nutrition, endocrine status, gender, pregnancy, lactation, and pathological conditions including osteoporosis and renal failure.

Use of methods to measure bone lead by either L X-ray or K X-ray should be regarded as largely research or clinical investigation techniques. As they now exist, these techniques do not have current clinical application other than clinical investigation. Several years of refinement of methods are likely to be needed before these techniques are broadly applicable.

Because current treatment methods of lead toxicity do not adequately remove lead from bone (i.e., young children after treatment may have bone lead concentrations similar to adults in the general and occupationally lead-exposed populations), lead remaining in bone can become an internal source of lead and release lead under adverse metabolic conditions.

Lead has a long half-life in bone: over 10 years for many types of bone. This can serve as a biomarker of lead exposures that occurred many years earlier.

Mobilization of skeletal lead stores during pregnancy may expose both the mother and fetus to sufficient quantities of biologically active lead to produce both learning, behavioral deficits in the fetus. Mobilization of skeletal lead in geriatric populations as a result of osteoporosis or normal skeletal demineralization may produce deleterious target organ system effects in latter stages of life.

Nutritional factors such as dietary calcium intake appear to play important roles in mediating lead absorption form the gastrointestinal tract and mobilization of lead from the skeleton.

Renal osteodystrophy is a common complication of

renal failure that occurs at greater frequency in lead-exposed workers.

Statistical analyses of lead blood pressure and cardiovascular disease showed a small but attributable risk associated with lead.

Studies in experimental animals have demonstrated the presence of lead-binding proteins in major target organs such as the kidney and brain that appear to share a number of characteristics with receptors for other biologically active molecules. These molecules may play a central role in delineating populations at special risk for lead toxicity.

Lead has been shown to produce a number of learning and behavioral deficits in humans that appear to have a number of serious societal consequences. Measurements of lead in bone may greatly aid in assessing behavioral deficits in humans by better defining dosimetry.

More research is urgently needed to evaluate the effects of lead to relatively unstudied target organ systems for lead such as the kidney, immune, reproductive, and skeletal systems. There is a critical need for new classes of biological indicators for assessing lead exposure, lead effects, and susceptibility to lead toxicity at low exposure levels.