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A critical review of biomarkers used for monitoring human exposure to lead: advantages, limitations and future needs

Uma avaliação crítica sobre biomarcadores utilizados para o monitoramento biológico de exposição ao chumbo: vantagens, limitações e perspectivas futuras

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Abstract *Lead concentration in whole blood (Blood-Pb) is the primary biomarker used to monitor exposure to this metallic element. However, the difficulty in assessing the exact nature of Pb exposure is dependent not so much on problems with current analytical methodologies, but rather on the complex toxicokinetics of Pb within various body compartments. If we are to differentiate more effectively between Pb that is stored in the body for years and Pb from recent exposure, information on other biomarkers of exposure may be needed. None of the current biomarkers of internal Pb dose has yet been accepted by the scientific community as a reliable substitute for a Blood-Pb measurement. This review focuses on the limitations of biomarkers of Pb exposure, and the need to improve the accuracy of their measurement. We present here only the traditional analytical protocols in current use and we attempt to assess the influence of confounding variables on Blood-Pb levels. Finally, we discuss the interpretation of Blood-Pb data with respect to both external and endogenous Pb exposure, past or recent exposure, as well as the significance of lead determinations in human specimens including hair, nails, saliva, bone, blood, urine, feces, and exfoliated teeth.*

Key words *Lead, Biomonitoring, Biomarkers of exposure*

Resumo *A concentração de chumbo (Pb) no sangue total (Pb-B) vem sendo comumente utilizada para monitorar a exposição a este elemento químico. Entretanto, a dificuldade em avaliar a natureza exata da exposição ao Pb é dependente não só de problemas inerentes a metodologias analíticas inapropriadas, bem como da toxicocinética complexa do Pb em compartimentos de nosso corpo. Se quisermos diferenciar mais efetivamente entre o Pb que está estocado no corpo por anos daquele proveniente de uma exposição recente, deverão ser obtidas informações pela análise de outros biomarcadores de exposição. Entretanto, nenhum dos biomarcadores de dose interna para Pb é aceito pela comunidade científica como substituto ao Pb-B. O foco desta revisão está nas limitações de biomarcadores de exposição ao Pb e nas necessidades para melhorar a exatidão nas determinações. Procuramos apresentar somente os protocolos analíticos em uso corrente e tentamos avaliar a infuência de variáveis de confusão nos níveis de Pb-B. Finalmente, fizemos uma discussão sobre a interpretação dos dados de Pb-B com respeito a fontes de exposição, sejam elas endógenas e exógenas, recente ou passada, bem como a importância das determinações de Pb no cabelo, unhas, saliva, ossos, sangue (plasma e sangue total), urina, fezes e dente decíduo.*

Palavras-chave *Chumbo, Biomonitoramento, Biomarcadores de exposição*

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Introduction

Over the last two decades, atmospheric concentrations of lead (Pb) have decreased significantly around the globe, as more and more nations have chosen to remove tetraethylead from gasoline (Thomas *et al.*[1]). However, humans may also be exposed to Pb through contaminated food, water, and house dust, and through remaining industrial activities, such as metal recycling and the battery industry. In the US, for example, although the use of lead in house paint peaked in 1940 and was banned in 1978, 40% of the nation's housing stock is estimated still to contain lead-based paint (Wakefield[2]).

After Pb enters the body, it can travel along several pathways, depending on its source and, by extension, its bioavailability. The fraction of Pb that is absorbed depends mainly on the physical and chemical form, particularly particle size and the solubility of the specific compound. Other important factors are specific to the exposed subject, such as age, gender, nutritional status and, possibly, genetic background (National Research Council[3], ATSDR[4]). One of the earliest toxicokinetics studies reported that Pb, once absorbed into the blood compartment, has a mean biological half-life of about 40 days in adult males (Rabinowitz *et al.*[5]). The half-life in children and in pregnant women was reported to be longer, due to bone remodeling (Gulson *et al.*[6], Manton *et al.*[7]). However, another study was unable to confirm this finding (Succop *et al.*[8]). Like many other "bone-seeking" elements, Pb from blood is incorporated into calcified tissues such as bone and teeth, where it can remain for years (Rabinowitz[9], O'Flaherty[10]). According to Rabinowitz[9], the "half-life" of Pb in bone (Bone-Pb) ranges from 10 to 30 years. However, the use of the term half-life to describe the biological clearance of Pb from bone implicitly makes assumptions about the kinetics of the process by which Pb is released. Some workers prefer to use the term "residence time", to avoid implying something more precise than what can be directly determined (D. Chettle, personal communication). From calcified tissue stores, Pb is slowly released, depending on bone turnover rates, which in turn are a function of the type of bone, whether compact (slow turnover) or trabecular (rapid turnover) (O'Flaherty[10]). Brito *et al.*[11] reported that the release rate of Pb from bone varies with age and intensity of exposure. This group also examined estimates of exchange rates amongst compartments (Brito[12]). The transfer of Pb from blood to other compartments was shown to be very much more rapid than the one-month estimate reported previously by others, with the overall clearance rate from blood (sum of rates from blood to cortical bone, to trabecular bone and to other tissue, implying a, "half-value of 10 to 12 days" (Brito[12]). This highlights the difference between the overall clearance viewed from outside, when no allowance can be made for recirculation, and actual transfer rates.

