Biomarkers of Lead Exposure: Platforms and Analysis

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Contents

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Abstract

In this chapter, the main lead exposure biomarkers reported in the scientific literature are summarized, along with their pros and cons and situations in which they should be recommended. The population is exposed daily to lead concentrations present in the environment. Pregnant women and children are the groups most affected by lead exposure and may suffer a number of health effects. Lead exposure surveillance is crucial both for environmental and occupational exposure. Knowing how to select the most suitable biomarker for each exposure scenario is essential to allow useful and effective biomonitoring. Different types of biological matrices can be used to assess biomarkers of lead exposure, including teeth, bone, nails, whole blood, plasma, urine, saliva, and hair. Blood Pb remains the biomarker of choice for recent lead exposure, while long-term exposure can be determined using the mean of serial blood Pb levels. Pb in teeth, and bone can be good biomarkers for investigating cumulative exposure. Pb in urine and saliva reflects recent exposure and has the advantage of being a noninvasive method. However, some factors which vary greatly among individuals need to be taken into account. Nails can serve as a good tool for initial screening.

Keywords

Lead · Exposure · Environment · Occupational · Biomonitoring · Teeth · Bone · Nails · Blood · Plasma · Urine · Saliva · Hair

Abbreviations

ALA	Aminolevulinic acid
ALAD	Aminolevulinic acid dehydratase
BLRV	Blood lead reference values
CDC	Centers for Disease Control and Prevention
EDTA	Ethylenediaminetetraacetic acid
ET AAS	Electrothermal atomic absorption spectrometry
GABA	γ-Aminobutyric acid
GF AAS	Graphite furnace atomic absorption spectrometry
HCl	Hydrochloric acid
HNO$_3$	Nitric acid
H$_2$O$_2$	Hydrogen peroxide
H$_2$SO$_4$	Sulfuric acid
ICP-MS	Inductively coupled plasma mass spectrometry
IAEA	International atomic energy agency
LEPAC	Lead Exposure and Prevention Advisory Committee
NHANES	National Health and Nutrition Examination Survey
Na$_2$EDTA	Disodium ethylenediaminetetraacetic dihydrate
NH$_4$OH	Ammonium hydroxide
Pb	Lead

SDGs	Sustainable development goals
TMAH	Tetramethylammonium hydroxide
UNICEF	The United Nations Children's Fund
XRF	X-ray fluorescence

Introduction

The population is exposed daily to low lead levels present in the environment and also to high concentrations in lead hot spots or during occupational activities, particularly informal activities, which often lack the proper occupational hygiene (Fig. 1). Pregnant women and children are the groups most affected by exposure to chemical contaminants and may suffer a number of health effects, including cognitive and antisocial behavior in children and adolescents. The United Nations Children's Fund (UNICEF) released the 2020 report, "The Toxic Truth: Children's Exposure to Lead Undermines a Generation of Potential Future," reporting that lead exposure spans the 17 sustainable development goals (SDGs), with 1 in 3 children in the world (approximately 800 million) presenting blood lead levels above the reference values accepted by the World Health Organization (WHO) and the US Centers for Disease Control and Prevention (CDC), calling for urgent global actions to combat this public health problem.

Fig. 1 Different lead exposure sources
Legend to Figure 1 This figure shows different types of lead exposure to which humans can be exposed

Inorganic lead is widely distributed in the environment. The entry of lead into the human body can occur via ingestion and/or inhalation. More specifically, in occupational exposures, inhalation can occur through fumes from soldering lead alloys or metals. After entering the body, lead is distributed through the blood and binds to the bones and soft tissue. The Pb^{2+} ion has an affinity for proteins, compromising the functioning of structures containing them. These characteristics can affect hemoglobin production and cause damage to the central and peripheral nervous systems, nephropathy and vascular problems, etc. (Hoet 2005; Olympio et al. 2009, 2010c).

Given these deleterious effects, lead exposure surveillance is crucial both for environmental and occupational exposure (Olympio et al. 2017), and knowing how to select the most suitable biomarker for each exposure scenario is essential to allow useful and effective biomonitoring. The primary aim (e.g., (1) to investigate recent or remote exposure, (2) to study general population or worker exposure, or (3) to include children or adults) dictates whether the biomarker of choice will yield the best evidence.

Biological Matrices and Lead Exposure Biomarkers

Different biological matrices can be used to assess biomarkers of lead exposure (Fig. 2). In this chapter, each of these biomarkers will be discussed in detail.
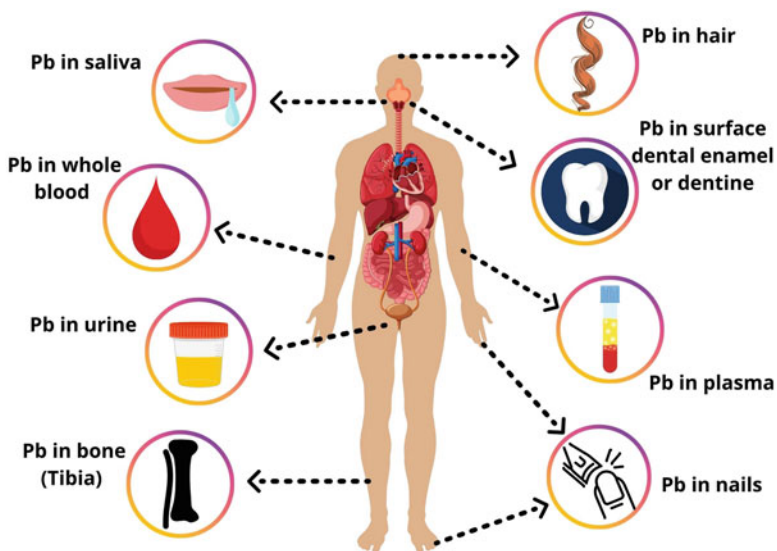

Fig. 2 Biomarkers of lead exposure
Legend to Figure 2 This figure shows all the biomarkers of lead exposure covered in this chapter

Teeth

Remote exposure biomarkers can be used to evaluate remote or chronic lead exposure, for example, to assess whether adolescents with antisocial behavior were exposed to Pb during central nervous system development (Olympio et al. 2009). Teeth lead concentrations are widely recognized biological markers of childhood lead exposure because deciduous teeth accumulate lead during their embryonic and postnatal life (NRC 1993).

