TABLE OF CONTENTS

CHAPTER 9

QUANTITATIVE EVALUATION OF LEAD AND BIOCHEMICAL INDICES OF LEAD EXPOSURE IN PHYSIOLOGICAL MEDIA

Contents (Continued)

9. QUANTITATIVE EVALUATION OF LEAD AND BIOCHEMICAL INDICES OF LEAD EXPOSURE IN PHYSIOLOGICAL MEDIA

The sine qua non of a complete understanding of an agent's effects on an organism, e.g., dose-effect relationships, is the quantitative evaluation of the agent in some indicator medium and/or physiological parameters associated with exposure to this agent. This said, two questions follow:

1) What are the most accurate, precise and efficient ways to carry out such measurements?

2) Which measurements in the case of lead (lead itself or biological indicators), in which media, are most appropriate for the particular exposure issue being addressed?

Under the rubric of "analysis" are a number of discrete steps, all of which are important contributors to the relative quality of the final analytical result: (1) collection of sample and its transmission to the point of analysis; (2) laboratory manipulation of the sample, physically and chemically, prior to delivery to a given instrumentation system; (3) the instrumental analysis and quantitation of the level; and (4) establishment of relevant criteria for accuracy and precision, to include internal and external quality assurance checks. Each of these steps is discussed in this chapter.

From a historical perspective, it is clear that the definition of "satisfactory analytical method" for lead has been changing over the years in ways paralleling (1) the evolution of more sophisticated instrumentation/procedures, (2) the greater awareness of such factors as background contamination and loss of element from sample, and (3) growth of a statistical framework in which to evaluate analytical data. For example, newer methods of lead analysis such as anodic stripping voltammetry, background-corrected atomic absorption spectrometry, and isotope dilution mass spectrometry (particularly the last named) are inherently more sensitive and specific than older, classical approaches. Increasing use of the newer methods would tend to result in lower values being measured in a given sample for lead. Whether this analytical change can be discerned in the face of such variables as temporal changes in exposure to lead of sample source is another matter.

Following closely upon the above is the issue of minimal criteria for analytical quality to be applied to an element such as lead, which is also ubiquitously distributed as a contaminant. Obviously, the constraints placed upon a laboratory attempting analysis of geochemical samples of pristine

origin, or extremely low lead levels in biological samples such as plasma, i.e., ultra-clean, ultra-trace analysis, are quite severe and very few laboratories in the world can lay credible claim to such capability.

Ideally, similar standards should be adhered to as closely as possible across the rest of the analytical spectrum. With many clinical, epidemiological, and experimental studies, however, this may be unrealistic interms of realities and goals of the studies. For one thing, laboratory performance is but one part of the quality equation, the problems of sampling being equally important but relatively less subject to tight control. The necessity of rapidly obtaining a blood sample in cases of suspected lead poisoning or the field collection of hundreds (or thousands) of blood samples in urban populations limits the number of sampling safeguards to those which are realistically achievable. Sampling in this context will always be accompanied by a certain amount of analytical "suspicion." Furthermore, a certain amount of biological lead analysis data is employed in a framework of relativity, such as in experimental studies concerned with the relative increase in tissue burden(s) of lead with increases in dosing or effect severity. In addition, any major compromising of an analytical protocol may be statistically discernible. Thus, analysis of biological media for lead must be done with protocols minimizing the relative risk of inaccuracy. Specific accuracy and precision characteristics of a method used in a particular report should be noted, to permit some judgment on the part of the reader about the impact of methodology on the results reported.

The choice of measurement (Question 2, above) and medium for analysis is dictated by both the type of information desired and by technical/logistical considerations. As noted elsewhere, whole blood lead reflects recent or ongoing exposure while mineralizing tissue such as deciduous teeth push back the exposure time frame to months and years. While urine lead values are not particularly good correlates of lead exposure under steady state conditions in populations at large, such measurements are of considerable clinical value in tandem with the use of chelating agent challenge in assessing chelatable lead burdens in an individual. The acquisition of blood samples by venipuncture versus finger puncture will be governed by such factors as cost and feasibility, contamination risk, the biological quality of the finger punch sample, etc. Biological indicators which strongly correlate with lead burden may be more desirable in that they evidence actual response and, taken together with

blood lead data, provide a less risky diagnostic tool for assessment of lead exposure.

A detailed discussion of the relative diagnostic value of various indicators is beyond the scope of this section and the issue is reviewed elsewhere in this document.

9.1 DETERMINATIONS OF LEAD IN BIOLOGICAL MEDIA

9.1.1 Sampling And Sample Handling Procedures For Lead In Biological Media

There are two aspects of lead analysis in biological media which place a premium on careful collection and handling of lead-containing samples: (1) lead occurs at trace levels in most indicators of subject exposure, even under conditions of high lead exposure; (2) such samples must be obtained against a backdrop of pervasive contamination, the full extent of which may still be unrecognized by many laboratories.

The reports of Speeke et al. (1976), Patterson and Settle (1976), Murphy (1976), Berman (1976), and Settle and Patterson (1980) review detailed aspects of the problems of sampling and subsequent sample handling in the laboratory, and it is clear from these discussions that the usual, feasible precautions taken in the course of sample acquisition and detailed below for clinical and epidemiological studies should not be taken by any means as absolute, but rather what is practical and feasible in most cases. In light of the above, furthermore, it may also be the case in many studies that the inherent sensitivity or accuracy of a given methodology/instrumentation is less the determining factor in overall analysis than the quality of sample collection and subsequent handling.

9.1.1.1 Blood Sampling--Samples for blood lead determination may be collected by venipuncture (venous blood) or finger tip puncture (capillary blood). Collection of capillary vs. venous blood will be mandated by a number of factors, including the feasibility of obtaining samples on a screening basis for many subjects and the relative difficulty of securing subject compliance, particularly in the case of children and their parents. Furthermore, capillary blood may be collected as discret volumes into small volume, capillary tubes, or as spots on filter paper disks. With capillary tubes, obtaining good mixing with anticoagulant to avoid clotting is an important consideration, as is the problem of lead contamination of the tube, while the use of filter paper requires the selection of paper with uniform composition, low lead content and uniform blood dispersal characteristics.

Whether venous or capillary blood is collected, much care must be exercised in cleaning the puncture site as well as the selection of lead-free receiving containers. Cooke et al. (1974) employed a virgorous scrubbing with low-lead soap solution and deionized water rinsing, while Marcus et al. (1975) carried out preliminary cleaning with ethanolic citric acid solution followed by 70-percent ethanol rinsing. The vigor in cleaning the puncture site is probably as important as any particular choice of cleaning agent. Marcus et al. (1977) drew attention to the fact that in one procedure for puncture site prepping, where the site is covered with wet paper hand towels, contamination will occur in the case of paper towels from recycled paper, owing to significant lead retention in paper prepared this way.

Capillary and venous blood lead levels, in theory, should be virtually identical, although the available literature indicates that some differences, mainly reflecting problems of sampling, do arise int he case of capillary blood. Also, lower values of capillary vs. blood lead may reflect "dilution" of the sample by extracellular fluid owing to excessive compression of the puncture site. Joselow and Bogden (1972) compared a micro method with finger puncture and spotting onto filter paper with a macro procedure using venous blood and the procedure of Hessel (1968) for flame atomic absorption spectrometry, obtaining a correlation coefficient of $r = 0.9$ (range, 20-46 µg/dl). In a similar manner, Cooke et al. (1974) only obtained an r value of 0.8 (no range given), while Mitchell et al. (1974) obtained a value of 0.92 (10-92 µg/dl). Mahaffey et al. (1979) found that capillary blood levels in a test comparison were ca. 20 percent higher than corresponding venous blood levels in the same subjects, presumably reflecting sample contamination. Similar elevations have been described by DeSilva and Donnan (1980). Carter (1978) has found that blood samples with lower hemoglobin may spread onto filter paper differently than non-amenia samples, requiring correction in quantitation to obtain values which are reliable. With pediatric subjects in whom iron-deficiency anemia may commonly occur, this complication should be kept in mind.

Capillary blood collection done with filter paper, over the capillary blood tube alternative, included the use of Whatman No. 4 (Cernik and Sayers, 1971; Cernik, 1974) and Schleicher and Schull No. 903 (Joselow and Bogden, 1972.)

Important considerations in venous sampling are the relative freedom of the blood container from interior surface lead, and lead in the anticoagulant

used. For studies focused on "normal" ranges, such tubes may still add some lead to blood and still meet certification requirements. The "low-lead" heparinized blood tubes commercially available (blue stopper Vacutainer, Becton-Dickinson) were found to contribute less than 0.2 µg/dl to whole blood (Rabinowitz and Needleman, 1982). Nackowski et al. (1977) surveyed a large variety of commerically available blood tubes in terms of lead and other metal contamination. Lead uptake by blood over time from the various tubes was seen to be minimal with the "low-lead" Vacutainer tubes as well as all but 4 of the remaining types of tubes. In the large survey of Mahaffey et al. (1979), 5 ml Monoject (Sherwood) or 7 ml lavender-top Vacutainer (Becton-Dickinson) tubes were seen to be satisfactory. For more precise work, 1) tubes are best re-cleaned in the laboratory and lead-free anticoagulant added (although this would involve less convenience in sampling efficiency than the evacuated tubes) and 2) verification of blank levels by the analyst for every batch of samples processed should be carried out.

9.1.1.2 Urine Sampling--Urine samples require collection with lead-free containers and caps as well as the addition of a low-lead bacteriocide if samples are to be stored for any period. If possible, 24-hour samples should be obtained, as such collection would level any effect of variation in excretion over the time period. If spot sampling is done, lead levels should be expressed per unit creatinine.

9.1.1.3 Hair Sampling--The usefulness of hair lead analysis depends on the manner of sampling. Hair samples should be removed from subjects in some consistent fashion either a predeterimined length from the skin or using the entire hair, and should be placed in air-tight containers for shipment or storage. For segmental analysis, the entire hair length will be required.

9.1.1.1 Mineral Tissue--Mineralizing tissue such as deciduous teeth represents a matrix which, like hair, is the opposite of blood in stability. A number of precautions do attend such sample collection.

One factor in deciduous teeth collection is consistency in the type of dentition collected across various subjects. In the study of Fosse and Justesen (1978), no difference in lead content between molars and incisors was seen, while Chatman and Wilson (1975) reported comparable whole tooth levels for cuspids, incisors, and molars. On the other hand, Mackie et al. (1977) and Lockeretz (1975) saw levels varying with tooth type, showing a statistical difference (Mackie et al., 1977) in going from 2nd molar (lowest) to incisor (highest). One factor in this difference in data is that the former two

studies found rather low overall lead levels across groups, while the Mackie et al. (1977) report concerned higher values, suggesting that tooth type differences in content may be magnified at higher relative levels of exposure.

Delves et al. (1982), in a detailed study of lead level variation in incisors, have found that lead levels may even vary within a specific type of dentition: comparing pairs of central incisors or pairs of central and lateral incisors all from the same child. These data suggest the desirablity of acquiring 2 teeth per subject, e.g., lateral incisors, to get an average lead value.

Teeth containing fillings are best eliminated from analysis, while extensive decay may also compromise the analytical utility of the tooth. In the report of Mackie et al. (1977), such teeth were discarded if the extent of decay exceeded ca. 30 percent.

9.1.1.5 Sample Handling in the Laboratory--With blood samples, there is the potential problem of the effect of storage on the lead content. It is clear that dilute aqueous solutions of lead will surrender a sizable portion of the lead content to the container surface, glass or plastic (Issaq and Zielinski, 1974; Unger and Green, 1977), but a comparable effect or the extent of such an effect with blood is not clear. Unger and Green (1977) claim that lead loss from blood to containers parallels that seen with aqueous solutions, but their data do not describe this. Moore and Meredith (1977) used isotopic lead spiking (^{203}Pb) with and without carrier in various containers at various temperatures to monitor lead stability in blood over time. The only material loss occurred with soda glass at room temperature by 16 days. Nackowski et al. (1977) found that "low lead" blood tubes, while quite satisfactory in terms of sample contamination, began to show transfer of lead to container wall by 4 days. Meranger, et al. (1981) studied movement of lead, spiked to various levels, to containers of various composition as a function of temperature and time. In all cases, lead loss to containers was significant. There are problems with the above reports. Spiked samples probably are not incorporated into the same biochemical environment as lead inserted in vivo. The Nackowski et al. (1977) study did not indicate whether the blood samp'e were kept frozen or refrigerated between testing intervals.