Physiological differences between children and adults account for much of the increased susceptibility of small children to the deleterious effects of Pb: whereas in adults 94% of Pb body burden is stored in bones and teeth, this proportion is only 70% in children (Barry[13]). Besides, the continuous growth of young children implies constant bone remodeling for skeletal development (O'Flaherty[10]). This contributes to a state in which Pb stored in bone is continually released back into the blood compartment, as what has been described as "endogenous contamination" (Gulson *et al.*[6]). This process is particularly significant for pregnant women, since pregnancy also causes an increase in bone remodeling. The apparently limited success of various Pb hazard control measures in decreasing Blood-Pb levels in exposed children and pregnant women may reflect a constant bone resorption process (Rust *et al.*[14]). Interestingly, Popovic *et al.*[15] recently reported very different long-term Pb kinetics between men and women, with pre-menopausal women appearing to retain Pb more avidly or release Pb more slowly compared to postmenopausal women and men.

Biomonitoring of human exposure to lead

Biomonitoring for human exposure to Pb reflects an individual's current body burden, which is a function of recent and/or past exposure. Thus, the appropriate selection and measurement of biomarkers of lead exposure is of critical importance for health care management purposes, public health decision-making, and primary prevention activities in the future.

It is well known that Pb affects several enzymatic processes responsible for heme synthesis. Pb directly inhibits the activity of the cytoplasmic enzyme δ-aminolevulinic acid dehy-

dratase (ALA-D), resulting in a negative exponential relationship between ALA-D and Blood-Pb. There is also depression of coproporphyrinogen oxidase, resulting in increased coproporphyrin activity. Pb also interferes with the normal functioning of the intramitochondrial enzyme ferrochelatase that is directly responsible for the chelation of Fe by protoporphyrin. Failure to insert Fe into the protoporphyrin ring results in depressed heme formation and an accumulation of protoporphyrin; this in turn chelates Zn in place of Fe, to form zinc protoporphyrin (ZPP). These effects also result in modifications of some other metabolite concentrations in urine (ALA-U), blood (ALA-B) and plasma (ALA-P), coproporphyrin in urine (CP). The activities of pyrimidine nucleotidase (P5'N) and nicotinamide adenine dinucleotide synthase (NADS) are also modified in blood, after Pb exposure. Levels of these various metabolites in biological fluids have been used in the past to diagnose Pb poisoning, when direct Pb levels were difficult to obtain in tissues or body fluids (Leung[16]) or as information complementary to Blood-Pb test results. They are more accurately described as biomarkers for toxic *effects* of Pb. In this review, we focus on markers that are more accurately defined as biomarkers of Pb *exposure*, i.e., Pb concentrations in biological tissues and fluids. Biomarkers for the toxic effects of Pb have been reviewed in some detail elsewhere (Sakai[17]).

Throughout the last five decades, whole blood has been the primary biological fluid used for assessment of Pb exposure, both for screening and diagnostic purposes, and for biomonitoring purposes in the long term. While Blood-Pb measurements are reflective of recent exposure, past exposures may also be represented in them, as a result of Pb mobilization from bone back into blood (Gulson *et al.*[6]). In those subjects lacking excessive exposure to Pb, from 45 to 75% of the Pb in blood may have come from bone (Gulson *et al.*[18], Smith *et al.*[19]). In exposed children, however, it has been reported that the Bone-Pb contribution to blood can be 90% or more (Gwiazda *et al.*[20]). Thus, reductions in Blood-Pb levels after environmental Pb remediation may be buffered somewhat by contributions from endogenous lead sources (Lowry *et al.*[21]; Rust *et al.*[14]). Remediation efforts typically result in reductions of Blood-Pb levels in exposed children of no more than 30%, when evaluated within several months after intervention (US EPA[22]). Roberts *et al.*[23] reported that in children with Blood-Pb levels between 25-29 μg/dL who not treated with chelation drugs, the time required for Blood-Pb to decline to <10 μg/dL is about 2 years. Some workers have suggested that the efficacy of Pb hazard remediation efforts should be evaluated over extended periods, to allow adequate time for mobilization and depletion of accumulated skeletal Pb stores, and a reduction in the absolute contribution to blood lead levels from these stores (Gwiazda *et al.*[20], Lowry *et al.*[21]). Thus, the mean of serial Blood-Pb levels should be a more accurate index of long-term Pb exposure.