Dentine lead can be used to assess exposure to different environmental risk factors for lead exposure. Dentine lead levels can be used to investigate the long-term effects of early lead exposure, including during childhood and prenatal periods (Arora et al. 2014). Dentine is characterized by three different types: (a) primary dentine, which forms before tooth eruption; (b) secondary dentine, which develops after eruption from the odontoblasts living within the pulp and is laid down in layers within the pulp cavity; and (c) reparative or tertiary dentine, which results from thermal, chemical, bacterial, or mechanical trauma to the odontoblasts. This type is darker in color and dense in structure and has few tubules. According to Arora et al. (2014), measurements of Pb in dentine can be reliably used to reconstruct Pb past exposure history but also have the potential to estimate timing of exposure up to the moment the tooth is shed, allowing the investigation of critical windows of susceptibility. Pb levels in dentine at birth were significantly associated with umbilical cord blood Pb, while prenatal dentine Pb was associated with maternal patella Pb. In assessments of postnatal exposure, Pb levels in 3-month dentine were significantly associated with Pb concentrations in children's blood, and mean Pb concentrations in secondary dentine correlated with cumulative blood lead.

Dentine lead determination is usually based on the collection of naturally shed deciduous teeth. Each child should supply at least one tooth (Fergusson et al. 2008; Arora et al. 2014), which can be stored dry at room temperature. Information on the reason the tooth was shed and age at shedding are important (Arora et al. 2014).

Another way of using teeth to measure lead exposure is by evaluating surface dental enamel lead levels through in vivo microbiopsies (Gomes et al. 2004; Olympio et al. 2010a, b, c, 2018a). Lead determination in surface dental enamel is a reliable way of measuring remote exposures to Pb in a rapid, safe, and painless procedure (Olympio et al. 2018a). Biopsy depths depend on the pH of the extraction solution and on its residence time on the surface dental enamel. Exposure to acid for a short period removes a shallower layer of dental enamel. The measurements of lead concentrations in tooth enamel using chemical and physical methods for different biopsy depths converge to values similar to those made using the conventional cylinder approach (Olympio et al. 2018a). This information is crucial, as deeper biopsies contain less lead than superficial ones, since surface enamel has a steep lead gradient (Brudevold et al. 1977). Notably, the surface dental enamel of permanent teeth that have erupted is not susceptible to environmental lead intake, making it a good biomarker of remote lead exposure. A statistically significant association between lead exposure and antisocial behavior in adolescents has been confirmed by longitudinal and cross-sectional studies, irrespective of the biological sample

(blood, bone, or tooth enamel) used (Dietrich et al. 2001; Needleman et al. 2002; Wright et al. 2008, 2009; Olympio et al. 2009, 2010a, b, c; Arbuckle et al. 2016) or the cultural environment of the population cohorts investigated. However, given the lack of reference values for lead in teeth, levels can only be compared against the same study groups to which the same methodology has been applied.

For collecting surface dental enamel samples, teeth need to be cleansed and dried, and an adhesive tape placed firmly onto the labial surface of the subject to delimit the biopsy site. This biopsy procedure has been described by Olympio et al. (2010c, 2018b) and Gomes et al. (2004).

Bone

Bone lead, usually tibial, can also be used to evaluate cumulative exposure for chronic lead absorption. Epidemiologic studies have used bone lead levels as a biomarker of lead exposure in environmental exposure populations, such as children with attention-deficit hyperactivity disorder (Needleman et al. 1996, 2002; Lin et al. 2019) or the older population (Specht et al. 2018). Bone lead can be an important source of steady-state blood lead concentration, even considering environmental exposure. Lead can accumulate to produce a skeletal burden that remains the predominant source of blood lead concentration even after exposure ceases. Therefore, previous exposures may contribute to total blood lead concentration, especially during pregnancy and senescence, reflecting the long half-life of removal from the bloodstream (Vigeh et al. 2011; NRC 1993). During periods of bone activity or demineralization, such as pregnancy, lactation, or osteoporosis events, mobilization of Pb stored in the bones into circulating blood occurs (Hu et al. 1991; Shahida et al. 2021). Furthermore, lead exposure may be a risk factor for the development of osteoporosis, since Pb may replace Ca from the bones and affect osteoblast function (Shahida et al. 2021).

Bone lead levels can also be evaluated in occupationally exposed populations, especially in cases of prolonged exposure (Hu et al. 1991; Landrigan 1991; Vigeh et al. 2011; Barry et al. 2019). Bone Pb measurements are normally performed using X-ray fluorescence equipment, and the measurement site is the middle of the tibia. A K-shell X-ray fluorescence (KXRF) device is the most commonly used, and each analysis takes about 30 min (Specht et al. 2018; Barry et al. 2019; Lin et al. 2019). Before measurement, the subject's legs are cleaned using alcohol and ethylenediaminete-traacetic acid (EDTA) to remove any Pb contamination. The study participant is placed in a sitting position with their right leg immobilized during the analysis (Hu et al. 1991; Landrigan 1991; Specht et al. 2018; Barry et al. 2019; Lin et al. 2019).