In the report of Lerner (1975) blood samples (35 originally) were collected from a single subject into lead-free tubes and after freezing were forwarded in blind fashion to a certified testing laboratory over a period of 9 months. Four samples were lost while one was rejected as being grossly

contaminated (ca. 4 S.D.s of mean). Of the remaining 30 samples, the mean was 18.3 with an S.D. of ± 3.9. The analytical method used had a precision (S.D.) of ± 3.5 µg Pb/dl at normal levels of lead, suggesting that the overall stability of the samples in terms of lead content was rather good. In the report of Boone et al. (1979), samples frozen for periods of less than a year showed no effect of storage, while Piscator (1982) noted no change in low levels (< 10 µg/dl) when samples were stored at -20°C for 6 months.

Based on the above data, blood samples to be stored for any period of time should be frozen rather than refrigerated, with care taken to prevent breaking of the tube during freezing. Teeth and hair samples stored in containers to minimize contamination are indefinitely stable.

The actual site of analysis should be as lead-free as possible. Failing the unlikely availability of an "ultra-clean" facility such as that described by Patterson and Settle (1976), the next desirable level of laboratory cleanliness is the "Class 100" facility, defined as one in which there are fewer than 100 airborne particles >0.5 µ, employing high efficiency particulate air (HEPA) filtering and laminar air flow (with movement away from sample handling areas). Totally inert surfaces in the working area and an antechamber for contaminated clothes hanging, appliance cleaning, etc. are other features.

All plastic and glass ware coming into contract with samples should be rigorously cleaned of lead and stored away from dust contact, and materials such as ashing vessels, etc., should be such as to permit minimal lead leaching. In this connection, Teflon ware is more desirable than glass or other plastics (Patterson and Settle, 1976).

Reagents, particularly for chemical degradation of biological samples, should be both certified as such and periodically tested for retention of quality. Several commerical grades of reagents are available, although precise work may require doubly-purified materials from the National Bureau of Standards. Storage of these reagents should be such as to minimize surface contamination around the top of the containers.

For a more detailed discussion of appropriate laboratory practices, one may consult the 2-volume NBS monograph, Sp. Publ. #422, Accuracy in Trace Analyses: Sampling, Sample Handling and Analysis, 1976.

9.1.2 Methods of Lead Analysis

Detailed technical discussion of the array of instrumentation available for measurements of lead in blood and other media is outside the purpose of

this report. The discussion is structured more appropriately to those aspects of methodology dealing with relative sensitivity, specificity, accuracy, and precision. While there is increasing acceptance of international standardized units for expressing lead levels in various media (SI units) units familiar to clinicians and epidemiologists are retained. To connect μg Pb/dl blood to SI units (μmoles/liter) multiply by 10/207, 0.048.

Many reports over the years have purported to offer satisfactory analysis of lead in biological media, many with rather meager adherence to criteria for accuracy and precision and many showing lack of demonstrable utility across a rather wide spectrum of analytical applications. Therefore, discussion in this section is confined to "definitive" and reference methods for lead analysis, except for a brief treatment of the traditional but now widely supplanted colorimetric method.

Using the definition of Cali and Reed (1976), a definitive method is one in which all major or significant parameters are related by solid evidence to the base or derived units of the SI with a high degree of confidence bounds to the limits of uncertainty. A reference method, by contrast, is one (1) of demonstrated accuracy, (2) validated by a definitive method, and (3) arrived at by concensus through testing of performance by a number of laboratories.

In the case of lead in biological media, the definitive method is isotope-dilution mass spectrometry (IDMS). IDMS accuracy stems from the fact that all manipulations are on a weight basis involving simple procedures, and measurements entail only ratios and not the absolute determinations of the istopes involved, greatly reducing instrumental corrections or errors. Reproducible results to a precision of one part in 10^4 or 10^5 are routine with specially designed instruments.

In terms of reference methods for lead in biological media, such a label cannot technically be attached to atomic absorption spectrometry in its various instrumentation/methodology configurations or the electrochemical technique, anodic stripping voltammetry, although these have been termed such in so far as their precision and accuracy can be verified or calibrated against IDMS.

Other methods which are recognized for trace metal analysis in general are not fully applicable to biological lead or have inherent shortcomings. X-ray fluorescence analysis lacks the requisite sensitivity for media with low lead content and the associated sample preparation may occasion a high contamination risk. A notable exception may be X-ray fluorescence analysis of

teeth or bone in situ as discussed below. Neutron activation analysis is the method of choice with many elements, but is restricted for lead measurement, since the associated radiochemical transformation with neutron bombardment produces a species with a half-life of 0.85 seconds.

9.1.2.1 Lead Analysis In Whole Blood--The first generally accepted technique for quantitating lead in whole blood and other biological media involved spectrophotometric measurement based on the binding of lead to a chromogenic agent to yield a chromophoric complex. Typically, the complexing agent has been dithizone, 1,5-diphenylthiocarbazone, giving a complex with lead which is spectrally measured at 510 nm.

Two variations of the spectrophotometric technique when measuring rather low levels of lead have been the USPHS (National Academy of Sciences, 1972) and APHA (American Public Health Association, 1955) procedures. In both, venous blood or urine are wet ashed using concentrated nitric acid of low lead content followed by treatment of the ash with hydroxylamine and sodium citrate to a pH of 9-10. Cyanide ion is added and the solution extracted with dithizone in chloroform. Back extraction removes the lead into dilute nitric acid, the acid layer is treated with ammonia, then cyanide, and re-extracted with dithizone in chloroform. The extracts are read in a spectrophotometer at 510 nm. Bismuth interference is handled (APHA variation) by removal with dithizone at pH 3.4. According to Lerner (1975), the analytical precision (S.D.) in the "normal" range is ca. ± 3.5 µg Pb/dl.

The most accurate and precise method for lead measurement in blood and other media, as noted before, is isotope dilution mass spectrometry. As typified by the report of Machlan et al. (1976), whole blood samples are accurately weighed and a weighed aliquot of ^{206}Pb isotope solution added. After sample decomposition with ultra-pure nitric + perchloric acids, samples are evaporated, residues taken up in dilute lead-free hydrochloric acid and lead isolated using anion exchange columns. Column eluates are evaporated with the above acids and lead deposited onto high purity platinum wire from dilute perchloric acid. The $^{206}Pb/^{208}Pb$ ratio was then determined by thermal ionization mass spectrometry. Samples without added isotope and reagent blanks were also carried through the procedure. In terms of precision, the 95 percent confidence level for lead samples overall was within 0.15 percent. Because of expense and high requirements for operator expertise, time, and level of laboratory cleanliness, IDMS is mainly of practical value in the

development of standard reference materials (SRMs) and for the verification of other analytical methods.

Atomic absorption spectrometry (AAS) is widely used for lead measurements in whole blood, with sample analysis involving macro analysis of venous blood with chemical degradation, micro analysis of liquid samples with or without degradation, and samples applied to filter paper. It is thus the most flexible in terms of prior sample collecting or manipulation. By means of a flame or electrothermal excitation, ionic lead in some matrix is first vaporized and then converted to the atomic state, followed by resonance absorption from either a hollow cathode or electrodeless discharge lamp generating lead absorption lines at 217.0 and 283.3 nm. After monochrometer separation and photomultiplier enchancement of the differential signal, it is measured electronically.

The earliest methods of atomic absorption spectrometric analysis involved the aspiration of ashed samples of blood, usually subsequent to extraction into an organic solvent to enchance sensitivity by preconcentration, into a flame. Some methods did not involve digestion steps prior to solvent extraction (Kopito et al., 1974). Of these various macro flame AAS methods, that of Hessel (1968) continues to be used with some frequency.

At present, lead measurement in blood by AAS entails any of several micro methods which permit greater sensitivity, precision, and economy of sample and time.

The flame micro method of Delves (1970), termed the Delves Cup procedure, usually involves delivery of discrete, small samples of unmodified whole blood to nickel cups, with subsequent drying and peroxide decomposition of organic content prior to positioning in the flame. The marked enhancement of sensitivity over conventional flame aspiration is due to immediate, total consumption of sample and the generation of a localized population of atoms. In addition to discrete blood volumes, blood-containing filter paper disks have been used (Joselow and Bogden, 1972; Cernik and Sayers, 1974; Piomelli et al., 1980). Several modifications of the Delves method include that of Ediger and Coleman (1972), where dried blood samples in the cups are pre-ignited to destroy organic matter by placement near the flame in precise, repeatable fashion, and the variation of Barthel et al. (1973), where blood samples are mixed with dilute nitric acid in the cups followed by sample drying (200°C) in an oven and charring at 450°C on a hot plate.

The Delves Cup procedures requires correction for background spectral interference, which is usually achieved by instrumentation equipped with a dueterium arc lamp or with dual-channel capabiltity, with one channel operating at a non-resonance absorption line. While the 217.0 nm line of lead is less subject to such interference, precise work is best done with correction.

This micro method as applied to whole blood lead appears to have an operational sensitivity of 1.0 µg Pb/dl, or somewhat below when competently employed, and a relative precision of ca. 5 percent in the range of levels encountered in industrialized areas.

Micro AAS methods employing electrothermal (furnace) excitation in lieu of a flame can be ca. 10-fold more sensitive than the Delves procedure, and a number of reports describing whole blood lead analysis have appeared (see annual bibliographies in Atomic Spectroscopy, Perkin-Elmer Corp.). Because of increased sensitivity, the "flameless" AAS technique permits the use of small blood volumes (1-5 µl) with samples undergoing drying and dry ashing in situ. Physicochemical and spectral interferences are inherently severe with this approach, requiring careful background correction. In one Flameless AAS configuration, background correction exploits the Zeeman effect, where correction is made at the specific absorption line of the element and not over a band-pass region as is the case with the deuterium arc. While control of background interference up to 1.5 molecular absorbence is claimed with the Zeeman system (Koizumi and Yasuda, 1976), it is technically preferable to employ charring prior to atomization. Hinderberger et al. (1981) used dilute ammonium phosphate solution to minimize chemical interference in their furnace AAS method.

Precision can be a problem in the flameless technique without careful attention to the problem of sample diffusibility over and into the graphite matrix of the receiving receptacle -- tube, cup, or rod. With the use of diluted samples and larger applied volumes, the relative precision of this method can approach that of the Delves technique (Delves, 1977).

In addition to the various atomic absorption spectral methods noted above, electrochemical techniques have been applied to blood lead analysis. Electrochemical methods, in theory, differ from AAS methods in that the latter are "concentration" methods regardless of sample volumes available, while electrochemical analysis involves bulk consumption of sample and hence would have infinite sensitivity, given an infinite sample volume. This intrinsic

property is of little practical advantage given sample, instrumentation design, and blank limits.

The most widely used electrochemical method for lead measurement in whole blood and other biological media is anodic stripping voltammetry (ASV) and it is probably the most sensitive, involving as it does an elctrochemical preconcentration (deposition) step in the overall analysis (Matson and Roe, 1966; Matson, 1970). In this method, samples such as whole blood, 50-100 µl, are usually wet ashed and reconstituted in dilute acid or mode electro-available with metal exchange reagents. Using freshly prepared composite electrodes of mercury film deposited on carbon, lead is plated out from the solution for a specific time and at a selected negative voltage. The plated lead is then reoxidized in the course of anodic sweeping, generating a current peak which may be chart recorded, or displayed on commerical instruments, as units of concentration (µg/dl).

One alternative to the time and space demands of wet ashing blood samples is the use of metal exchange reagents which displace lead from binding sites in blood by competitive binding (Morell and Giridhar, 1976; Lee and Meranger, 1980). In one commerical preparation, this consists of a solution of calcium, chromium, and mercuric ions.

The working detection limit of ASV for blood is comparable to that of the AAS micro methods while the relative precision is best with prior sample degradation, ca. 5 percent, but less when the blood samples are run directly with the ion exchange reagents (Morrell and Geridhar, 1976), particularly at the low end of "normal" blood lead values. While AAS methods require attention to various spectral interferences to achieve satisfactory performance, electrochemical methods such as ASV require consideration of such factors as agents which complex lead and alter its red-ox potential properties and the effects of co-reducible metals. Chelants used in therapy, particularly penicillamine, may interfere, as does blood copper in cases where this element is elevated, as in pregnancy and such disease states as leukemia, lymphoma, and hyperthyroidism (Berman, 1981). At very low levels of lead in blood, then, ASV may pose more problems than atomic absorption spectrometric techniques.