Data collected as part of the US National Health and Examination Survey (NHANES) give the 95th percentile for blood Pb of 7.0 μg/dL for children aged 1-5 years, and 5.20 μg/dL for adults aged 20 years and older (CDC[24]). While current populations blood Pb levels in the US have dropped markedly compared to 30 years ago, new concerns have been raised regarding possible adverse health effects in children at Blood-Pb levels below 10 μg/dL: perhaps there is no safe threshold but, rather, a continuum of toxic effects (Canfield *et al.*[25]). In light of these concerns, the CDC Advisory Committee on Childhood Lead Poisoning Prevention formed a working group to review of the evidence for adverse health effects at Blood-Pb levels <10μg/dL in children. Although this working group concluded that several studies in the literature had demonstrated a statistically significant association between Blood-Pb levels <10 μg/dL as well as some adverse health effects in children, the effects were very small and could conceivably have been influenced by residual confounding factors. The working group's report called for further studies to examine the relationship between lower Blood-Pb levels and health outcomes, to provide a more complete understanding of this issue (CDC[26]).

Many studies have reported statistically significant associations between Blood-Pb levels and various health effect outcomes. Some, however, have been statistically weak, with the magnitude of the effect relatively small. According to Hu *et al.*[27], such weaknesses of association may occur because Blood-Pb is not a sufficiently sensitive biomarker of exposure or dose at the target organ(s), or because the relationships involved are biologically irrelevant and are only found because of an uncontrolled confounding factor. Furthermore, in view of the kinetics of Pb distribution within the body

(cycling between blood, bone, and soft tissues), differentiation of low-level chronic exposure from a short high-level exposure is not possible on the basis of a single Blood-Pb measurement (Hu *et al.*[27]). Consequently, there is renewed interest in alternative biomarkers that may aid with better diagnosis of the extent of lead exposure. Such alternatives include Pb determinations in plasma/serum, saliva, bone, teeth, feces, and urine. However, none has gained convincing acceptance as an alternative to Blood-Pb. This is due in part to data based on erroneous or dubious analytical protocols that do not take into account the confounding variables.

Plasma/serum lead

Plasma-Pb likely represents a more relevant index of exposure to, distribution of, and health risks associated with Pb than does Blood-Pb. Indeed, from a physiological point of view, we can assume that the toxic effects of Pb are primarily associated with Plasma-Pb, because this fraction is the most rapidly exchangeable one in the blood compartment. In recent years, increased attention is being paid to the monitoring of the concentration of Pb in plasma (or serum). However, research on associations between Plasma-Pb and toxicological outcomes is still quite rare, and a significant gap in knowledge remains.

Plasma/serum Pb levels in non-exposed and exposed individuals reported in older publications range widely from 0.02 to 14.5 µg/L (Versieck & Cornelis[28]). This is probably due to the use of inappropriate collection methods, analytical instrumentation, and/or methods for Pb determination. The development and use of more sensitive analytical instrumentation, especially inductively coupled plasma mass spectrometry (ICP-MS), has resulted in determinations of Pb in plasma and serum specimens with much lower detection limits and with better accuracy. More recent data, also based on ICP-MS methods, have shown Plasma-Pb levels below 1.0 µg/L in non-exposed individuals (Schutz *et al.*[29]).

The use of advanced analytical techniques is not the only essential requirement to assure accurate and reliable Plasma-Pb data. Contamination of the specimen may occur at the pre-analytical phase, i.e., during collection, manipulation or storage. Use of Class-100 biosafety cabinets and clean rooms for specimen preparation and analysis is mandatory. Moreover, all analytical reagents used must be of the highest-purity grade. These conditions are far more rigorous than are typically required for clinical Blood-Pb measurements as performed in a commercial laboratory. After the blood specimen has been collected, the serum/plasma separation must be performed as soon as possible, since there is high potential for Pb to move from the dominant Blood-Pb subcompartment repository, i.e., the erythrocytes, into the plasma via hemolysis, leading to erroneously high results for Plasma-Pb. Plasma hemolysis can be estimated by analyzing measurement of hemoglobin levels in the specimen, since these levels are likely to become abnormally elevated with hemolysis (Smith *et al.*[30]). Materials for specimen collection and storage, and the anticoagulant, must be of the highest quality, since these can be another source of Pb contamination.