Nail

Nail lead levels are a biomarker suitable for investigating subchronic environmental lead exposure. The collection of nail samples is noninvasive, and the samples are easily transported and stored, making the process economically viable. Disclosing

exogenous exposure, nail tests constitute a good tool for initial screening (Oliveira et al. 2021). A toenail can represent an exposure period of 2–12 months, versus 5–6 months for fingernails (Ab Razak et al. 2015; Sakamoto et al. 2015). Fingernail lead levels seem to serve as an indicator of lead exposure sources in contact with the individual, but not as a reliable biomarker of internal dose (Olympio et al. 2020; Oliveira et al. 2021). External contamination is a major concern in the pretreatment of nail samples. Therefore, comparison of the results of different studies is hampered by the heterogeneity of methods of analysis and preparation (Błażewicz et al. 2017). Oliveira et al. (2021) concluded that lead levels in nails did not show a significant difference for a range of exposure levels to the element, as measured in the blood. Only weak correlations between nail and blood lead levels were detected. Although the correlation between mean nail lead levels and the appearance of nails was not statistically significant, nails that were abnormal in appearance contained higher nail lead levels, suggesting external contamination, even after washing of samples.

For the collection procedure, research participants should ideally refrain from cutting their nails for 15 days to ensure a sufficient sample of nail clippings (Oliveira et al. 2021). Clippings measuring at least 1 mm in height should be collected from the thumbnails of both the left and right hands using a pair of stainless-steel nail clippers. Nail polish should be removed using acetone (Sakamoto et al. 2015). After collection, the samples can be stored in ribonuclease (RNase)-free microfuge tubes, decontaminated for trace elements and then kept at room temperature until sample preparation and analysis (Olympio et al. 2020; Oliveira et al. 2021).

Blood

Blood lead of exposed populations remains the biomarker of choice for lead exposure (Barbosa Jr et al. 2005; Olympio et al. 2018a), when the aim is recent exposure evaluation. Blood lead levels generally reflect recent exposure that has occurred 20–30 days before measurement. However, long-term Pb exposure can be assessed by measuring the mean of serial blood Pb levels over longer periods, providing a better assessment of temporal fluctuations in Pb absorption (Barbosa Jr et al. 2005). Blood lead concentrations can be assessed in children and adults to investigate possible sources of contamination or for environmental monitoring in population-based studies. Blood lead levels may aid investigation of associated risk factors in environmentally exposed children and adults. In this case, blood lead can be evaluated in conjunction with the exposure sources, albeit the diet, air, dust, soil, or home and school environments (Leroux et al. 2018; Olympio et al. 2018a).

In occupationally exposed populations, blood lead as a biomarker is an important tool to assess the level of workers' recent exposure (Mohammadyan et al. 2019; Ferreira et al. 2019). Occupational exposures are often responsible for high blood lead levels reported among adults. Therefore, the use of this biomarker may identify risks of lead poisoning and investigate health risks related to this exposure (Rinsky et al. 2018; Ferreira et al. 2019).

The collection of whole blood is the best practice to measure lead exposure. Initial screening capillary tests can be used but need confirmatory testing using a venous sample (CDC 2021). Blood should be drawn from the patient's arm by a certified phlebotomist. The Centers for Disease Control and Prevention recommendation (CDC 2018) is to invert the tube eight to ten times after drawing the blood sample to prevent clotting and ensure good distribution of the additives throughout the blood sample. Vials should be duly labeled after collection. No fasting or special diets are required before blood collection for Pb determination. Contamination of blood samples during the collection process by the external environment is a major concern because lead is ubiquitous. The main precautions are cleaning the collection area and covering the surface with a sterile disposable pad, using prescreened or known lead-free supplies, including sterile collection devices labeled "metal-free," "for trace elements," or "for lead testing," because only the "sterile" designation does not indicate the device is free of contamination by metals. If possible, include one or more field blanks (empty collection tubes) to evaluate potential metal contamination in the analytical phase. If the study involves the collection of blood for other analyses, always take the lead sample first to avoid contamination (CDC 2018).

Plasma

Plasma Pb levels reflect the fraction of circulatory Pb that is more freely available for exchange with tissues. For this reason, some studies consider it a relevant index of exposure and health risks associated with Pb (Barbosa Jr et al. 2005). Evidence suggests that plasma and blood Pb ratios vary between and within individuals, where this disparity may be attributable to underlying differences in toxicokinetics (Smith et al. 2002). According to Sommar et al. (2014), plasma lead testing should be considered for exposed individuals, but not for low exposures in the general population. The lack of reference values hinders comparisons of plasma lead levels.

Plasma collection should follow the same procedures and precautions described earlier for blood. Blood samples should be collected by venipuncture into a tube containing anticoagulants for plasma separation. After collection, the sample should be centrifuged at room temperature. The plasma fraction needs to then be transferred to a metal-free microtube or polyethylene tube and immediately frozen (Smith et al. 2002; Barbosa Jr et al. 2006). After blood collection, plasma separation must be performed as soon as possible because there is high potential for hemolysis, during which Pb moves from erythrocytes into the plasma, leading to erroneously high results for plasma Pb (Smith et al. 2002).

Urine

Lead in urine reflects recent Pb absorption and is the most widely accepted biomarker for trace metal exposure after blood (Esteban and Castaño 2009; Gil et al. 2011; Molina-Villalba et al. 2015). Urinary lead levels depend on the elimination of

Pb by the kidney, which can fluctuate and may not rise significantly following chronic Pb poisoning (Nouioui et al. 2019). Variations in the volume of urine produced both within and between individuals may result in substantial variations in lead concentration, and creatinine correction has therefore been used to adjust for variations in hydration status (Aylward et al. 2014; Salles et al. 2021). Compared to other matrices, urine is more accessible and available in large volumes. In addition, urine collection is a low-cost, noninvasive method that can be applied to all participants easily. Also, the technique allows low lead concentration determinations (Zhang et al. 2017; Salles et al. 2021).