It should be noted that correction of whole blood lead values for hematocrit, although carried out in the past, is not appropriate. Kochen and Greener (1973) have demonstrated that there is no correlation between hematocrit and whole blood lead level, in humans and animals, suggesting that

erythrocytes serve as a carrier of blood lead, with a binding capacity in excess of those levels associated with even very heavy exposure.

9.1.2.2 Lead In Plasma--While virtually all of the lead present in whole blood is bound to the erythrocyte (Robinson et al., 1958; Kochen and Greener, 1973), lead in plasma figures in movement to affected tissues and a number of reports have addressed the issue, using a variety of methods. It is of importance that every precaution be taken to use non-hemolyzed blood samples for plasma isolation.

Rosen et al. (1974) used flameless atomic absorption spectrometry and microliter samples of plasma to measure plasma lead, with background correction for the smoke signal generated for the unmodified sample.

Cavalleri et al. (1978) used a combination of solvent extraction of modified plasma with preconcentrating and flameless atomic absorption. These authors noted that the micro method used by Rosen et al. (1974) permitted less precision and accuracy than their technique, involving as it did a significantly smaller amount of lead delivery to the furnace accessory.

DeSilva (1981) used a technique similar to that of Cavalleri et al. (1978), solvent extraction and flameless AAS, but collected samples in heparinized tubes, claiming that the use of EDTA as anticoagulant disturbs the cell-plasma distribution of lead enough to yield erroneous data. Much more care was given in this procedure to the issue of background contamination. In both cases, increasing levels of plasma lead were measured with increasing whole blood lead, suggesting an equilibrium ratio in contradiction to the data of Rosen et al. (1974), who found a fixed level of 2-3 µg Pb/dl plasma over a wide range of blood lead. But in the DeSilva (1981) study, the actual levels in plasma were much lower than reported by Cavalleri et al. (1978).

Using istope-dilution mass spectrometry and sample collection/manipulation in an "ultra-clean" facility, Everson and Patterson (1980) measured the plasma lead levels in 2 subjects, a control and a lead-exposed worker. The control had a plasma lead level of 0.002 µg Pb/dl, several orders of magnitude lower than that seen with studies using other less precise analytical approaches. The lead-exposed worker had a plasma level of 0.2 µg Pb/dl. Several other reports in the literature, using isotope-dilution mass spectrometry noted somewhat higher values of plasma lead (Manton and Cook, 1979; Rabinowitz et al., 1974), which Everson and Patterson have ascribed to problems of laboratory contamination. However, utilizing tracer lead data to minimize the

impact of contamination results in a value of 0.15 µg/dl (Rabinowitz, et al., 1974).

Concerning appropriate plasma lead methodology, levels are extremely low, how low varying with the methods employed to measure such concentrations. While the data of Everson and Patterson (1980) were obtained from only 2 subjects, it seems unlikely that use of more subjects would result in a plasma lead range extending upward to levels seen with ordinary methodology in ordinary laboratory surroundings. The above discussion is necessary to the question of what is to be apropriate methodology for plasma analysis, and the Everson and Patterson (1980) report indicates that some level of doubt will surround results obtained with conventional methods.

Although not the primary focus of their study, the values obtained by Everson and Patterson (1980) for whole blood lead, unlike the data for plasma, are within the range for unexposed (11 µg Pb/dl) and exposed (80 µg Pb/dl) subjects generally reported with other methods. This would suggest that, for the most part, reported values do indeed reflect in-vivo blood lead rather than sampling problems or methods inaccuracy.

9.1.2.3 Lead In Teeth--When carrying out analysis of shed deciduous or extracted permanent teeth, some reports have employed the whole tooth after surface cleaning to remove contaminating lead (e.g., Moore et al., 1978; Fosse et al., 1978; Mackie et al., 1977), while others have measured lead in dentine (e.g., Shapiro et al., 1973; Needleman et al., 1979; Al-Naimi et al., 1980). Several reports (Grandjean et al., 1979; Shapiro et al., 1973) have also described the analysis of secondary, or circumpulpal, dentine, the portion of the tooth having the highest relative fraction of lead.

Dentine separation in the procedure of Needleman et al. (1979) involves embedding the tooth in wax, followed by thin central sagittal sectioning, the dentine being isolated from the sawed sections by careful chiseling.

The mineral and organic composition of teeth and their components requires the use of thorough chemical decomposition techniques, including wet ashing and/or drying ashing steps, sample pulverizing or grinding, etc. In the procedure of Steenhout and Pourtois (1981), teeth are dry ashed at 450°C, powdered and dry ashing repeated. The powder is then dissolved in nitric acid. Fosse and Justesen (1978) reduced tooth samples to a coarse powder by crushing in a vise, followed by acid dissolution. Oehme et al. (1978) crushed samples to a fine powder in an agate mortar and dissolved the samples in

nitric acid. Mackie et al. (1977) and Moore et al. (1978) dissolved samples directly in concentrated acids. Chatman and Wilson (1975) and Needleman et al. (1974) carried out wet ashing with nitric acid followed by dry ashing at 450°C. Oehme et al. (1978) found that acid wet ashing of tooth samples gave better results if carried out in a heated Teflon bomb at 200°C.

With regard to instrumental methods of lead measurements in teeth, atomic absorption spectrometry and anodic stripping voltammetry have been employed more frequently than other methods.

With the atomic absorption spectrometry methods, the high mineral content of teeth tends to argue for preliminary isolation of lead from this matrix prior to analysis. In the method of Needleman et al. (1974) and Chatman and Wilson (1975), ashed residues in nitric acid are treated with ammonium nitrate and ammonium hydroxide to a pH of 2.8, followed by dilution and extraction with a methylisobutylketone solution of ammonium pyrrolidinecarbodithioate. Analysis is by flame AAS using the 217.0 nm lead absorption line. A similar procedure was employed by Fosse and Justesen (1978).

Anodic stripping voltammetry has been successfully used in tooth lead measurement (Shapiro et al., 1973; Needleman et al., 1979, Oehme et al., 1978). As typified by the method of Shapiro et al. (1973), samples of dentine are dissolved in a small volume of low-lead concentrated perchloric acid and diluted (5.0 ml) with lead-free sodium acetate solution. With deoxygenation, samples were analyzed in a commercial ASV unit, using a plating time of 10 minutes at a plating potential of -1.05 V. Anodic sweeping was at a rate of 60 mV/sec. and a variable current of 100-500 μA.

Since lead content of teeth is higher than in most sampling media of biological origin, the relative precision of analysis with appropriate accomodation of the matrix effect, such as the use of matrix-matched standards, in the better studies indicates a value of ca. 5-7 percent.

All of the above methods involve shed or extracted teeth and as a consequence represent retrospective determination of lead exposure. In the procedure of Bloch et al. (1976), tooth lead is measured in-situ using an X-ray fluorescence technique. Multiple measuring by this approach is attractive in terms of detecting the "on-going" rate of increase in blody lead burden and when combined with serial blood sampling provides data for blood lead-tooth lead relationships, and would be very useful in prospective studies. A collimated beam of radiation from ^{57}Co is allowed to irradiate the upper cenral incisor teeth of the subject. Using a relatively safe 100-second

irradiation time and measurement of $K_{\alpha 1}$ and $K_{\alpha 2}$ lead lines via a germanium diode and a pulse height analyzer for signal processing, lead levels of 15 parts per million or higher can be measured.

9.1.2.4 Lead In Hair--Hair constitutes a non-invasive sampling method with virtually no problems with sample stability on extended storage. However, the advantages of accessibility and stability are offset by the problem of assessing external contamination of the hair surfce by atmospheric fallout, hand dirt, lead in hair preparations, etc., and is probably of less value, overall, than measurements using other media.

The various methods which have been employed for removal of external lead have been reviewed (Chatt et al., 1980; Gibson, 1980; Chattopadhyay et al., 1977). Cleaning techniques, obviously, should be vigorous enough to remove surface lead but not such as to remove the endogenous fraction. To date, it remains to be demonstrated that any published cleaning procedure is reliable enough to permit acceptance of reported levels of lead in hair. Such a demonstration would have to employ lead isotopic studies with both surface and endogenous isotopic lead removal being monitored as a function of some particular cleaning technique.

9.1.2.5 Lead In Urine--Analysis of lead in urine is complicated by the relatively low concentrations of this element occurring in the medium (lower than in blood in many cases) as well as the complex mixture of mineral elements present. Lead levels are higher, of course, in cases where lead mobilization or therapy with chelants is carried out, but in these cases, samples must be analyzed so as to account for lead bound to chelants such as EDTA. This requires either sample ashing or standards containing the chelant.

Although analytical methods have been published which carry out direct analysis of lead in this medium, samples are probably best wet ashed prior to analysis, using the usual mixtures of nitric + sulfuric and/or perchloric acids.

Both atomic absorption spectrometric and anddic stripping voltammetric methods have been applied to urine lead analyses, the former entailing either direct analysis of ashed residues or a preliminary chelation-extraction step.

With flame AAS, ashed urine samples must invariably be extracted with a chelant such as ammonium pyrrolidinecarbodithioate in methylisobutylketone to achieve reasonably satisfactory results. Direct analysis, furthermore, creates mechanical problems with burner operation, owing to the high mineral content,

and resulting in considerable down time. The procedure of Lauwerys et al. (1975) is typical of flame AAS methods with preliminary lead isolation.

Owing to the relatively greater sensitivity of graphite furnace (flameless) AAS, this variation of the method has been applied to urine analysis in scattered reports and where it appears that adequate performance for direct sample analysis requires steps to minimize matrix interference. Typical of the better direct analysis methods is that of Hodges and Skelding (1981). Urine samples are mixed with iodine solution and heated, followed by dilution with a special reagent containing ammonium molybdate, phosphoric acid and ascorbic acid. Small aliquots (5 µl) are delivered to the furnace accessory of an AAS unit containing a graphite tube pretreated with ammonium molybdate. The relative precision of the method is reported to be about 6 percent. In the method of Legotte et al. (1980), such tube treatment and sample modifications were not employed and the average precision figure was 13 percent.

Compared to various atomic absorption spectrometric methods, anodic stripping voltammetry has been less frequently employed for urine lead analysis and it would appear from available electrochemical methods in general that such techniques applied to urine require further development. Franke and de Zeeuw (1977) employed differential pulse anodic stripping voltammetry as a screening tool for lead and other elements in urine. Jagner et al. (1979) described analysis of urine lead using potentiometric stripping, where the element is preconcentrated at a thin-film mercury electrode as in conventional ASV, but where reoxidation occurs, after disconnecting the circuity, with either oxygen or mercuric ions if deoxygenated samples are used.

As noted in Section 9.1.1.2, spot sampling of lead in urine should be expressed per unit creatinine, if it is not possible to obtain 24 h. collection.

9.1.2.6 Lead In Other Tissues--Bone sample of experimental animal or human autopsy origin require preliminary cleaning procedures for removal of muscle and connective tissue, with care being taken to minimize sample contamination while doing so. As is the case with teeth, samples must be chemically decomposed prior to analysis.

Satisfactory instrumental methods for bone lead analysis comprise a much smaller literature than is the case for other media.

Wittmers et al. (1981) have described the measurement of lead in dry-ashed (450°C) bone samples using flameless atomic absorption spectrometry.

Ashed samples were weighed and dissolved in dilute nitric acid containing lanthanum ion, the latter used to suppress interference from bone elements. Small volumes (20 µl) and the high calcium content required that atomization be done at 2400°C. to avoid condensation of calcium within the furnace. Quantitation was by the method of additions. Relative precision was 6-8 percent at relatively high lead content (60 µg/g ash) and 10-12 percent at levels of 14 µg/g ash or less.

Ahlgren et al. (1980) have described the application of X-ray fluorescence analysis to in-vivo lead measurement in the human skeleton, using tibia and phalanges. In this technique, irradiation is carried out with dual ^{57}Co gamma ray source. The generated $K_{\alpha 1}$ and $K_{\alpha 2}$ lead lines are detected with a lithium-drifted germanium detector. The detection limit is 20 parts per million.