Commercial evacuated blood tubes, prepared specifically for blood Pb measurements, are available with less than 5 µg/L Pb (Esernio-Jenssen *et al.*[31]), but it is nevertheless desirable for the analyzing laboratory to characterize the background Pb contamination in each new lot of tubes, to ensure that reported concentrations are not compromised by contamination. The choice of anticoagulant is important, because EDTA, as a strong metal-chelating agent, may be difficult to obtain without some background contamination and/or may give misleadingly high Plasma-Pb results due to selective extraction of Pb bound to erythrocytes. The use of heparin has different associated problems because heparinized blood is more prone to form fibrin clots after several hours. These issues were evaluated by Smith *et al.*[32] in some detail; they compared commercial Vacutainer® type tubes with ultracleaned collection tubes containing either EDTA or heparin. As there are no commercial blood collection tubes available that are certified for ultra-low Pb measurements, the analyzing laboratory should prepare pre-cleaned polyethylene tubes containing ultra low-Pb anticoagulants.

There are many reports of Plasma-Pb measurements where validation data are either weak or absent altogether. For example, some simply cite successful participation of the analyzing laboratory in quality assurance (QA) programs for *whole blood* Pb operated by the CDC and the College of American Pathologists (Hernandez-Avila *et al.*[33]), while others neglect

to cite any kind of QA (Dombovari *et al.*[34]). Participation in QA schemes that are designed specifically for *whole blood* lead, while commendable, does not address the much more challenging analysis for Plasma-Pb. The problem is compounded by the lack of certified reference materials for either serum or plasma Pb (Cake *et al.*[35]). For these reasons, production of plasma or serum reference materials that have Pb concentrations certified close to current human values is urgently needed to support method validation.

Saliva lead

Saliva has been proposed as a diagnostic specimen for various purposes, as it is easily collected (Silbergeld[36]). However, in the absence of consistent and dependable saliva Pb measurements, it is not generally accepted as a reliable biomarker of Pb exposure. Saliva shows large variations in its ion content throughout the day, coupled with changes in salivary flow rates before, during, and after meals. Variations also arise depending on the manner in which saliva collection is stimulated (or not), and on the nutritional and hormonal status of the individual.

There are some data to suggest an association between Pb levels in saliva and those in either plasma or blood (Pan[37], Omokhodion & Crockford[38]). Moreover, it has been argued that Pb in saliva is the direct excretion of the Pb fraction in diffusible plasma, i.e., the fraction not bound to proteins (Omokhodion & Crockford[38]). Despite the associations reported in the literature, the older saliva Pb concentrations are quite high, and the values vary from study to study. On the other hand, recent data suggest much lower saliva Pb levels, in both exposed and unexposed subjects (Koh *et al.*[39], Wilhelm *et al.*[40]). According to Wilhelm *et al.* [40], Pb content in the saliva of unexposed children is usually below 0.15 µg/dL.

Uncontrolled variation in salivary flow rates, lack of standard or certified reference materials, and absence of reliable reference values for human populations are major factors that limit the utility of saliva Pb measurements. In addition, the very low levels of Pb present in saliva serve to limit the range of suitable analytical techniques thereby further diminishing the utility and reliability of this biomarker for prediction of Pb exposure.

Hair lead

Hair is a biological specimen that is easily and non-invasively collected, with minimal cost, and it is easily stored and transported to the laboratory for analysis. These attributes make hair an attractive biomonitoring substrate, at least superficially. Because lead is excreted in hair, many have suggested it for assessing Pb exposure, particularly in developing countries where specialized laboratory services may be unavailable and resources limited (Schumacher *et al.*[41]). However, there is an extensive debate ongoing about the limitations of hair as a biomarker of metal exposure generally. Here we limit the discussion to Pb exposure, although many of the issues for Pb, such as pre-analytical concerns for contamination control, sampling and reference ranges, also apply to other metals.

The ability to distinguish between Pb that is endogenous, i.e., absorbed into the blood and incorporated into the hair matrix, and Pb that is exogenous, i.e., derived from external contamination, is a major problem. During the washing step, it is assumed that exogenous Pb is completely removed whereas endogenous Pb is not. However, no consensus exists about how removal of the former is best accomplished.

Some publications that describe the use of hair for assessing Pb exposure reference a hair washing method proposed by the International Atomic Energy Agency (IAEA) in 1978. The approach entailed washing of hair specimens with acetone/water/acetone (Ryabukin[42]). However, a recent study (Morton *et al.*[43]) has demonstrated that the IAEA method failed to remove exogenous Pb from hair.

Another issue is the significant variation in the Pb concentration profile among various subpopulations according to age, gender, hair-color, and smoking (Wolfsperger *et al.*[44]). Moreover, geographical, racial/ethnic, and ecological factors can also affect Pb distribution in hair within a given population. Thus, it is difficult to establish reference ranges, because confounding factors impose restrictions on the interpretation of individual results. No consensus exists on the length of the hair specimen to be collected, or the amount, or the position on scalp. Variations in Pb content between single hairs from the same individual can be as high as ±100%, particularly in the distal region (Renshaw *et al.*[45]).