Ideally, 24-hour urine collection should be performed, but this is often logistically difficult. Hence, participants are instructed to provide the first urine in the morning by collecting only the normal urine flow and not the initial discharge (CDC 2018; Zhang et al. 2017; Salles et al. 2021). Externally threaded containers are preferred to minimize contamination of the specimen and prevent leaks. Lot screened polypropylene (PP) cryovials or tubes are preferred for aliquots. Colored plastics and containers containing O-rings should be avoided because of the increased risk of trace element contamination from pigments. In other cases, containers used should be pre-cleaned, and the Pb transfer of the material constituting the container for the sample should be prescreened before sample collection (Salles et al. 2021). After urine collection, urine samples should be transported in dry ice and all urine specimens stored in a freezer (preferably at $\leq -20°C$). If study participants perform collection of the urine sample at their homes, they must receive instructions for proper collection and storage (CDC 2018; Salles et al. 2021).

Saliva

Saliva is an useful alternative sample matrix for human biomonitoring. The use of this biomarker for lead offers the advantage of easy collection, noninvasive methods and dispenses with the need for medical staff. Environmental or occupational lead exposure can be assessed using this biomarker (Shawahna et al. 2021). However, several factors must be taken into account, including variations in ion content, changes in salivary flow, differences in saliva stimulation during collection, and the nutritional and hormonal status of the individual (Barbosa Jr et al. 2005; Michalke et al. 2015). Saliva reflects recent lead exposure, although results associating levels of Pb in saliva with lead concentrations in blood and plasma are conflicting in the literature. Nevertheless, use of Pb saliva as a biomarker may serve as an additional and/or alternative measurement for lead biomonitoring (Costa de Almeida et al. 2009; Gil et al. 2011; Michalke et al. 2015). Barbosa Jr et al. (2006) suggest that salivary lead may not be used as a surrogate of plasma or blood or as a biomarker to diagnose lead exposure for low to moderately lead-exposed populations. Other studies have reported low concentration of Pb in saliva and found no correlations between blood and saliva Pb levels (Costa de Almeida et al. 2010; Gil et al. 2011). The magnitude of blood Pb levels strongly influences the direction of the correlation with saliva (Gil et al. 2011). Given the lack of reference

values for lead in saliva, this biomarker should only be used to compare groups from the same study.

Whole saliva is mainly collected by five different methods: the draining method, the spitting method, the suction method, the rinsing method, and the absorbent method. Using the draining method or "passive drool," the subject allows saliva to drip off the lower lip into a tube. The participant should expectorate residual saliva into the tube only at the end of the collection period. Using the spitting method, saliva is allowed to accumulate in the mouth, and the subject spits it out into a test tube every 60 s. The suction method uses a small aspirator to withdraw saliva from the mouth. The rinsing method is a liquid-based collection system consisting of a mouth-rinsing solution. Finally, the absorbent method uses a swab, cotton roll, or gauze sponge, and after 2–5 min in the mouth, this is removed from the oral cavity and saliva transferred into a collection vial by centrifugation (Michalke et al. 2015). However, this last method described is not indicated for the evaluation of lead in saliva, due to the risk of lead contamination in the swab, cotton, or gauze. Also, before collection, the study participants should be asked to wash their mouths using water and fill a metal-free collection tube with approximately 5 mL of saliva. After collection of unstimulated salivary sample, these should subsequently be stored at −20 or −80°C (Costa de Almeida et al. 2009; Gil et al. 2011; Shawahna et al. 2021).

Hair

Human hair analysis can be used to investigate exposure to lead from various sources (Strumylaite et al. 2004; Nomura and Oliveira 2010; Michalak et al. 2014). The use of hair lead as a biomarker of environmental exposure to toxic elements has become common practice. Hair is inert and easier to sample and can be stored without technical problems (Nomura and Oliveira 2010; Michalak et al. 2014). This biomarker is also useful for assessing occupational exposure, particularly for medium to high levels of Pb pollution (Nouioui et al. 2019). The metal body burden of lead in hair reflects a record of relatively long periods because human hair can incorporate metals into its structure, providing a chemical calendar (Michalak et al. 2014; Nouioui et al. 2019; Li et al. 2021).

The main disadvantages of this matrix include the difficulty distinguishing endogenous (actual internal dose absorbed into the blood) from exogenous (external contamination) lead, and there is no consensus on the best method of removing exogenous Pb. Other disadvantages are variations between subpopulations of different race, age, and sex and in hair color and hair care, length of hair collected, and amount and position on the scalp, as well as variations in sample preparation and laboratory methodologies (Wolfsperger et al. 1994; Harkins and Susten 2003; Esteban and Castaño 2009; Peña-Fernández et al. 2017). Few studies provide reference values for correct interpretation and correlation between lead levels in the hair, blood, and other target tissues, a problem adding to the controversy over use of this matrix (Esteban and Castaño 2009; Peña-Fernández et al. 2017). Therefore, Pb in hair may not indicate internal exposure, and data may be insufficient to predict

Biomarker	Advantages	Disadvantages
Teeth Pb	Fast, safe and painless method.	No reference values. Heterogeneity of analysis methods = difficult comparison.
Bone Pb	Painless and noninvasive method. No storage.	Requires specific equipment. Gender variation. Pb mobilization during pregnancy, lactation and osteoporosis events.
Plasma Pb	Reflect the fraction of circulatory Pb. Good biomarker to investigate health risk associated with Pb.	Invasive method. Requires a phlebotomist. Requires an adequate infrastructure for transport and storage.
Nail Pb	Painless and noninvasive method. Economically viable. Easily transported/ stored. Good for initial screening.	Risks of external contamination in the pretreatment of samples. Not a good biomarker for internal dose. Heterogeneity of washing and preparation methods.
Blood Pb	Biomarker of choice for recent exposure. Existence of reference values. Easy to compare.	Invasive method. Requires a phlebotomist. Requires an adequate infrastructure for transport and storage.
Urine Pb	Accessible in large volumes in a noninvasive method. Economically viable. Existence of reference values.	Variation in the volume within and between individuals. Depend on the elimination of Pb by the kidney. Requires adjust for variations in hydration status.
Saliva Pb	Easy collection. Painless and noninvasive method.	Variations in salivary flow and stimulation during collection. Could be affected by nutritional and hormonal status.
Hair Pb	Noninvasive method. Easily transported/ stored.	Difficulty distinguishing endogenous and exogenous Pb. Variations between races, ages, sexes, hair care and colors.