Soft organs differ from other biological media in the extent of anatomic heterogeneity as well as lead distribution, e.g., brain and kidney. Hence, sample analysis involves either discrete regional sampling or the homogenizing of the organ. The efficiency of the latter can vary considerably, depending on the density of the homogenate, the efficiency of rupture of the formed elements, etc. Glass-on-glass homogenizing is to be avoided because of liberation of lead from glass matrix with abrasion.

Atomic absorption spectrometry, in its flame or flameless variations, appears to be the method of choice in many studies. In the procedure of Slavin et al. (1975), tissues were wet ashed, the residues taken up in dilute acid and analyzed with the furnace accessory of an AAS unit. A large number of reports representing slight variations of this basic technique have appeared over the years (see Annual Bibliographies of Atomic Spectroscopy, Perkin-Elmer Corp.). Flame procedures, being less sensitive than the graphite furnace method, require more sample than may be available or are restricted to measurement in tissues where levels are relatively high, e.g., kidney. In the method of Farris et al. (1978), samples of brain, liver, lung or spleen (as discrete segments) are lyophilized and solubilized at room temperature with nitric acid. Following neutralization, lead is extracted into methylisobutylketone with ammonium pyrrolidinecarbodithioate and aspirated into the flame of an AAS unit. The reported relative precision was 8 percent.

9.1.3 Quality Assurance Procedures In Lead Analysis

Regardless of technical differences among the different methodologies for lead analysis, one can define the quality of such techniques as being of: (1)

poor accuracy and poor precision; (2) poor accuracy and good precision; and (3) good accuracy and precision. In terms of available information, the major focus in assessing quality has been on blood lead determinations.

According to Boutwell (1976), the use of quality control testing for lead measurement rests on 4 assumptions: (1) the validity of the specific procedure for lead in some matrix has been established; (2) the stability of the factors going to make up the method has been both established and manageable; (3) the validity of the calibration process and the calibrators with respect to the media being analyzed has been established; and (4) surrogate quality control materials of reliably determined analyte content can be provided.

The above assumptions, when translated into practice, revolve around steps employed within the laboratory, using a battery of "internal checks" and the further reliance on "external checks" such as a formal, well-organized milti-laboratory proficiency testing program.

Analytical quality protocols can be further divided into start-up and routine procedures, the former entailing the establishment of detection limits, "within-run" and "between-run" precision, recovery of analyte, etc. With adoption of a new method desirable for some particular analytical advantage, the method is usually compared in the laboratory or outside the laboratory for relative performance. For example, Hicks et al. (1973) and Kubasik et al. (1972) reported that micro techniques for measuring lead in whole blood were found to have a satisfactory correlation with results using conventional flame procedures. Matson (1970) noted a good agreement between anodic stripping voltammetry and both atomic absorption spectral and the dithizone colorimetric techniques.

The problem with such a means for method comparison and using ordinary samples is that the reference method is assumed to be accurate for the particular level of lead in that given matrix at that point in time. Good correlation data obtained in this fashion may simply indicate that two inaccurate methods are simultaneously performing with the same level of precision proficiency.

Preferable approaches for assessing accuracy are the use of certified samples as to lead content, obtained by a definitive method, or by direct comparison using a given group of samples and different techniques with a definitive procedure.

Eller and Haartz (1977) compared precision and accuracy of 5 available methods for measuring lead in blood: dithizone spectrometric, extraction + tantalum boat AAS, extraction + flame aspiration AAS, direct aspiration AAS, and the graphite furnace AAS techniques. Porcine whole blood certified by the NBS using isotope-dilution mass spectrometry at 1.00 µg Pb/g (± 0.023) was tested and all methods were found to be equally accurate, while precision of the tantalum boat technique was found to be the least among the methods. The obvious limitation of these data is that they relate to a rather high blood lead content, suitable for use in exposure of lead workers of some other occupational context, but less appropriate for clinical or epidemiological investigations.

Boone et al. (1979) compared the analytical performance of 113 laboratories using various methods and 12 whole blood samples (bovine blood from animals fed a lead salt) certified as to lead content using isotope-dilution mass spectrometry at the NBS. Lead content ranged from 13-102 µg Pb/dl and the methods included anodic stripping voltammetry and 5 variations of AAS.

The order of agreement with NBS values, i.e., relative accuracy, was extraction > ASV > tantalum strip > graphite furnace > Delves Cup > carbon rod. The AAS methods all tended to show bias, being positive at values less than 40 µg Pb/dl and negative at levels greater than 50 µg Pb/dl. ASV tended to show less of a positive bias problem, although it was not bias-free with either of the blood lead ranges. In terms of relative precision, the ranking is ASV> Delves Cup>tantalum strip>graphite furnace>extraction>carbon rod. The overall ranking in accuracy and precision indicates ASV>Delves Cup>extraction>tantalum strip> graphite furnace>carbon rod. As the authors caution, the above data should not serve to indicate that any established laboratory using one particular technique would not perform better than this; rather, it should be used as assistance for newer facilities choosing among methods.

There are a number of steps in quality assurance with the routine measurement of lead that are both necessary and should be used in an ongoing program. With respect to internal checks of routine performance, these include calibration, precision and accuracy testing. With biological matrices, the use of matrix-matched standards is quite important, as is an understanding of the range of linearity and variation of calibration curve slopes from day to day. It is common practice to analyze a given sample in duplicate, further replication

being carried out if the first two determinations vary beyond a predetermined amount. A second desirable step is the analysis of samples collected in duplicate but analyzed "blind", to avoid analyst bias.

Monitoring of accuracy within the laboratory is limited to the availability of control samples having a certified lead content and in the same medium as the samples being analyzed. Standard reference materials (SRMs) such as orchard leaves and lyophilized bovine liver are of help in some cases, but such materials are not available for important media such as whole blood generally. Recourses can be had to commercially available whole blood samples, prepared and certified by the marketing facility (TOX-EL, A.R. Smith Co., Los Angeles, CA; Kaulson Laboratories, Caldwell, NJ). With these sources, attention must be paid to the reliability of methods used by their reference laboratories. The use of such materials, from whatever source, must be done in a way to minimize analyst bias, so that the attention given control specimens reflects that given routine samples.

Finally, the most important form of quality assurance is the ongoing assessment of laboratory performance by proficiency testing programs using externally provided specimens for analysis. Earlier interlaboratory surveys of lead measurement in blood (and urine) indicated that a number of laboratories had exhibited unsatisfactory performance, even at relatively high levels of lead (Keppler et al., 1970; Donovan et al., 1971; Berlin et al., 1973), although it may have been the case that there were problems in the preparation and state of the blood samples during and after distribution (WHO, 1977).

These earlier programs for proficiency indicated that: (1) many laboratories were able to achieve a good degree of precision within their own facilities; (2) that the greater the number of samples routinely analyzed by a facility, the better the performance; and (3) that 30 percent of the laboratories routinely analyzing blood lead reported values differing by more than 15 percent from the true level (Pierce et al., 1976).

In the more recent, but very limited, study of Paulev et al. (1978), 5 facilities participated in a survey, using samples to which known amounts of lead were added. For lead in both whole blood and urine, the interlaboratory coefficient of variation was reported to be satisfactory, ranging from 12.3 to 17.2 percent for blood and urine samples. Aside from its limitation of scope, this study used "spiked" vs. in-vivo lead, so that extraction techniques used

in most of the laboratories surveyed would have given misleadingly better results in terms of actual recovery.

Maher et al. (1979) described the outcome of a proficiency study involving up to 38 laboratories analyzing whole blood pooled from a large number of samples submitted for blood lead testing. The Delves Cup micro technique was the most heavily represented, followed by chelation-extraction + flame AAS and graphite furnace AAS. Anodic stripping voltammetry was used by only ca. 10 percent of the laboratories, so that the results really portray AAS methods. All laboratories had about the same degree of accuracy, with no evidence of consistent bias, while the interlaboratory coefficient of variation was ca. 15 percent. A subset of this group, certified by the American Industrial Hygiene Association for air lead, showed a corresponding precision figure of ca. 7 percent. Over time, the subset of AIHA-certified laboratories remains about the same in proficiency while the other facilities show continued improvement in both accuracy and precision. This study indicates that program participation does help the relative performance of a laboratory doing blood lead determinations.

The most comprehensive proficiency testing program is that carried out by the Centers for Disease Control, USPHS. This consists of two operationally and administratively distinct subprograms, that conducted by the Center for Environmental Health (CEH) and the other by the Licensure and Proficiency Testing Division, Laboratory Improvement Program Office (LIPO). CEH is directed at facilities involved in lead poisoning prevention and screening while LIPO is concerned with laboratories seeking certification under the Clinical Laboratories Improvement Act of 1967 as well as regulations of the Occupational Safety and Health Administration. Both the CEH and LIPO protocols involve the use of bovine whole blood certified as to content by the means of the reference laboratories (6 in the CEH program, 20-23 in that of LIPO) and with an ad-hoc target range of ± 6 µg Pb/dl for values of 40 µg Pb/dl or less and ± 15 percent for higher levels. Samples (3) are provided monthly with CEH, for a total of 36 yearly, while LIPO participants receive 3 samples quarterly, 12 samples yearly. Use of a fixed range rather than a standard deviation has the advantage of allowing the monitoring of overall laboratory improvement.

For Fiscal Year 1981, 114 facilities were in the CEH program, 92 of these participating for the entire year. Of these, 57 percent each month reported all 3 samples within the target range, and 85 percent on average reported 2

out of 3 samples correctly. Of the facilities reporting throughout the year, 95 percent had a 50 percent or better performance, i.e., 18 blood samples or better.

If one compares these summary data for FY 1981 with earlier annual reports, it would appear that there has been considerable improvement in the number of laboratories achieving higher levels of proficiency. For the interval FY 1977-FY 1979, there was a 20 percent increase in the number correctly analyzing >80 percent of all samples and a 33 percent decrease in those reporting <50 percent correct. In the last several years, FY 1979- FY 1981, overall performance appears to have more or less stabilized.

With the LIPO program for 1981 (Dudley and Boone, 1982), the overall laboratory performance, averaged across all quarters, was 65 percent of the laboratories analyzing all samples correctly and ca. 80 percent performing well with 2 of 3 samples. Over the 4 years of this program, an increasing ability to correctly analyze lead in blood appears to have been demonstrated.

Current OSHA criteria for certification of laboratories doing occupational blood lead measurements require 8 of 9 samples be correctly analyzed in the previous quarter (U.S. OSHA, 1982). These criteria appear to reflect the ability of a number of laboratories to perform at this level.

It should be noted that most proficiency programs, including the CEH and LIPO surveys, are appropriately concerned with blood lead levels encountered in such cases as pediatric screening for excessive exposure to lead or in occupational exposure. As a consequence, there does appear to be an underrepresentation of lead values in the low end of "normal" range. In the CEH distribution for FY 1981, 4 samples were below 25 µg Pb/dl, or 11 percent. The relative performance comparison of the 114 facilites with these samples indicates outcomes much better than with the whole sample range.

9.2 DETERMINATION OF ERYTHROCYTE PORPHYRIN (FREE ERYTHROCYTE PROTOPORPHYRIN, ZINC PROTOPORPHYRIN)

9.2.1 Methods Of Erythrocyte Porphyrin Analysis

With lead exposure, the final step in heme biosynthesis -- insertion of iron into protoporphyrin IX to form heme -- is inhibited, leading to an accumulation of the porphyrin, with zinc (II) occupying the position normally filled by iron. Depending on the particular method of analysis, zinc protoporphyrin (ZPP) itself or the metal-free form, free erythrocyte protoporphyrin (FEP) is measured. FEP generated as a consequence of chemical manipulation

should be kept distinct from the metal-free form biochemically produced in the porphyria, erythropoietic protoporphyria.

Porphyrins are labile to photochemical decomposition and hence samples must be collected and handled with protection from light and analyzed as soon thereafter as possible. Hematocrits must also be obtained to normalize levels in blood samples from anemic subjects to the normal hematocrit values.

In terms of methodological approaches for EP analysis, virtually all methods now in use exploit the ability of porphyrins to undergo intense fluorescence when excited at the appropriate wavelength of light. Such fluorometric techniques can be further classed as to laboratory or wet chemical micro methods or micro methods using recently developed instrumentation, the hematofluorometer. The latter involves direct measurement in whole blood. Since the mammalian erythrocyte contains all of the EP in whole blood, either packed cells, or whole blood may be used, although the latter is more analytically expedient.