Recently, the Agency for Toxic Substances and Disease Registry (ATSDR) established an

expert advisory panel to review current knowledge on the use of hair analysis for trace metals in biomonitoring (ATSDR[46]). The general consensus was that many scientific issues need to be resolved before hair analysis can become a useful tool in understanding environmental exposures. Although hair analysis may be able to answer some specific questions about environmental exposure to a few substances, it often raises more questions than it answers. The scientific community currently does not know the range of Pb contamination levels typically found in human hair. Without reliable data, in the peer-reviewed literature, on baseline or background hair contamination levels in the general population, health agencies cannot determine whether results from a given site are unusually high or low, unless the data have been collected from comparison populations (ATSDR[46]).

In addition to the pre-analytical issues and the absence of reliable reference ranges, the quality of analytical techniques used for determining Pb, as well as other trace metals, in hair has been questioned. In a recent interlaboratory study of commercial laboratories that specifically market the test for trace metals in hair, interlaboratory agreement was judged very poor, with wide discrepancies observed for Pb as well as other elements (Seidel[47]). Other reports have also cautioned against the use of hair analysis for trace-metal assessment.

Urinary and fecal lead

The determination of lead in urine (Urine-Pb) is considered to reflect Pb that has diffused from plasma and is excreted through the kidneys. Collection of urine for Pb measurements is non-invasive and is favored for long-term biomonitoring, especially for occupational exposures. However, a spot urine specimen is particularly unreliable, because it is subject to large biological variations that necessitate a creatinine excretion correction. Urine-Pb originates from plasma Pb that is filtered at the glomerular level; thus, according to some authors (Tsaih *et al.*[48]) urinary Pb levels that are adjusted for glomerular filtration rate may serve as a proxy for Plasma-Pb. Hirata *et al.*[49] found a better correlation between the concentration of Plasma-Pb and Urine-Pb than between Blood-Pb and Urine-Pb, for Pb workers with low levels of Pb exposure. Manton *et al.*[7], using high-precision Pb isotope ratio measurements, found the concentration of Urine-Pb to be about 10% of that in whole blood; however, the correlations were not particularly robust. In contrast, correlations with isotopic ratios were excellent. According to Tsaih *et al.*[48], cortical bone contributes a mean of 0.43 μg Pb per day excreted in urine, whereas trabecular bone contributes as much as 1.6 μg Pb per day. Cavalleri *et al.*[50] observed different Pb kinetics between exposed and non-exposed subjects after the administration of $CaNa_2EDTA$. In unexposed subjects, Blood-Pb levels remained stable even after 5 h of $CaNa_2EDTA$ administration. However, Plasma-Pb levels in the unexposed group decreased by as much as one-half, while Urine-Pb increased by a factor of 10. In the Pb-exposed group, the same amount of chelation therapy resulted in Plasma-Pb levels increasing by a factor of 2 while Blood-Pb levels decreased by a factor of 2, with a higher Urine-Pb excretion. Thus, it seems that in non-exposed subjects a major contribution for Urine-Pb is derived from the Pb fraction in soft tissues that is in equilibrium with that in plasma compartment. We could speculate that the larger the amount of erythrocyte-bound Pb, the weaker the binding forces, and that a significant fraction of Pb is released from red blood cell membranes into plasma, and is then filtered by the kidney. Since the amount of Pb excreted is very high, the kidneys are unable to remove it rapidly from the blood stream; this may account for the temporal elevation of Plasma-Pb levels.

The availability of reliable urine quality control materials and reference materials certified for Pb content, and participation in external quality assessment schemes for urine Pb, constitute important factors in assuring the accuracy of analytical results. However, the tendency for urate salts to precipitate out of urine during transit and storage can be a complicating factor in the analysis. Moreover, because only a few papers have examined associations between Urine-Pb and other biomarkers, the use of urinary Pb measurements is essentially limited to long-term occupational monitoring programs, monitoring patients during chelation-therapy and, until very recently, to clinical evaluation of potential candidates for chelation therapy.

Measurement of Fecal-Pb content over several days is one possible approach to estimating the overall magnitude of childhood Pb intake. According to Gwiazda *et al.* [20], Fecal Pb

content should give an integrated measure of Pb exposure/intake from all sources, dietary and environmental, inside and outside the home (by isotopic composition). However, a limitation to the use Fecal-Pb is that the collection of complete fecal samples over multiple days may not be feasible. As stated by Gwiazda *et al.*[20], Fecal-Pb reflects unabsorbed, ingested Pb plus Pb that is eliminated via endogenous fecal (biliary) routes; inter-individual variations in these physiologic processes may show up as variation that is wrongly attributable to environmental Pb exposure.