Fig. 3 Advantages and disadvantages associated with the use of each biomarker of lead exposure Legend to Figure 3 This figure shows all the advantages and disadvantages of using different biomarkers of lead exposure

health effects (Harkins and Susten 2003). Especially among young girls, collecting blood, despite being more invasive, is often more acceptable than having their hair cut because children can be against cutting their hair (Personal Communication: Gouveia et al. 2014).

To collect this matrix, it is advisable to cut the scalp hair samples with stainless-steel scissors from the head as near as possible to the scalp. Different studies report collection in different regions of the head, including occipital, temporal, frontal, and cranial regions, although the occipital region is the most common site (Strumylaite et al. 2004; Michalak et al. 2014; Peña-Fernández et al. 2017; Nouioui et al. 2019; Li et al. 2021). After collection, hair samples need to be sealed in new polyethylene bags for transport and storage (Li et al. 2021). A summary of all biomarkers of lead exposure presented in this chapter considering their advantages and disadvantages is presented in the figure below (Fig. 3).

Elemental Analysis

Lead has been included in numerous human biomonitoring studies (Leroux et al. 2018; Olympio et al. 2009, 2018a, b; Oliveira et al. 2021; Salles et al. 2021). The determination of trace elements requires qualified analysts and adequate infrastructure to ensure reliable results. All steps, from sampling, storage, conservation, and

preparation, must be carefully carried out in order to avoid errors, since samples can be easily contaminated by other elements abundant in the Earth's crust (Si, Al, Fe, Ca, Mg, Na, K, Mn, and Ti) and those that are always present in the work environment, mainly in the form of dust (Zn, Pb, Cd, Hg, Cu, As, Ni) (Tölg and Tschöpel 1994). Spectrometric methods, such as inductively coupled plasma mass spectrometry (ICP-MS) and graphite furnace atomic absorption spectrometry (GF AAS), offer the sensitivity needed for trace-level quantification of Pb in biological samples. On the other hand, X-ray fluorescence (XRF) provides direct elemental determination of solids without complex sample preparation.

Studies that have used different matrices to determine lead in the human body and methods of sample preparation and analysis are presented in Table 1.

Sample Preparation

Among all of the analytical operations, sample preparation is the most critical step and the one where most mistakes occur and which is most time-consuming.

Sample preparation involves physical and chemical operations to convert the samples into a suitable form for introduction into the measuring instrument employed and to minimize interference in the quantification of the analyte. Considered a critical step in the analytical sequence because of the costs involved, time required, and high incidence of errors, sample preparation step must be strategically planned to avoid analyte losses, contamination, and costly procedures.

Throughout the analytical work, the materials used for sample preparation must be cleaned in order to prevent contamination. Plastic bottles and glassware materials must be cleaned by soaking in 10% v v^{-1} nitric acid for 24 h, rinsed with ultrapure water, and dried at room temperature. All reagents used for sample preparation and analysis must be of analytical reagent grade, the acids double sub-distilled and high purity and deionized water (resistivity 18.2 MΩ cm^{-1}). Finally, to avoid contamination from the air, the minimum that must be available is a laminar flow cabinet, but the most effective approach is to prepare the sample and solutions in a clean room.

The sample preparation of urine, blood, and plasma can be done by simple dilution with deionized water; solution of ammonium hydroxide, ethylenediamine-tetraacetic acid (EDTA), nitric acid, Triton X-100, and butanol (Tanvir et al. 2020; Goullé et al. 2005); or complete decomposition with nitric acid and hydrogen peroxide under heating, following an addition dilution step with deionized water (Vorkamp et al. 2021). In the case of simple dilution, nitric acid is required to keep elements stable in the solution and Triton X-100 to solubilize the lipids of biological samples through micelle formation, lysing cells, and reducing the clogging of the introduction system of the equipment used (Freire et al. 2018). The dilution factors are important in correcting possible instrumental interference (Olympio et al. 2018a). Samples must be diluted by several factors prior to instrumental analysis. When the dilution is lower than 1: 50, instrumental calibration is usually performed using a matrix-matching approach due to high matrix interference. Aqueous calibration can be easily used for dilutions greater than or equal to 1: 50 (Olympio et al. 2018a).

Table 1 Details on different methods of preparation and analysis for different biological matrices used to determine biomarkers of lead