Owing to the relatively high sensitivity of fluorometric measurement for FEP or ZPP, laboratory methods for spectrofluorometric analysis require a relatively small sample of blood; hence, micro techniques are currently the most popular in most laboratories. These involve either liquid samples or blood collected on filter paper, the latter of use particularly in field sampling.

In the micro procedure of Piomelli and Davidow (1972), small volumes of whole blood, analyzed directly or after collection on filter paper, are treated with a suspension of Celite in saline followed by a 4:1 mixture of ethyl acetate: glacial acetic acid. After agitation and centrifugation, the supernatant is extracted with 1.5N HCl. The acid layer is analyzed fluorometrically using an excitation wavelength of 405 nm and measurement at 615 nm. Blood collected on filter paper discs is eluted with 0.2 ml H_2O first. The filter paper method was found to work just as well as liquid samples of whole blood. Protoporphyrin IX is employed as quantitation standard.

In the variation of Chisolm and Brown (1975), volumes of 20 µl of whole blood are treated with ethyl acetate/acetic acid (3:1) and briefly mixed. The acid extraction step is done with 3 N HCl followed by a further step of dilution with more acid. In this procedure, protoporphyrin IX is used as the working standard with coproporphyrin used to monitor the calibration of the fluorometer and any variance with the protoporphyrin standard.

The above micro fluorometric methods all involve double extraction. In the single-extraction variation of Orfanos et al. (1977), liquid samples of whole blood (40 µl) or blood on filter paper are treated with acidified ethanol, the mixtures agitated and centrifuged and the supernatants analyzed directly in fluorometer cuvettes. For blood samples on filter paper, blood is first leached from the paper with saline by soaking for 60 minutes. Coproporphyrin is used as the quantitating standard. The correlation coefficient with the Piomelli and Davidow procedure (vide supra) over the range 40-650 µg EP/dl RBCs was r = 0.98.

Lamola et al. (1975) analyzed the zinc protophyrin (ZPP) as such in their procedure. Small volumes of blood, 20 µl, are worked up in a detergent (dimethyl dodecylamine oxide)-phosphate buffer solution and fluorescence measured at 594 nm with excitation at 424 nm. In the variation of Joselow and Flores (1977), 10 µl of whole blood is diluted 1,000-fold, along with protoporphyrin (Zn) standards, with the detergent-buffer solution. It should be noted that it is difficult to obtain the ZPP standard in pure form, and Chisolm and Brown (1979) report the use of protophyrin IX plus zinc salt for such standards.

Hanna et al. (1976) compared 4 micromethods for EP analysis: Double extraction with ethyl acetate/acetic acid and HCl (Piomelli and Davidow, 1972), single extraction with ethanol, single extraction with acetone (Chisolm et al., 1974), and direct solubilization with detergent (Lamola et al., 1975). Of these, the ethyl acetate and ethanol procedures were satisfactory with complete extraction occurring with the ethylacetate/acetic acid method.

The levels of precision with these wet micro methods appears to differ with the specifics of analysis. Piomelli (1973) reported a coefficient of variation (C. of V.) of 5 percent, compared to Herber's observation of 2-4 percent (Herber, 1980) and 6-11 percent for total C. of V., which included precision of samples, standards, and day-to-day variation. The Lamola et al. (1975) method for ZPP measurement was found to have a C. of V. of 10 percent (same day, presumably), while Herber (1980) reported for this method a day-to-day C. of V. of 9.3-44.6 percent.

Herber (1980) found that the wet chemical micro method of Piomelli (1973) had a detection limit of 20 µg EP/dl whole blood, while that of Lamola et al. (1975) was sensitive to 50 µg EP/dl whole blood.

The recent development of direct instrumental measurement of ZPP, using the hematofluorometer, has added a dimension to the use of EP measurement in

lead exposure in terms of field screening of large groups of subjects. As originally developed by Bell Laboratories (Blumberg et al., 1977) and now produced commercially, the apparatus employs front-face optics, in which excitation of the fluorophore is at an acute angle to the sample surface, with emitted light emerging from the same surface and being detected. Routine calibration requires a stable fluorescing material with spectra comparable to ZPP and the triphenylmethane dye Rhodamine B is employed for the purpose. Absolute calibration requires adjusting the microprocessor-controlled readout system to read the known concentration of ZPP in reference blood samples, the latter calibration being done as frequently as possible.

Hematofluorometers are designed for the measurement of EP in samples containing oxyhemoglobin, i.e., capillary blood. Venous blood, therefore, must first be oxygenated, usually by moderate shaking for ca. 10 minutes (Blumberg et al., 1977; Grandjean and Lintrup, 1978). A second problem with hematofluorometer use, in contrast to wet chemical methods, is interference by bilirubin (Karacic et al., 1980; Grandjean and Lintrup, 1978), and this would be a problem with relatively low levels of EP. At levels normally encountered in lead workers or subjects with anemia and/or non-occupational lead exposure, the degree of such interference is not considered significant (Grandjean and Lintrup, 1978). Karacic et al. (1980) have found that carboxyhemoglobin, COHb, may pose a potential problem, but the relevance of this to cases where EP levels of subjects exposed to lead are obtained has not been fully elucidated. Background fluorescence in cover glass may be a problem, and should be tested in advance. Finally, the accuracy of the hematofluorometer is affected by hemolyzed blood.

Competently employed, the hematofluorometer appears to be reasonably precise. Blumberg et al. (1977) reported a C. of V. of 3 percent over the entire range of ZPP values measured, using a prototype apparatus. Karacic et al. (1980) found the relative standard deviation to vary from 1 percent (0.92 mmol ZPP/mol Hb) to 5 percent (0.41 mmol ZPP/mol Hb) depending on concentration. Grandjean and Lintrup (1978) obtained a day-to-day C. of V. of 5 percent using blood samples refrigerated for up to 9 weeks. Herber (1980) obtained a total C. of V. of 4.1-11.5 percent.

A number of investigators have compared EP measured by the hematofluorometer with the laboratory or wet chemical techniques, ranging from single, intralaboratory comparison to interlaboratory performance testing, the latter including the EP proficiency testing program of the Centers for Disease Control.

Working with the prototype instrumentation, Blumberg et al. (1977) obtained correlation coefficients of r = 0.98 (range 50-800 µg EP/dl RBCs) and 0.99 (range, up to 1,000 µg EP/dl RBCs) for comparison with the Granick and Piomelli methods, respectively. Equally good correlation results have been achieved with the studies of Grandjean and Lintrup (1978), Castoldi et al. (1979), and Karacic et al. (1980).

Several reports (Culbreth et al., 1979; Scoble et al., 1981; Smith et al., 1980) have described the application of high-performance liquid chromatography (HPLC) to the analysis of either free or zinc protoporphyrin in whole blood. In one of the methods (Scoble et al., 1981), the protoporphyrins as well as coproporphyrin and Mesoporphyrin IX are determined on-line fluorometrically in less than a claimed time of 6 minutes using 0.1 ml of blood sample. The HPLC approach remains to be tested in inter-laboratory proficiency programs.

9.2.2 Interlaboratory Testing Of Accuracy And Precision In EP Measurement

In a relatively early attempt to assess interlaboratory proficiency in EP measurement, Jackson (1978) reported results of a survey of 65 facilities analyzing 10 whole blood samples by one or more available methods for EP, in the form of direct measurement with the hematofluorometer or one of the wet chemical methods. In this survey, the instrumental methods had a low bias compared to the extraction techniques but tended to show better interlaboratory correlation.

At present, the ongoing EP proficiency testing program of CDC constitutes the most comprehensive assessment of laboratory performance. Every month, 3 samples of whole blood prepared at the University of Wisconsin Laboratory of Hygiene are forwarded to participants, 36 samples/year. Reference means are determined by a group of reference laboratories with a target range of ± 15 percent across the whole range of EP values. For Fiscal Year 1981 (1981 Data Summary, Erythrocyte Protoporphyrin: Proficiency Testing, CDC), 198 laboratories participated, of which 139 were involved for the entire year. Three of the 36 samples were not included. Of the year-long participants (139 facilities), 93.5 percent had better than half the samples within the target range, 84.2 percent performed satisfactorily with 70 percent or more of the samples, and about half (50.4 percent) of all laboratories had 90 percent or better correct results.

Comparatively, the participants as a group showed greater proficiency than in the previous year. Of the various methods in current use, the hematofluorometer direct measurement technique is most heavily represented. For the January, 1982, survey, for example, 154 participants used the hematofluorometer, followed by 30 using the Piomelli and 7 using the Chisolm/Brown methods as the 3 major techniques.

9.3 MEASUREMENT OF URINARY COPROPORPHYRIN

The elevation of urinary coproporphyrin (CP-U) with lead intoxication served as a useful indicator of such intoxication in children and lead workers for many years, although analysis of CP-U has declined considerably in recent times with the development of other testing methods, such as measurement of erythrocyte protoporphyrin. It still possesses the advantage of showing active intoxication (Piomelli and Graziano, 1980).

The standard method of CP-U determination is the fluorometric procedure of Schwartz and coworkers (1951). Urine samples are treated with acetate buffer and aqueous iodine, the latter converting coproporphyrinogen to CP. The porphyrin is paritioned into ethyl acetate and back-extracted (4 X) with 1.5 N HCl. Coproporphyrin is employed as quantitating standard. Working curves are linear below 5 µg CP/dl urine.

In the absorption spectrometric technique of Haeger-Aronsen (1960), iodine is also used to convert coproporphyrinogen to CP. The extractant is ethyl ether, from which the CP is removed to 0.1 N HCl. Absorption is read at 3 wavelengths, 380, 430 and the Soret maximum at 402 nm, and quantitation carried out using an equation involving the 3 wave lengths.

9.4 MEASUREMENT OF DELTA-AMINOLEVULINIC ACID DEHYDRATASE ACTIVITY

Delta-aminolevulinic acid dehydratase (5-aminolevulinate hydrolase; porphobilinogen synthase; E.C. 4.2.1.24; ALA-D) is an allosteric sulfhydryl enzyme that mediates the conversion of two units of d-aminolevulinic acid to prophobilinogen, a precursor in the heme biosynthetic pathway to the porphyrins. Inhibition of the activity of this enzyme by lead is the enzymological basis of its diagnostic utility in assessing lead exposure, using erythrocytes.

A number of sampling precautions attend the measurement of this enzyme's activity. ALA-D activity is modified by the presence of zinc as well as lead and consequently blood collection tubes which have high background zinc content, mainly in the rubber stoppers, must be avioded completely or care taken to avoid stopper-blood contact. Nackowski et al. (1977) observed that the presence

of zinc in blood collection tubes is a pervasive problem, and it appears that plastic-cup tubes are the only practical means of avoiding the problem. To guard against zinc in the tube itself, it would appear prudent to determine the extent of zinc leachibility by blood and to use one tube lot, if possible. Heparin is the anticoagulant of choice, since the lead binding agent, EDTA, or other chelants, would affect the lead-enzyme interaction.

The relative stability of the enzyme in blood is such that determinations of activity should be carried out as soon after the collection as possible. Even with refrigeration, analysis of activity should be done within 24 hours (Berlin and Schaller, 1974). Furthermore, porphobilinogen is light-labile and requires the assay be done with restriction of light.

Various procedures for ALA-D activity measurement are chemically based on measurement of porphobilinogen generated from the substrate, d-ALA, the former being condensed with p-dimethylaminobenzaldehyde (Ehrlich's reagent) to yield a chromophore measured at 553 nm in a spectrophotometer.

In the European Standardized Method for ALA-D activity measurement (Berlin and Schaller, 1974), developed with the collaboration of 9 laboratories and developed for use with blood samples having relatively low lead content, triplicate blood samples (0.2 ml) are hemolyzed, along with a blood blank, with water for 10 minutes at 37° C. Samples are then mixed with d-ALA solution followed by a 60-minute incubation. The enzyme reaction is terminated by addition of a solution of mercury (II) in trichloroacetic acid followed by centrifugation and filtration. Filtrates are mixed with modified Ehrlich's reagent (p-dimethylaminobenzalehyde in trichloroacetic/perchloric acid mixture) with reaction for 5 minutes, followed by chromophore measurement in a spectrophotometer at 555 nm. Activity is quantified in terms of µmol d-ALA/min./l erythrocytes. It should be noted that the amount of phosphate for Solution A in this report should be 1.78g, not the 1.38g stated.