Nail lead

Like hair, nails possess many superficial advantages as a biomarker for Pb exposure, especially because specimen collection is non-invasive and simple, and because nail specimens are very stable after collection, not requiring special storage conditions. Nail-Pb is considered a reflection of long-term exposure, because this compartment remains isolated from other metabolic activities in the body (Takagi *et al.*[51]). Since toenails are less affected by exogenous environmental contamination than are fingernails, they have been preferred for Pb exposure studies. Toenails have a slower growth rate than fingernails (up to 50% slower, especially in winter) and thus may provide a longer integration of Pb exposure.

Lead concentration in nails depends on the subject's age (Nowak & Chmielnicka[52]), but it seems do not depend on subject's gender (Rodushkin & Axelsson[53]).

Gulson[54] reported high variability in Pb levels measured in the same fingernails and toenails of various subjects, even after rigorous washing procedures; such lack of reproducibility suggests that nail specimens offer only limited scope in assessing exposure to Pb.

Bone lead

Since bone accounts for more than 94% of the adult body burden of lead (70% in children) (O'Flaherty[10]), many researchers accept that a cumulative measure of Pb dose may be the most important determinant of some forms of toxicity (cumulative measure means an exposure that is integrated over many years, rather than based on a single Blood-Pb measurement) (Landrigan & Todd[55] 1994; Hu *et al.*[27]). In support of this hypothesis, recent studies have shown that Bone-Pb, but not Blood-Pb, is significantly related to declines in hematocrit and hemoglobin among moderately Pb-exposed construction workers, and to decreased birth weight among mothers and increased odds of clinically relevant hypertension (Hu *et al.*[56], Gonzalez-Cossio *et al.*[57]). According to Hu *et al.* [27] other adverse health outcomes that are likely to be better associated with Bone-Pb levels include impairment of cognitive performance and growth in children, and kidney failure, gout, elevated blood pressure, reproductive toxicity, and adverse cardiovascular events in adults.

As pointed by Hu *et al.*[27] two major paradigms relate to skeletal lead: Bone-Pb as an indicator of cumulative lead exposure (Bone-Pb as a repository), and Bone-Pb as a source of body burden that can mobilized into the circulation (Bone-Pb as a source). Hernandez-Avila *et al.*[33] reported a strong association between Bone-Pb levels and Serum-Pb levels of adults exposed to Pb. That study indicated the potential role of the skeleton as an important source of endogenous labile Pb that may not be adequately discerned through measurement of Blood-Pb levels. The same authors argue that skeletal sources of Pb accumulated from past exposures should be considered, along with current sources, when exposure pathways are being evaluated. In an attempt to characterize the source of Pb exposure, Gulson *et al.*[18] measured the $^{206}Pb/^{204}Pb$ isotopic ratios in immigrant Australian subjects, Australian-born subjects and environmental samples. The immigrant population exhibited Pb isotopic ratios from 17.7 to 18.5, distinct from the ratio in Australian-born subjects (approximately 17.0). This difference allowed a distinction to be drawn between current exposure, acquired from Australian sources, and older bone-stored Pb that was not acquired from Australian sources.

Differing bone types have differing Bone-Pb mobilization characteristics. For example, the tibia principally consists of cortical bone, while the patella is largely trabecular bone. Pb in trabecular bone is more biologically active than is Pb in cortical bone, and trabecular bone has a shorter turnover time. The endogenous contribution of Pb from bone stores is an important health consideration. The O'Flaherty kinetic model can be used to indicate the quan-

tity of Pb delivered from bone as a function of bone turnover and Pb exchange (O'Flaherty[10]). A recent revised version of this model (Fleming *et al.*[58]) suggests that a smeltery worker with a tibia Pb concentration of 100 μg/g can expect a continuous endogenous contribution to Blood-Pb of 16 μg/dL. A pregnant woman with a tibia Pb concentration of 50 μg/g can end up with a contribution of 8-μg/dL Blood-Pb; this figure does not take into account the increased rate of bone turnover associated with pregnancy. Individuals who are not exposed to Pb in the workplace typically display tibia Pb levels up to about 20 μg/g (Roy *et al.*[59]).

Over the last decade, bone Pb measurements based on non-invasive *in vivo* X-ray fluorescence (XRF) methods have become increasingly accepted. The technique uses fluorescing photons to remove an inner-shell electron from a Pb atom, leaving it in an excited state. The result is emission of X-ray photons that are characteristic of Pb. Measurements are performed by using one of four kinds of XRF: two involve fluorescence of the K-shell electrons of Pb (KXRF), and the other two involve fluorescence of the L-shell electrons (LXRF) (Todd *et al.*[60]). Several groups, mainly in North America have reported the development of *in vivo* measurement systems; the majority have adopted the KXRF approach based on excitation with a ^{109}Cd isotope and backscatter geometry, because of its advantages: it provides a robust measurement with a better detection limit and a lower effective (radiation) dose (as compared to LXRF) (Todd & Chettle[61]). The radiation dose is not a limiting factor in using this technique with humans, as demonstrated by Todd & Chettle[61].