Matrix	Sample preparation	Instrumental analysis	LOD	References
Urine	**Dilution:** 20-fold with (0.4 v v^{-1}) HNO$_3$ 65% and (0.005% v v^{-1}) Triton X-100	ICP-MS	n/a ; 0.13 µg L^{-1}	Freire et al. (2018) and Salles et al. (2021)
Bone	sample analyzed without previous treatment	XRF	7.6–38.63 mg kg$^-$1	Zhang et al. (2021)
Blood	**Dilution:** tenfold with 5 gL^{-1} of 25% ammonia, 0.5 g L^{-1} Triton X-100, and 0.5 g l$-$1 ethylenediaminetetraacetic acid (EDTA) disodium salt dihydrate in Millipore water	ICP-MS	0.07 µg L^{-1}	Sommar et al. (2014)
Blood	**Dilution:** 0.5% (v v^{-1}) double-distilled HNO$_3$ and 0.005% (v v^{-1}) Triton X-100	ICP-MS	0.05 µg L^{-1}	Costa de Almeida et al. (2010)
Blood	**Dilution:** 50-fold with a solution (0.01% (v v^{-1}) Triton X-100, 0.5% (v v^{-1}) nitric acid)	ICP-MS	10000 µg L^{-1}	Olympio et al. (2018a)
Blood	**Dilution:** sixfold with (0.2% Triton X-100 and 5% nitric acid)	GF AAS	n/a	Leroux et al. (2018)
Fingernail	**Wash:** 1% Triton X-100 and acetone **Digestion:** 10–20 mg sample, 1 ml of 25% (w v^{-1}) TMAH solution (room temperature overnight) **Dilution:** tenfold with (1% v v^{-1}) HNO$_3$	GF AAS ICP-MS	0.13 µg g^{-1} (ETAAS) 0.015 µg lg^{-1} (ICP-MS)	Batista et al. (2008)
Fingernail	**Wash:** acetone, HCl 0.1 mol L^{-1}, and high purity deionized water. **Digestion:** 200 µL of (65% v v^{-1}) HNO$_3$ in a thermostatic bath at 90 °C for 30 min **Dilution:** tenfold with deionized water	ICP-MS	1.9 µg g^{-1} 144.3 µg g^{-1}	Oliveira et al. (2021)
Fingernail	**Wash:** (0.5 % w v^{-1}) Triton X-100 and HNO$_3$ **Digestion:** 300 µL of 65 % (w v^{-1}) HNO$_3$ and 100 µL of (30 % w v^{-1}) H$_2$O$_2$ in a shaking water bath at 90 °C for 30 min or analyzing these by direct solid sampling	GF AAS	70 µg L^{-1}	Olympio et al. (2020)

Table 1 (continued)

Matrix	Sample preparation	Instrumental analysis	LOD	References
Hair	**Wash:** cleaned with mild detergent and deionized water **Digestion:** 0.1 g with 4 mL (70%) HNO_3 and 2.5 mL H_2SO_4 (98%) and then heated in a sand bath	ICP-MS	n/a	Li et al. (2021)
Hair	**Wash:** cleaned with 0.1 mol L^{-1} HCl and dried by wrapping with filter paper for approximately 24 h in an oven (45 °C). A single hair strand is then cut into 36 segments of 5 mm	GF AAS	40 µg g^{-1}	Nomura and Oliveira (2010)
Plasma	**Digestion:** 100 µL of sample, 500 µL of HNO_3 **Dilution:** tenfold dilution with a 2% HNO_3 solution	ICP-MS	0.06 µg L^{-1}	Tanvir et al. (2020)
Tooth	sample analyzed without previous treatment	GF AAS	n/a	Olympio et al. (2010a, b, c) and (2018b)
Saliva	**Dilution:** 25 mL of the acid digested salivary sample, 0.75 mL of Milli-Q water, and 1.5 mL of acid diluent (1% v/v concentrated nitric acid)	ICP-MS	n/a	Shawahna et al. (2021)
Saliva	**Dilution:** tenfold with a solution containing ammonia (0.04 mol L^{-1}), (Na_2EDTA; 200 mg L^{-1}), and Triton X-100 (100 mg L^{-1})	ICP-MS	0.02 µg L^{-1}	Barbosa Jr et al. (2006)

Legend to Table 1 This *table presents a list of studies using different matrices to determine lead levels in the human body and describes methods of preparation and analysis*
n/a: not available

In the case of fingernails and hair, a cleaning step is required to remove surface contaminants that can lead to overestimation of results in the analyte determination. The cleaning step must be carried out with care so as not to remove the analytes present in the samples through leaching. In the case of fingernail, the literature survey revealed many methods of washing for these samples using different reagents, such as acetone and hydrochloric acid, solutions containing Triton X-100 and nitric acid (Olympio et al. 2020), and Triton X-100 and acetone (Batista et al. 2008). Digestion procedures can be performed using a microwave oven system (Oliveira et al. 2021) or shaking water bath with nitric acid and hydrogen peroxide (Olympio et al. 2020), as well as by using tetramethyl ammonium hydroxide solution, incubated at room temperature overnight (Batista et al. 2008), followed by dilution of the sample prior to analysis.

The cleaning of hairs can be performed using different procedures: the IAEA protocol (acetone + water + acetone) (Bermejo Barrera et al. 2000), a combination of the IAEA protocol with hydrochloric acid washing (acetone + water + acetone + hydrochloric acid) (Nomura and Oliveira 2010), and mild detergent and deionized water (Li et al. 2021) to distinguish endogenous and exogenous content. Digestion in a microwave oven system is the most common method to oxidize hair samples. Generally, nitric acid, or a mixture of nitric acid and hydrogen peroxide in various proportions, has been employed (Astolfi et al. 2020). For direct analysis of solids, dispensing with the step of sample digestion and minimizing sample manipulation and consequent contamination or analyte loss, only the cleaning step is necessary.

Measures of lead in dental enamel can be performed by surface enamel acid etch microbiopsies with 10 μL 1.6 mol L^{-1} hydrochloric acid in 70% (v v^{-1}) glycerol. The biopsy solution should be transferred to a centrifuge tube containing 200 μL of high purity water and then analyzed without previous treatment (Olympio et al. 2010c).

The Pb fraction in saliva is about 100 times lower than the dose detectable in other biological markers. These very low analyte concentrations require an analytical technique with extremely highly sensitivity, such as ICP-MS (Caporossi et al. 2010). In this case, sample dilution can be done by dilution with concentrated nitric acid (Wilhelm et al. 2002) or with ethylenediaminetetraacetic disodium dihydrate (200 mg L^{-1}) and Triton X-100 (100 mg L^{-1}) (Barbosa Jr et al. 2006).

Unlike other samples that need a digestion or dilution step, lead measurement in the bones can be carried out directly by portable XRF. This technique allows in vivo noninvasive Pb quantification within minutes (Specht et al. 2019; Zhang et al. 2021). For this approach, the area of the body to be analyzed (e.g., legs) must be cleansed before analysis. This cleansing can be done with alcohol and ethylene diamine tetraacetic acid (EDTA) cotton swabs to remove any contamination (Specht et al. 2018; Zhang et al. 2021).