In a micro scale variation, Granick et al. (1973a) used only 5 µl of blood with termination of the assay by trichloroacetic acid.

In comparing various reports dealing with the relationship of lead exposure and d-ALA-D inhibition, attention should be paid to the units of activity measurement employed with the several techniques. The Standard procedures of Berlin and Schaller (1974) expresses activity as µmol ALA/min/l cells, while the method of Tomokuni (1974) expresses µmol porphobilinogen/h/ml cells. Similarly, when comparing the Bonsignore et al. (1965) procedure to

that of Berlin and Schaller (1974), a conversion factor of 3.8 is necessary in going from Bonsignore to European Standard Method units (Trevisan et al., 1981).

Several factors have been shown to affect d-ALA-D activity. Rather than single measurement of enzyme activity in blood, Granick et al. (1973b) measured activity before and after treatment with dithiothreotol, an agent which reactivates the enzyme by lead complexing. The ratio of activated/unactivated activity vs. blood lead levels accommodates inherent differences in enzyme activity among individuals due to genetic and other reasons. Other agents for such activation include zinc (Finelli et al., 1975) and zinc + glutathione (Mitchell et al., 1977). Wigfield and Farant (1979) found that enzyme activity is related to assay pH in a way that reduced activity from such a pH-activity relationship can be misinterpreted as lead inhibition. These workers find that pH shifts away from optimal, in terms of activity, with increasing blood lead content and as the incubation step proceeds.

9.5 MEASUREMENT OF DELTA-AMINOLEVULINIC ACID IN URINE AND OTHER MEDIA

Delta-aminolevulinic acid (d-ALA) levels increase with elevated lead exposure, owing to the inhibitory effect of lead on the activity of ALA dehydratase and/or the increase of ALA synthase activity by feedback derepression, with the result that this intermediate in heme biosynthesis rises in the body and eventually results in increased urinary excretion. The measurement of this metabolite in urine provides an indication of the level of lead exposure.

The ALA content of urine samples is stable for ca. two weeks or more if urine samples are acidified with tartaric or acetic acid and kept refrigerated.

Values of ALA-U measured are adjusted for urine density, if concentration is percent volume, or is measured per unit creatinine. As noted in the case of urinary lead measurement, 24 h. collection is more desirable than spot sampling.

Five manual and one automated procedures for urinary ALA measurement are most widely in use. The procedures of Mauzerall and Granick (1956) and Davis and Andelman (1967) are the most involved, requiring the initial chromatographic separation of ALA. The approach of Grabecki et al. (1967) omits chromatographic isolation while the automated variation of Lauwerys et al. (1972) omits prechromatography but includes the use of an internal standard. In the method of Tomokuni and Ogata (1972), chromatography is omitted but solvent extraction is employed to isolate the pyrrole intermediate.

In the method of Mauzerall and Granick (1956), ALA is condensed with a β-dicarbonyl compound, acetylacetone, at pH 4.6, to yield a pyrrole intermediate (Knorr Condensation reaction), which is then further reacted with p-dimethylaminobenzaldehyde in perchloric/acetic acid. The samples are then read in a spectrophotometer at 553 nm at 15 minutes after mixing. In this method, there is separation of both porphobilinogen and ALA from urine using a dual column configuration of a cation exchange and an anion exchange resin, the latter retaining the porphobilinogen and the former used to separate ALA from urea. The detection limit is 3 μmoles/liter urine.

In the modification of this method by Davis and Andelman (1967), disposable cation/anion resin cartridges were used, in a sequential configuration, to expedite chromatographic separation and increase sample analysis rate. Commercial disposable columns based on the design (Bio-Rad) are now available and appear satisfactory.

In these two approaches, the problem of aminoacetone, a metabolite occurring in urine and reacting to give an interferent, is not taken into account. With the technique of Marver et al. (1966), a chromatographic step subsequent to the condensation reaction to form the pyrrole is inserted, using Dowex-1, which separates the ALA derivative from that of the aminoacetone.

In the methods of Tomokuni and Ogata (1972), ALA is condensed with ethylacetoacetate and the resulting pyrrole extracted with ethyl acetate. The extract is then treated with Ehrlich's reagent and the resulting chromophore measured spectrophotometrically.

Lauwerys et al. (1972) developed an automated ALA analysis method for lead worker screening, in which ALA is added in known amount as an internal standard and the pre-chromatgraphy avoided. They report a high correlation ($r = 0.98$, no range available) with the procedure of Mauzerall and Granick (1956).

Roels et al. (1974) compared the relative proficiency of 4 methods -- those of Mauzerall and Granick (1956), Davis and Andelman (1967), the Lauwerys et al. (1972) automated version, and the Grabecki et al. (1967) method, which omits chromatographic separation and is normally used with occupational screening. The chromatographic methods gave identical results over the range of 0-60 mg ALA/l urine, while the automated method showed a positive bias at levels <6 mg/l. The Grabecki et al. (1967) technique was least satisfactory of any procedure. These workers also noted that commercial ion-exchange columns gave low variability (<10 percent) in results.

Della Fiorentina et al. (1979) combined the Tomokuni and Ogata (1972) extraction method with a correction equation for urine density. Up to 25 mg ALA/l, the C. of V. was ≤4 percent along with a good correlation (r = 0.937) with the Davis and Andelman (1967) technique. While there is a time saving in avoiding prechromatography, it is necessary to prepare a curve relating urine density to a correction factor for quantitative measurement.

Although ALA analysis is normally done with urine as the indicator medium, Haegar-Aronsen (1960) reported a similar coloremetric method for blood and McGee et al. (1977) described a gas-liquid chromatographic method for ALA in plasma and urine. Levels of ALA in plasma are much lower than that in urine. In the latter method, ALA is isolated from plasma, reacted with acetyl-acetone and partitioned into a solvent -- trimethylphenylhydroxide -- which also serves for pyrolytic methylation in the injection port of the gas-liquid chromatograph, the methylated pyrrole being more amenable to chromatographic isolation than the more polar precursor. For quantitation, an internal standard -- 6-amino-5-oxohexanoic acid -- is used. The sample requirement is 3 ml plasma. Levels measured ranged from 6.3-73.5 ng ALA/ml plasma, and yield values which are ca. 10-fold lower than the coloremetric techniques (O'Flaherty et al., 1980).

9.6 MEASUREMENT OF PYRIMIDINE-5'-NUCLEOTIDASE ACTIVITY

Erythrocyte pyrimidine-5'-nucleotidase (5'-ribonucleotide phosphohydrolase, E.C. 3.1.3.5, Py5N) catalyzes the hydrolytic dephosphorylation of the pyrimidine nucleotides uridine- and cytidinemonophosphate to uridine and cytidine (Paglia and Valentine, 1975). Enzyme inhibition by lead in humans and animals results in incomplete degradation of reticulocyte RNA fragments, accumulation of the nucleotides, and increased cell hemolysis (Paglia et al., 1975; Paglia and Valentine, 1975; Angle and McIntire, 1978; George and Duncan, 1982).

There are two methods for measurement of Py5N activity. One is quite laborious in terms of time and manipulation, while the other is shorter but requires the use of radioisotopes and radiometric measurement.

In the method of Paglia and Valentine (1975), heparinized venous blood is filtered through cotton or a commercial cellulose preparation to separate erythrocytes from platelets and leukocytes. Cells are given multi-saline washings, packed lightly and subjected to freeze hemolysis. The hemolysates are dialyzed against a saline-Tris buffer containing $MgCl_2$ and EDTA to remove nucleotides and other phosphates. The assay system consists of dialyzed

hemolysate, $MgCl_2$, Tris buffer at pH 8.0, and either UMP or CMP; incubation is for 2 hours at 37°C. Activity is terminated by treatment with 20 percent trichloroacetic acid followed by centrifugation. The supernatant inorganic phosphate, P_i, is measured by the classic method of Fiske and Subbarow (1925), the phosphomolybdic acid complex being measured spectrophotometrically at 660 nm. A unit of enzyme activity is expressed as μmol P_i/hour/g hemoglobin.

Hemolysates appear to be stable (90 percent) with refrigeration at 4°C. for up to 6 days, provided that mercaptoethanol is added at the time of assay. Like the other method, activity measurement requires the determination of hemoglobin.

In the simpler approach of Torrance et al. (1977), which can be feasibly applied to much larger numbers of samples, erythrocytes are separated from leukocytes and platelets with a 1:1 mixture of microcrystalline and alpha-cellulose, followed by saline washing and hemolysis with a solution of mercaptoethanol and EDTA. Hemolysates are incubated with a medium containing purified ^{14}C-CMP and $MgCl_2$ for 30 minutes at 37°C. The reaction is terminated by sequential addition of barium hydroxide and zinc sulfate solution. Proteins and unreacted nucleotide are precipitated, leaving the labeled cytidine in the supernatant. Aliquots are measured for ^{14}C activity in a liquid scintillation counter. Enzyme activity is expressed as nanomoles CMP/minute/g hemoglobin. The blank activity is determined for each sample by carrying out the precipitation step as soon as the hemolysate is mixed with the labeled CMP, i.e., t = 0. This procedure shows a good correlation, r = 0.94 (range, 135-189 enzyme units), with the method of Paglia and Valentine (1975). The two methods express a unit of enzyme activity differently, so that one must know the method in comparing enzyme activity.

9.7 REFERENCES

Ahlgren, L.; Haeger-Aronsen, B.; Mattson, S.; Schutz, A. (1980) In-vivo determination of lead in the skeleton after occupational exposure to lead. Brit. J. Ind. Med. 37: 109-113.

Al-Nami, T.; Edmonds, M. I.; Fremlin, J. H. (1980) The distribution of lead in human teeth, using charged particle activation analysis. Phys. Med. Biol. 25: 719-726.

American Public Health Association. (1955) Methods for Determining Lead in Air and Biological Materials. New York.

Angle, C. R.; McIntire, M. S. (1978) Low level lead and inhibition of erythrocyte pyrimidine nucleotidase. Environ. Res. 17: 296-302.

Barthel, W. F.; Smrek, A. L.; Angel, G. P., Liddle, J. A.; Landrigan, P. J.; Gehlbach, S. H.; Chisolm, J. J. (1973) Modified Delves cup atomic absorption determination of lead in blood. J. Assoc. Off. Anal. Chem. 56: 1252-1256.

Berlin, A.; Del Castilho, P.; Smeets, J. (1973) European inter-comparison programmes. In: Environemntal Health Aspects of Lead. Commission of the European Communities. Centre for Information and Documentation, Luxembourg. May 1973.

Berlin, A.; Schaller, K. H. (1974) European standardized method for the determination of d-aminolevulinic acid dehydratase activity in blood. Z. Klin. Chem. Klin. Biochem. 12: 389-390.

Berman, E. (1976) The Challenge of Getting the Lead Out. In: Accuracy in Trace Analyses: Sampling, Sample Handling, Analysis. (P. D. LaFleur, ed.). Vol. II. NBS Special Publication No. 422, National Bureau of Standards, Washington, DC.; pp. 715-720.

Berman, E. (1981) Heavy Metals. Lab. Med. 12: 677-684.

Bloch, P.; Garavaglia, G.; Mitchell, G.; Shapiro, I. M. (1976) Measurement of lead content of children's teeth in situ by X-ray fluorescence. Phys. Med. Biol. 20: 56-63.

Blumberg, W. E.; Eisinger, J.; Lamola, A. A.; Zuckerman, D. M. (1977) Zinc protoporphyrin level in blood determined by a portable hematofluorometer: a screening device for lead poisoning. J. Lab. Clin. Med. 89: 712-723.

Bonsignore, D.; Calissano, P.; Cartasegna, C. (1965) Un semplice metodo per la determinazione della d-amino-levulinico-deidratasi nel sangue. Med. Lav. 56: 199-205.

Boone, D. J.; Hearn, T. L.; Lewis, D. S. (1979) Comparison of interlaboratory results for blood lead with a definitive method. Clin. Chem. 25: 389-393.

Boutwell, J. H. (1976) Accuracy and quality control in trace element analyses. In: Accuracy in Trace Analyses: Sampling, Sample Handling, Analysis (P. D. LaFleur, ed.). Vol. I. NBS Special Publication No. 422, National Bureau of Standards, Washington, DC; pp. 35-40.