Calibration is usually performed with Pb-doped plaster-of-Paris phantoms (Todd *et al.*[60]). Method accuracy has been evaluated through comparison of XRF data from cadaver specimens with ETAAS data (Todd *et al.*[62]). However, XRF sensitivity and precision for Pb still constitute an analytical challenge. In addition to sample-to-sample reproducibility, XRF can also display a certain amount of imprecision associated with each calculated bone lead value (Ambrose *et al.*[63]). This uncertainty, estimated using a goodness-of-fit statistic from the curve fitting of the background, ranged from 3 to 30 μg/g Pb; clearly, this will represent a problem for measurements of low-level Pb (i.e., young children and non-exposed population). Another problem inherent to the XRF technique is photon scattering, due to overlying tissue or subject movement during the measurement period (Ambrose *et al.* [63]). Normalization of the Pb signal to the Ca backscatter signal appears to solve this problem. Precision depends on the amount of tissue overlying the bone: the greater the thickness of tissue, the poorer the precision. Todd & Chettle[61] comparing the K-shell with L-shell precisions with 3 mm and 6 mm of overlying soft tissue, reported that KXRF precision worsens by only 5%, whereas LXRF precision worsens by 49% for greater thickness. The precision of the LXRF method is much more severely affected by the strong attenuation of the Pb L X-rays.

Todd *et al.*[64] reported contiguous inhomogeneities in the distribution of Pb toward the proximal and distal ends of the tibia bones. They speculated that the region of lower Pb concentration has lower blood flow in the Haversian canals and, consequently, less Pb available for uptake into bone matrix during bone remodeling (Todd *et al.* [64]). Trabecular bone has a larger surface area and a greater volume of blood delivered per unit time, compared to cortical bone. In addition, there are more active osteons per gram in trabecular bone to accomplish resorption and deposition. Hernandez-Avila *et al.*[33] reported that, in individuals with no history to occupational Pb exposure, Bone-Pb (in particular trabecular Pb) exerts an additional independent influence on Plasma Pb after control for Blood-Pb.

Thus, an appropriate selection of the precise bone type to be analyzed for Pb content must be made before commencing. Moreover, further research on the relationship between various Bone-Pb sub-compartments and other Pb measures is warranted.

Tooth lead

Like bone, teeth accumulate Pb over the long term. However, there is some evidence that teeth are superior to bone as an indicator of cumulative Pb exposure, since the losses from teeth are much slower (Maneakrichten *et al.*[65]). Moreover, deciduous teeth are relatively easy to collect and analyze, since exfoliation generally occurs after the age of 6 years. Teeth are also very stable, for preservation purposes.

Chronic Pb exposure, from mouthing activity in early childhood, may be camouflaged by "dilution" effects during periods of rapid

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skeletal growth in young children and adolescents, and may not be detected by a single blood-Pb measurement. However, most of the published data based on Tooth-Pb have been based on whole tooth analysis, with no attempt to distinguish among tooth types (different teeth are formed at different ages), or to differentiate the Pb concentration in enamel from that in dentin (enamel contains much more Pb, by mass, than does dentin). Also not considered has been the influence of age and/or gender (Brown *et al.*[66]). Furthermore, use of deciduous teeth is only possible for children over 6 years in age. Recently, Gomes *et al.*[67] (2004) proposed a solution to the limitations mentioned above by using an *in vivo* enamel biopsy of children. In this approach, superficial minerals are leached from teeth and Pb determined by ETAAS. One important drawback to this approach is that, because an accumulation gradient for Pb has not yet been established for enamel, only biopsies of a given depth can be compared to one another. Another issue related to Tooth-Pb measurements is whether Pb that accumulates in the first few micrometers of the enamel surface was incorporated post-eruptively, e.g., from the mouth, saliva, food, rather than during the period when the tooth was mineralized inside the bone.

An interesting, and potentially valuable aspect of Tooth-Pb measurements is their capacity to elucidate the history of Pb exposure. Teeth are composed of several distinct tissues formed over a period of several years, and different parts of the tooth can bind Pb at different stages of the individual's life. Therefore, a section of tooth can yield historical information on the individual's prior exposure to Pb. For example, the enamel of all primary teeth, and parts of the enamel from some permanent teeth, are formed *in utero* and thus may provide information on pre-natal exposure to Pb. This information could be valuable in improving our understanding of dose-effect relationships for embryonic anomalies, particularly neurotoxic dysfunction. The dentine of the primary teeth provides evidence of exposure during the early childhood years, when hand-to-mouth activity is usually an important contributor to Pb body burden (Gulson[68]). However, enamel Pb levels may be useful for indirectly estimating the Pb composition of the mother's bone (Gulson[68]).