Instrumental Analysis

The analytical methods to quantify biomarkers in the blood, urine, hair, fingernails, tooth, and saliva should be based on a set of selection criteria such as sensitivity, accuracy, contamination issues, and robustness. Low levels of lead, method sensitivity, and sample amount available for analysis should be considered key parameters in the selection criteria for an analytical method (Vorkamp et al. 2021).

Digested and/or diluted samples can be analyzed by GF AAS using small amounts of sample (10–30 μL) (Leroux et al. 2018), while solid samples (hair and fingernail) can be analyzed by direct sampling without previous treatment (Nomura and Oliveira 2010). GF AAS has a detection limit in the μg L^{-1} range, allowing the quantitation of most inorganic parameters in biological matrices (Michalke et al. 2015). Unfortunately, this method has several disadvantages such as a relatively small linear working range, lower analytic frequency, and, most importantly, being a

mono-elementary analytic technique, although some studies involve simultaneous determinations in other applications (Luz and Oliveira 2011, 2019; Luz et al. 2013).

In the last few decades, the use of ICP-MS has been growing because of its simultaneous multielement measurement capability, coupled with much lower detection limits at elemental concentrations in the ng/L range. Moreover, the technique offers a wider linear dynamic range, allowing the determination of major and trace elements in the same sample aliquot. A particular challenge is avoiding interference, given that ICP-MS is subject to isobaric and polyatomic interferences. Recent advances in ICP-MS technology have allowed elemental determination in different applications at trace and ultra-trace levels in complex matrices (da Silva et al. 2021). Technologies, such as kinetic energy discrimination (KED), collision/reaction cells (CRC), and triple quadrupole, can help circumvent problems with matrix and spectral interferences. Although ICP-MS can achieve an ultralow detection limit, it also has disadvantages, such as high operating and maintenance costs, need for highly specialized operator, and laboratory infrastructure.

XRF has the advantage of being a nondestructive technique, enabling direct analysis of solids. Device software is generally equipped with programs that allow elemental determination without using standards, enabling analysis of materials for which standards are not commercially available. On the other hand, XRF is subject to substantial matrix interference.

Applications to Prognosis, Other Diseases, or Conditions

Therefore, in this chapter we review analytical platforms for investigating lead exposure. From a public health standpoint, there is a major concern of a possible "silent pandemic" (Grandjean and Landrigan 2006) of neurodevelopmental disorders resulting from children's continued exposure to low lead levels (Bellinger 2008).

The form in which neurodevelopmental toxicity is expressed depends on factors such as age at exposure, coexposure to other neurotoxicants, nutritional status, genotype, and the characteristics of the home environment (Hubbs-Tait et al. 2005; Weiss and Bellinger 2006; Olympio et al. 2018a; Silva et al. 2018). The health effects of lead poisoning vary for adults and children and can be related to different concentration ($\mu g\ dL^{-1}$) of lead in the blood (Fig. 4).

In 2012, the CDC introduced a blood lead "reference value" (BLRV) to identify children with higher levels of lead in the blood compared to most children. This level is based on the 97.5th percentile of blood lead values in US children (1–5 years from 2015 to 2016 and 2017 to 2018 NHANES cycles). Every 4 years, the CDC reanalyzes blood lead data from the most recent two NHANES cycles to determine whether the BLRV should be updated. Children with blood lead levels at or above the BLRV represent those in the top 2.5% with the highest blood lead levels. The Federal Advisory Committee, called the Lead Exposure and Prevention Advisory Committee (LEPAC), unanimously voted on May 14, 2021, in favor of recommending that the CDC update the reference value to 3.5 $\mu g\ dL^{-1}$ based on the NHANES data from the 2015–2016 and 2017–2018 cycles.
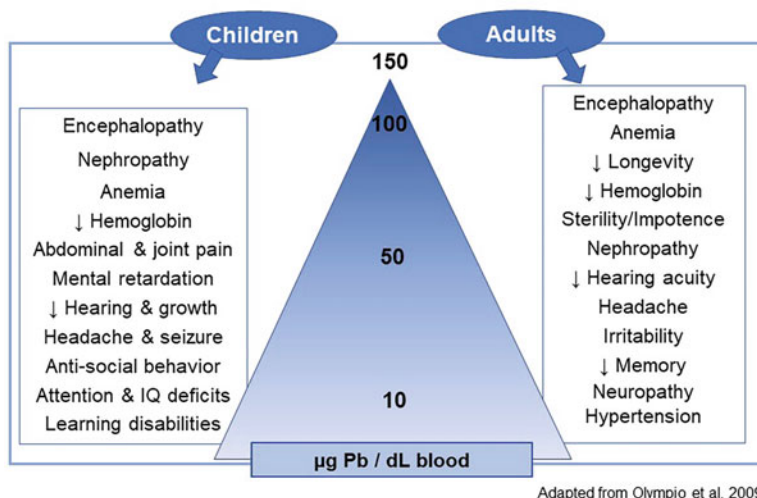
Adapted from Olympio et al. 2009

Fig. 4 Effects of lead poisoning in human health
Legend to Figure 4 This figure shows different degrees of lead poisoning and their effects on human health (Adapted from Olympio et al. 2009)

The CDC's BLRV is a screening tool, but the reference value is not health-based nor a regulatory standard. Once the exposure is confirmed, the person should be protected from the exposure source, the work or a given work process. Beyond the environmental exposure, countries present their occupational legislations for the maximum biological index permitted for lead in workers (Brasil 1994; OSHA 2006).