Cali, S. P.; Reed, W. P. (1976) The role of the National Bureau of Standards Standard Reference Materials in accurate trace analysis. In: Accuracy in Trace Analyses: Sampling, Sample Handling, Analysis (P. D. LaFleur, ed.). Vol. I. Special Publication No. 422, National Bureau of Standards, Washington, DC; pp. 35-40.

Carter, G. F. (1978) The paper punched disk technique for lead in blood samples with abnormal hemoglobin values. Brit. J. Ind. Med. 35: 235-240.

Castoldi, M. R.; Odone, P.; Buratti, M.; Alessio, L. (1979) Determination of erythrocyte zinc protoporphyrin: methodological problems. In: Proceedings of the International Conference Management Cont. Heavy Metals Environ. London: September, 1979; Commission of the European Communities, Luxembourg.

Cavalleri, A.; Minoia, C.; Pozzoli, L.; Baruffini, A. (1978) Determination of plasma lead levels in normal subjects and in lead-exposed workers. Brit. J. Ind. Med. 35: 21-26.

Cernik, A. A. (1974) Determination of blood lead using a 4.0 mm paper punched disc carbon cup sampling technique. Brit. J. Ind. Med. 31: 239-244.

Cernik, A. A.; Sayers, M. P. H. (1971) Determination of lead in capillary blood using a paper punched disc atomic absorption technique. Applications to the supervision of lead workers. Brit. J. Ind. Med. 28: 392-398.

Chatman, T.; Wilson, D. J. (1975) Lead levels in human deciduous teeth in Tennessee. Environ. Lett. 8: 173-183.

Chatt, A.; Secord, C. A.; Tiefenbach, B.; Jervis, R. E. (1980) Scalp hair as a monitor of community exposure to environmental pollutants. In: Hair, Trace Elements and Human Illness. (A. C. Brown and R. C. Crounse, eds.). Praeger, New York; pp. 46-73.

Chattopadhyay, A.; Roberts, T. M.; Jervis, R. E. (1977) Scalp hair as a monitor of community exposure to lead. Arch. Environ. Health 32: 226-236.

Chisolm, J. J., Jr.; Brown, D. H. (1975) Micro-scale photofluormetric determination of "free erythrocyte porphyrin" (protoporphyrin IX). Clin. Chem. 21(11): 1669-1682.

Cooke, R. E.; Glynn, K. L.; Ullman, W. W.; Lurie, N.; Lepow, M. (1974) Comparative study of a microscale test for lead in blood, for use in mass screening programs. Clin. Chem. 20(5); 582-585.

Culbreth, P.; Walter, G.; Carter, R.; Burtis, C. (1979) Separation of protoporphyrins and related compounds by reversed-phase liquid chromatography. Clin. Chem. 25: 605-610.

Davis, J. R.; Andelman, S. L. (1967) Urinary delta-aminolaevulinic acid (ALA) levels in lead poisoning. Arch. Environ. Health 15: 53-59.

Della Fiorentina, H.; Grogna, M.; Dewiest, F. (1979) Simplified determination of urinary d-aminolevulinic acid in a wide range of concentrations. Clin. Chem. 25: 581-583.

Delves, H. T. (1970) A micro-sampling method for the rapid determination of lead in blood by atomic absorption spectrophotometry. Analyst (London) 95: 431-438, May 1970.

Delves, H. T. (1977) Analytical techniques for blood-lead measurements. J. Anal. Toxicol. 1: 261-264.

Delves, H. T.; Clayton, B. E.; Carmichael, A.; Bubear, M.; Smith, M. (1982) An appraisal of the analytical significance of tooth-lead measurements as possible indices of environmental exposure of children to lead. Ann. Clin. Biochem. (in press).

DeSilva, P. E. (1981) Determination of lead in plasma and studies of its relationship to lead in erythrocytes. Brit. J. Ind. Med. 38: 209-217.

DeSilva, P. E.; Donnan, M. B. (1980) Blood lead levels in Victorian children. Med. J. Aust. II: 315-318.

Donovan, D. T.; Vought, V. M.; Rakow, A. B. (1971) Laboratories which conduct lead analysis on biologic specimens. Arch. Environ. Health 23(2): 111-113.

Dudley, D. M. T.; Boone, D. J. (1982) Critique: Blood lead analyses, 1981. Centers for Disease Control, USPHS, Atlanta, Ga. (in press).

Ediger, R. D.; Coleman, R. L. (1972) A modified Delves cup atomic absorption procedure for the determination of lead in blood. At. Abs. Newsletter 11(2): 33-36.

Eller, P. M.; Haartz, J. C. (1977) A study of methods for the determination of lead and cadmium. Am. Ind. Hyg. Assoc. J. 38: 116-124.

Environmental Sciences Associates, Inc. (1981) LoLevel blood lead control--assignment of values for metex-change kits A, C, D and H. Technical Report #81-2, March 5, 1981, Bedford, Massachusetts.

Everson, J.; Patterson, C. C. (1980) "Ultra-clean" isotope dilution/mass spectrometric analyses for lead in human blood plasma indicated that most reported values are artificially high. Clin. Chem. 26: 1603-1607.

Farris, F. F.; Poklis, A.; Griesmann, G. E. (1978) Atomic absorption spectroscopic determination of lead extracted from acid-solubilized tissues. J. Assoc. Off. Anal. Chem. 61: 660-663.

Finelli, V. N.; Klauder, D. S.; Karaffa, M. A. (1975) Interaction of zinc and lead on d-aminolevulinate dehydratase. Biochem. Biophys. Res. Commun. 65: 303-311.

Fiske, C. H.; Subbarow, Y. (1925) The colorimetric determination of phosphate. J. Biol. Chem. 66: 375-400.

Fosse, G.; Berg Justesen, N.-P. (1978) Lead in deciduous teeth of Norwegian children. Arch. Environ. Health 33: 166-175.

Franke, J. P.; DeZeeuw, R. A. (1977) Toxic metal analysis by differential pulse anodic stripping voltammetry in clinical and forensic toxicology. J. Anal. Toxicol. 1: 291-295.

George, J. W.; Duncan, J. R. (1982) Pyrimidine-specific 5' nucleotidase activity in bovine erythrocytes; effect of phlebotomy and lead poisoning. Am. J. Vet. Res. 43: 17-20.

Gibson, R. S. (1980) Hair as a biopsy material for the assessment of trace element status in infancy. A review. J. Human Nutr. 31: 405-416.

Grabecki, J.; Haduch, T.; Urbanowicz, H. (1967) Die enfachen Bestimmungs methoden der d-aminolavulinsaune im Harn. Int. Arch. Gewerbepathol. Gewerbehyg. 23: 226.

Grandjean, P.; Lintrup, J. (1978) Erythrocyte-Zn-protoporphyrin as an indicator of lead exposure. Scand. J. Clin. Lab. Invest. 38: 669-675.

Grandjean, P.; Nielsen, O. V.; Shapiro, I. M. (1979) Lead retention in ancient Nubian and contemporary populations. J. Environ. Pathol. Toxicol. 2: 781-787.

Granick, J. L.; Sassa, S.; Granick, S.; Levere, R. D.; Kappas, A. (1973a) Studies of lead poisoning. I. Microanalysis of erythrocyte protoporphyrin levels by spectrofluorometry in the detection of chronic lead intoxication in the subclinical range. Biochem. Med. 8: 135-148.

Granick, J. L.; Sassa, S.; Granick, S.; Levere, R. D.; Kappas, A. (1973b) Studies in lead poisoning. II. Correlation between the ratio of activated and inactivated d-aminolevulinic acid dehydratase of whole blood and the blood lead level. Biochem. Med. 8: 149-159.

Granick, S.; Sassa, S.; Granick, J. L.; Levere, R. D.; Kappas, A. (1972) Assays for porphyrins, d-aminolevulinic-acid dehydratase, and porphyrinogen synthetase in microliter samples of whole blood: Applications to metabolic defects involving the heme pathway. Proc. Natl. Acad. Sci., U.S.A. 69: 2381-2385.

Haeger-Aronsen, B. (1960) Studies on urinary excretion of d-aminolaevulinic acid and other haem precursors in lead workers and lead-intoxicated rabbits. Scand. J. Clin. Lab. Invest. 12 (Suppl. 47), pp. 37-41.

Hanna, T. L.; Dietzler, D. W.; Smith, C. H.; Cupta, S.; Zarkowsky, H. S. (1976) Erythrocyte porphyrin analysis in the detection of lead poisoning in children: evaluation of four micro methods. Clin. Chem. 22: 161-168.

Herber, R. F. M. (1980) Estimation of blood lead values from blood porphyrin and urinary 5-aminolevulinic acid levels in workers. Int. Arch. Occup. Environ. Health 45: 169-179.

Hessel, D. W. (1968) A simple and rapid quantitative determination of lead in blood. At. Absorp. Newsletter 7: 55.

Hicks, J. M.; Gutierrez, A. N.; Worthy, B. E. (1973) Evaluation of the Delves micro system for blood lead analysis. Clin. Chem. 19(3): 322-325.

Hinderberger, E. J.; Kaiser, M. L.; Koirtyohann, S. R. (1981) Furnace atomic absorption analysis of biological samples using the L'vov platform and matrix modification. At. Spectrosc. 2: 1-7.

Hodges, D. J.; Skelding, D. (1981) Determination of lead in urine by atomic absorption spectroscopy with electrothermal atomization. Analyst 106: 299-304.

Issaq, H. J.; Zielinski, Jr., W. L. (1974) Loss of lead from aqueous solutions during storage. Anal. Chem. 46: 1328-1331.

Jackson, K. W. (1978) Interlaboratory comparison of results of erythrocyte protoporphyrin analysis. Clin. Chem. 24: 2135-2138.

Jagner, D.; Danielsson, L.-G.; Aren, K. (1979) Potentiometric stripping analysis for lead in urine. Anal. Chim. Acta 106: 15-21.

Joselow, M. M.; Bogden, J. D. (1972) A simplified micro method for collection and determination of lead in blood using a paper disk-in-Delves cup technique. At. Absorp. Newsletter 11: 99-101.

Joselow, M. M.; Flores, J. (1977) Application of the zinc protoporphyrin (ZP) test as a monitor of occupational exposure to lead. Am. Ind. Hyg. Assoc. J. 38: 63-66.

Karacic, W.; Prpic Majic, D.; Telisman, S. (1980) The relationship between zinc protoporphyrin (ZPP) and "free" erythrocyte protoporphyrin (FEP) in lead-exposed individuals. Int. Arch. Occup. Environ. Health 47: 165-177.

Keppler, J. F.; Maxfield, M. E.; Moss, W. D.; Tietjen, G.; Linch, A. L. (1970) Interlaboratory evaluation of the reliability of blood lead analyses. J. Am. Ind. Hyg. Assoc. 31: 412-429.

Kochen, J. A.; Greener, Y. (1973) Levels of lead in blood and hematocrit: Implications for the evaluation of the newborn and anemic patient. Pediatr. Res. 7: 937-944.

Koizumi, H.; Yasuda, K. (1976) Determination of lead, cadmium and zinc using the Zeeman effect in atomic absorption spectrometry. Anal. Chem. 48: 1178-1182.

Kopito, L. E.; Davis, M. A.; Schwachman, H. (1974) Sources of error in determining lead in blood by atomic absorption spectrophotometry. Clin. Chem. 20: 205-211.

Kubasik, N. P.; Volosin, M. T.; Murray, M. H. (1972) Carbon rod atomizer applied to measurement of lead in whole blood by atomic absorption spectrophotometry. Clin. Chem. 18(5): 410-412.

Lamola, A. A.; Joselow, M.; Yamane, T. (1975) Zinc protoporphyrin (ZPP): A simple, sensitive, fluorometric screening test for lead poisoning. Clin. Chem. 21(1): 93-97.

Lauwerys, R.; Delbroeck, R.; Vens, M. D. (1972) Automated analysis of d-aminolevulinic acid in urine. Clin. Chim. Acta 40: 443-447.

Lauwerys, R.; Buchet, J. P.; Roels, H.; Berlin, A.; Smeets, J. (1975) Inter-comparison program of lead, mercury and cadmium analysis in blood, urine, and aqueous solutions. Clin. Chem. 21: 551-557.

Lee, S. W.; Meranger, J. C. (1980) Direct methods for the determination of lead in whole blood by anodic stripping voltammetry. Amer. J. Med. Technol. 46: 853-857.

Legotte, P. A.; Rosa, W. C.; Sutton, D. C. (1980) Determination of cadmium and lead in urine and other biological samples by graphite furnace atomic absorption spectrometry. Talanta 27: 39-44.