More recently, there has been some interest in using laser ablation – ICP-MS to examine Pb distribution in tooth profiles. This approach offers spatially resolved measurements of trace-element distribution that can be compared to a temporal axis via reference to the neonatal line, enabling researchers to use not only the Pb concentration of the entire tooth, but also the specific amount of Pb "stored" in each tooth layer, i.e., a time line of Pb exposure. Nevertheless, some serious challenges remain before this technique can be fully exploited (Uryu *et al.*[69]).

Conclusions

Thus far, an impressive body of data has been established based on the use of alternative biomarkers for monitoring exposure to Pb. However, it is still unclear to what extent such data are superior to the information obtained from Blood-Pb measurements. Clearly, many of the limitations identified in the foregoing sections must be resolved, before alternative biomarkers can be accepted as superior indicators of Pb exposure. At this time, Blood-Pb measurements are still the most reliable indicator of recent lead exposure, although serial blood-Pb measurements may offer a better assessment of temporal fluctuations in Pb absorption. If reliable and reproducible Plasma-Pb measurements can be obtained, these may offer better correlation with toxic effects. However, we do not yet know what a single Plasma-Pb value means, in terms of health effects; in the absence of a normal reference range, the clinical utility for individual assessment is problematic. Further research on this issue is clearly needed, especially for children and adults with low to moderate Pb exposure. Further efforts are also warranted in the further development and continued use of well-established analytical protocols, as well as in the estimation of random and systematic errors. Efforts are needed to create regional reference ranges of non-exposed populations for each biomarker; to acquire data related to long-term and short-term exposures; and to evaluate the influence of nutritional status and ethnicity (genetic polymorphisms).

A critical question that might be asked, with respect to an individual's Bone-Pb measurement, is what does it mean in terms of health risk or, perhaps, clinical management? To answer this question, we may need to distinguish between Bone-Pb measurements in children and pregnant women, i.e., those with high

bone turnover rate, compared to [non-pregnant] adults. In the former group, Bone-Pb may have little effect on Blood-Pb levels, but it may help us to estimate the extent to which Blood-Pb derives from endogenous sources, as well as its possible contribution to the labile Plasma-Pb pool.

List of abbreviations

ALA-D	δ-aminolevulinic acid dehydratase	**KXRF**	K-shell X-ray fluorescence
ALA-U	Aminolevulinic acid in urine	**LXRF**	L-shell X-ray fluorescence
ALA-B	Aminolevulinic acid in blood	**µg**	microgram
ALA-P	Aminolevulinic acid in plasma	**µg/L**	microgram per liter
ATSDR	Agency for Toxic Substances and Disease Registry	**µg/dL**	microgram per deciliter
		µg/g	microgram per gram
Blood-Pb	Lead levels in blood	**µg/mL**	microgram per mililiter
Bone-Pb	Lead levels in bone	**µg/24h**	microgram per day
CaNa2EDTA	Edetate calcium disodium	**µmol/L**	micromol per liter
Ca	Calcium	**NADS**	Nicotinamide Adenine Dinucleotide Synthase
^{109}Cd	Cadmium isotope 109		
CP	Coproporphyrin	**Nail-Pb**	Lead levels in nail
CDC	Centers for Disease Control and Prevention	**NHANES**	National Health and Examination Survey
EDTA	Ethylenediamine Tetraacetic Acid	**Pb**	Lead
EP	Erythrocyte Protoporphyrin	**^{204}Pb**	Lead isotope 204
ETAAS	Electrothermal Atomic Absorption Spectrometry	**^{206}Pb**	Lead isotope 206
		Plasma-Pb	Lead levels in plasma
ETV-ICP-MS	Electrothermal Vaporization Coupled to ICP-MS	**P5'N**	Pyrimidine nucleotidase
		QA	Quality Assurance
Fe	Iron	**Tooth-Pb**	Lead levels in tooth
Fecal-Pb	Lead levels in feces	**Urine-Pb**	Lead levels in urine
Hair-Pb	Lead levels in hair	**US EPA**	United States Environmental Protection Agency
HCl	Hydrocloric Acid		
IAEA	International Atomic Energy Agency	**XRF**	X-ray fluorescence
		WHO	World Health Organization
ICP-MS	Inductively Coupled Plasma Mass Spectrometry	**ZPP**	Zinc protoporphyrin

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Collaborators

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