Moreover, although effect biomarkers are not the focus of this chapter, in the event of a lead occupational exposure, in many cases effect biomarkers can be determined, such as blood and plasma aminolevulinic acid (ALA) levels or urinary ALA. Studies have also found many lead interference sites in the heme biosynthetic pathway. Thus, lead poisoning can be considered a chemical or acquired porphyria (Kauppinen 2005; Sassa 2006). Two thiol enzymes of the heme pathway are sensitive to lead: ferrochelatase and 5-aminolevulinic acid dehydratase (ALAD). In cases of lead poisoning, this sensitivity may cause ALA accumulation in tissues and urinary excretion (Warren et al. 1998). ALA competes with γ-aminobutyric acid (GABA), a neurotransmitter (Brennan and Cantrill 1979). Therefore, high levels of ALA in the blood and brain areas of patients carrying chemical and genetic porphyria could be associated with neurological disorders (Bechara 1996; Bechara et al. 2006).

However, focusing on lead exposure biomarkers is the action of choice because the only effective action for dealing with lead exposure is primary prevention to avoid the ubiquitous health effects of lead.

Dictionary of Terms

- **Dental enamel.** *Enamel is the thin outer covering of the* tooth*. This tough material is the hardest tissue in the human body. Enamel covers the crown, above the dentine. It is the part of the* tooth *that is visible outside of the gums.*
- **Dentine.** *The main supporting structure of the tooth, covered by dental enamel. It is 70% mineral and acellular, as hydroxyapatite crystals, and 30% organic as water, collagen, and mucopolysaccharide.*
- **Senescence.** *Biological aging. Natural process of gradual deterioration of functional characteristics in living organisms.*
- **Half-life.** *The time it takes for the concentration of a substance to fall to half of its initial value.*
- **Creatinine.** *Chemical compound made by muscles as part of regular, everyday activity. Filtered by the kidneys from the blood and excreted from the body in urine.*
- **Body burden.** *The total amount of a chemical substance in the body at any given time.*
- **Biopsy.** *The removal of a small tissue sample from any part of the body to examine it.*
- **Biopsy chemical methods.** *Calculations assume 17.4% of the dental enamel weight corresponds to phosphorus, and biopsy depth is then estimated by dividing the enamel mass (μg) per 2.95 g/cm^3 representing the density of dental enamel.*
- **Biopsy physical methods.** *Confocal Raman measurements acquired using a microscope with a frequency-doubled laser. The Raman spectrum of dental enamel is basically composed of hydroxyapatite peaks.*
- **Micelle.** *An aggregate of molecules with polar and nonpolar characteristics dispersed in a liquid.*
- **Accuracy.** *The proximity of measurement results or calculation to the correct value or a standard.*
- **Robustness.** *The ability to tolerate perturbations that might affect the statistical model, tests, and procedures. The resistance to errors.*
- **Aliquot.** *A portion of a solution. Material divided into small divisions.*
- **Isobaric interference.** *Different elements whose atoms share a common mass.*
- **Polyatomic interference.** *Combination of two or more atoms from different elements.*

Key Facts of Chemical Exposure

There are three common routes of exposure to a toxic substance: absorption by skin penetration, inhalation by the lungs, and ingestion by the oral route.

Occupational exposure can occur through contact with potentially toxic elements in the course of work activities.

Environmental exposure includes exposure to all chemicals present in the environment, including air, food, water, and soil.

Chronic exposure is characterized by repetitive and continuous contact with a substance over a long period (years).

Subchronic exposure is continuous exposure to a substance with an intermediate duration (months).

Acute exposure is related to a single exposure, single-dose administration, or a short contact with a chemical (hours).

Key Facts of Toxic Elements

Toxic elements are all elements that promote adverse effects at a given exposure level; some of these can also be denoted potentially toxic elements.

They are common to the environment but can be responsible for adverse health effects in organisms.

Toxic elements can be essential for the functioning of organisms or potentially harmful at a large enough concentration, while others may be potentially harmful, even at small concentrations.

The field of science that helps us understand the harmful effects exposure to toxic elements can cause is called toxicology.

Determinations of these elements in biological matrices help to establish the dose of the chemical to which a person is exposed and to investigate adverse effects.

Key Facts of Lead Poisoning

Lead is a neurotoxin and lead poisoning is recognized as a public health issue.

Pregnant women and children are the groups most vulnerable to lead poisoning.

The health effects of lead poisoning include cognitive and antisocial behavior in children and adolescents.

No level of lead exposure is considered safe to human health.

The use of lead exposure biomarkers is an approach enabling effective action for primary prevention to avoid lead poisoning.

Summary Points

- *Blood remains the biomarker of choice for recent lead exposure; despite being an invasive method, preparation for analysis can consist of a simple dilution process.*
- *Plasma lead reflects very recent exposure, as the half-life of plasma lead is shorter than 1 h. Plasma lead levels are highly elevated in the event of sudden absorption or acute exposure and rapidly diminish over time.*
- *The use of saliva as a biomarker for lead entails a noninvasive method with simple collection; however, changes in salivary flow, differences in stimulation during collection, and the nutritional and hormonal status of the individuals must be considered.*
- *Urinary lead levels reflect recent Pb absorption and depend on elimination by the kidney, which may fluctuate, and on variation between individuals, which should be adjusted by creatinine correction.*
- *Lead in nails reflects subchronic exposure and is a good tool for initial screening after removing surface contaminants; collection is noninvasive and economically*

viable, when other more appropriate biomarkers are precluded due to prohibitive costs or storage difficulties.

- *Hair offers the advantage of being inert, easier to sample, and stored without technical problems; however, the use of this matrix is controversial because of the lack of reference values, subpopulation variations, and difficulty distinguishing internal and external exposures.*
- *Teeth are recognized as biological markers of cumulative lead exposure, because of lead accumulation over embryonic and postnatal life; for example, surface dental enamel biopsies are a rapid, safe, and painless method. Given there is no reference value for lead in teeth, comparisons are valid only within the same study groups in which the same methodology was applied.*
- *Bone lead reflects cumulative exposure considering the lead concentration in skeletal burden after chronic Pb absorption; X-ray fluorescence instruments are used as a noninvasive method but are not accessible everywhere. There are variations between sexes and during pregnancy and osteoporosis.*

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