Lerner, S. (1975) Blood lead analyses--precision and stability. J. Occup. Med. 17: 153-154.

Lockeretz, W. (1975) Lead content of deciduous teeth of children in different environments. Arch. Environ. Health 30: 583-587.

Machlan, L. A.; Gramlich, J. W.; Murphy, J. J.; Bermes, I. L. (1976) The accurate determination of lead in biological and environmental samples by isotope-dilution mass spectrometry. In: Accuracy in Trace Analyses: Sampling, Sample Handling, Analysis (P. D. LaFleur, ed.). Vol. II, NBS Special Publication No. 422, National Bureau of Standards, Washington, DC; pp. 927-936.

Mackie, A. C.; Stephens, R.; Townshend, A.; Waldron, H. A. (1977) Tooth lead levels in Birmingham children. Arch. Environ. Health 32: 179-185.

Mahaffey, K. R.; Annest, J. L.; Barbano, H. E.; Murphy, R. S. (1979) Preliminary analysis of blood lead concentrations for children and adults: HANES II, 1976-1978. Trace Subst. Environ. Health 13: 37-51.

Maher, C. C.; Roettgers, D. M.; Conlon, H. J. (1979) Interlaboratory comparison of blood lead determinations. Am. Ind. Hyg. Assoc. J. 40: 230-237.

Manton, W. I.; Cook, J. D. (1979) Lead content of cerebrospinal fluid and other tissue in amyotrophic lateral sclerosis (ALS), Neurology 29: 611.

Marcus, M.; Hollander, M.; Lucas, R. E.; Pfeiffer, N. C. (1975) Micro-scale blood lead determinations in screening: Evaluation of factors affecting results. Clin. Chem. 21(4): 533-536.

Marcus, S. M.; Joselow, M. M.; Kemp, F.; Ziering, R.; Mihalovic, D.; Anderson, L. (1977) Warning: Spurious elevations of blood lead in micro puncture techniques. J. Ped. 91: 164.

Marner, H. S.; Tschudy, D. P.; Perlroth, M. G. (1966) The determination of amino ketones in biological fluids. Anal. Biochem. 14: 53-60.

Matson, W. R. (1970) Rapid sub-nanograms simultaneous analyses. Trace Substances in Environ. Health IV: 396-406.

Matson, W. R.; Roe, D. K. (1966) Trace metal analysis of natural media by anodic stripping voltammetry. Anal. Instrum. 4: 19-22.

Mauzerall, D.; Granick, S. (1956) The occurrence and determination of d-aminolevulinic acid and porphobilinogen in urine. J. Biol. Chem. 219: 435-446.

Meranger, J. C.; Hallebone, B. R.; Blanchette, G. A. (1981) The effect of storage times, temperatures and container types on the accuracy of atomic absorption determinations of Cd, Cu, Hg, Pb and Zn in whole heparinized blood. J. Anal. Toxicol. 5: 33-41.

Mitchell, D. G.; Aldous, K. M.; Ryan, F. J. (1974) Mass screening for lead poisoning: Capillary blood sampling and automated Delves-cup atomic-absorption analysis. N.Y. State J. Med. 74: 1599-1603, Aug. 1974.

Mitchell, R.; Drake, J. E.; Wittlin, L. A.; Rejent, T. A. (1977) Erythrocyte porphobilinogen synthase (delta-aminolaevulinate dehydratase) activity: a reliable and quantitative indicator of lead exposure in humans. Clin. Chem. 23: 105-111.

Moore, M. R.; Campbell, B. C.; Meredith, P. A.; Beattie, A. D.; Goldberg, A.; Campbell, D. (1978) The association between lead concentrations in teeth and domestic water lead concentrations. Clin. Chim. Acta 87: 77-83.

Moore, M. R.; Meredith, P. A. (1977) The storage of samples for blood and water lead analysis. Clin. Chim. Acta 75: 167-170.

Morrell, G.; Geridhar, G. (1976) Rapid micro method for blood lead analysis by anodic stripping voltammetry. Clin. Chem. 22: 221-223.

Murphy, J. J (1976) The role of the analytical blank in accurate trace analysis. In: Accuracy in Trace Analyses: Sampling, Sample Handling, Analysis (P. D. LaFleur, ed.). NBS Special Publication No. 422, National Bureau of Standards, Washington, DC; pp. 509-540.

Nackowski, S. B.; Putnam, R. D.; Robbins, D. A. (1977) Trace metal contamination of evacuated blood collection tubes. Amer. Ind. Hyg. Assoc. J. 38: 503-508.

National Academy of Sciences. (1972) Lead: Airborne Lead in Perspective. Washington, DC.

Needleman, H. L.; Davidson, I.; Sewell, E. M.; Shapiro, I. M. (1974) Subclinical lead exposure in Philadelphia school children: identification by dentine lead analyses. N. Engl. J. Med. 290: 245-248.

Needleman, H. L.; Gunnoe, C.; Leviton, A.; Reed, R.; Peresie, H.; Maher, C.; Barrett, P. (1979) Deficits in psychological and classroom performance of children with elevated dentine lead levels. N. Engl. J. Med. 300: 689-695.

O'Flaherty, E. J.; Hammond, P. B.; Lerner, S. I.; Hanenson, I. B.; Roda, S. M. B. (1980) The renal handling of d-aminolevulinic acid in the rat and in the human. Toxicol. Appl. Pharmacol. 55: 423-432.

Oehme, M.; Lund, W. (1978) The determination of copper, lead, cadmium and zinc in human teeth by anodic stripping voltammetry. Anal. Chim. Acta 100: 389-398.

Orfanos, A. P.; Murphey, W. H.; Guthrie, R. (1977) A simple fluorometric assay of protoporphyrin in erythrocytes (EPP) as a screening test for lead poisoning. J. Lab. Clin. Med. 89: 659-665.

Paglia, D. E.; Valentine, W. N. (1975) Characteristics of a pyrimidine-specific 5' nucleotidase in human erythrocytes. J. Biol. Chem. 250: 7973-7979.

Paglia, D. E.; Valentine, W. N.; Dahlgren, J. E. (1975) Effects of low-level lead exposure on pyrimidine-5'nucleotidase and other erythrocyte enzymes. J. Clin. Invest. 56: 1164-1169.

Patterson, C. C.; Settle, D. M. (1976) The reduction of orders of magnitude errors in lead analyses of biological materials and natural waters by evaluating and controlling the extent and sources of industrial lead contamination introduced during sample collecting and analyses. In: Accuracy in Trace Analyses: Sampling, Sample Handling, Analysis. Vol. I. (P. D. LaFleur, ed.). NBS Special Publication No. 422, National Bureau of Standards, Washington, DC; pp. 321-352.

Paulev, P.-E.; Solgaard, P.; Tjell, J. C. (1978) Interlaboratory comparison of lead and cadmium in blood, urine and aqueous solutions. Clin. Chem. 24: 1797-1800.

Pierce, J. O.; Koirtyohann, S. R.; Clevenger, R. E.; Lichte, F. E. (1976) The Determination of Lead in Blood. International Lead Zinc Research Organization, Inc., New York.

Piomelli, S.; Davidow, B. (1972) The FEP (free erythrocyte protoporphyrin) concentration: a promising screening test for lead poisoning. Pediatr. Res. 6: 366.

Piomelli, S. (1973) A micro-method for free erythrocyte porphyrins: The FEP test. J. Lab. Clin. Med. 81: 932-940.

Piomelli, S.; Graziano, J. (1980) Laboratory diagnosis of lead poisoning. Pediatr. Clin. North Amer. 27: 843-853.

Piomelli, S.; Corash, L.; Corash, M. B.; Seaman, C.; Mushak, P.; Glover, B.; Padgett, R. (1980) Blood lead concentrations in a remote Himalayan population. Science 210: 1135-1137.

Piscator, M. (1982) The importance of quality control for estimating dose-effect and dose-response relationships. In: Schramel, P.; Bratter, P., eds. Trace element analytical chemistry in medicine and biology. deGruyter and Co., New York, NY. In press.

Rabinowitz, M. B.; Needleman, H. L. (1982) Temporal trends in the lead concentrations of umbilical cord blood. Science 216: 1429-1431.

Rabinowitz, M.; Wetherill, G. W.; Kopple, J. D. (1974) Studies of human lead metabolism by using stable isotope tracers. Environ Health Perspect. 7: 145-152.

Robinson, M. J.; Karpenski, F. E.; Brieger, H. (1958) The concentration of lead in plasma, whole blood and erythroctyes of infants and children. Pediatr. 21: 793.

Roels, H.; Lauwerys, R.; Buchet, J.-P.; Berlin, A.; Smeets, J. (1974) Comparison of four methods for determination of d-aminolevulinic acid in urine and evaluation of critical factors. Clin. Chem. 20: 753-760.

Rosen, J. F.; Zarate-Salvador, C.; Trinidad, E. E. (1974) Plasma lead levels in normal and lead-intoxicated children. J. Ped. 84: 45-48.

Ryabukhin, Y. S. (1980) Nuclear-based methods for the analysis of trace element pollutants in human hair. J. Radioanal. Chem. 60: 7-30.

Schwartz, S.; Zieve, L.; Watson, C. J. (1951) An improved method for the determination of urinary coproporphyrin and an evaluation of factors affecting its analysis. J. Lab. Clin. Med. 37: 843-859.

Scoble, H. A.; McKeag, M.; Brown, P. R.; Kavarnos, G. J. (1981) The rapid determination of erythrocyte porphyrins using reversed-phase high performance liquid chromatography. Clin. Clim. Acta 113: 253-265.

Settle, D. M.; Patterson, C. C. (1980) Lead in albacore: guide to lead pollution in Americans. Science 207: 1167-1176.

Shapiro, I. M.; Dobkin, B.; Tuncay, O. C.; Needleman, H. L. (1973) Lead levels in dentine and circumpulpal dentine of deciduous teeth of normal and lead poisoned children. Clin. Chim. Acta 46: 119-123.

Slavin, S.; Peterson, G. E.; Lindahl, P. C. (1975) Determination of heavy metals in meats by atomic absorption spectrometry. At. Absorp. Newsl. 14: 57-59.

Smith, R.; Doran, D.; Mazur, M.; Bush, B. (1980) High performance liquid chromatographic determination of protoporphyrin and Zn protoporphyrin in blood. J. Chromatog. 181: 319-327.

Speecke, A.; Hoste, J.; Versieck, J. (1976) Sampling of Biological Materials. In: Accuracy in Trace Analyses: Sampling, Sample Handling, Analysis (P. D. LaFleur, ed.). Vol. I. NBS Special Publication No. 422, National Bureau of Standards, Washington, DC; pp. 35-40.

Steenhout, A.; Pourtois, M. (1981) Lead accumulation in teeth as a function of age with different exposures. Brit. J. Ind. Med. 38: 397-303.

Tomokuni, K. (1974) D-aminolevulinic acid dehydratase test for lead exposure. Arch. Environ. Health 29: 274-281.

Tomokuni, K.; Ogata, M. (1972) Simple method for determination of urinary d-aminolevulinic acid as an index of lead exposure. Clin. Chem. 18: 1534-1536.

Torrance, J.; West, C.; Beutler, E. (1977) A simple rapid radiometric assay for pyrimidine-5'-nucleotidase. J. Lab. Clin. Med. 90: 563-568.

Trevisan, A.; Buzzo, A.; Scarpa, F. M. (1981) Comparative study of methods for measurements of erythrocyte aminolevulinic acid dehydratase activity (Engl. Trans.). Med. Lav. 72: 113-117.

Unger, B. C.; Green, V. A. (1977) Blood lead analysis--lead loss to storage containers. Clin. Toxicol. 11: 237-243.

United States Occupational Safety and Health Administration. (1982) OSHA criteria for laboratory proficiency in blood lead analysis. Arch. Environ. Health 37: 58-60.

Wigfield, D. C.; Farant, J. P. (1979) Factors influencing the pH-activity relationship of d-aminolevulinic acid dehydratase from human blood and their relevance to blood lead assay. J. Anal. Toxicol. 3: 161-168.

Wittmers, L. E., Jr.; Alich, A.; Aufderheide, A. C. (1981) Lead in Bone. I. Direct analysis for lead in milligram quantities of bone ash by graphite furnace atomic absorption spectroscopy. Amer. J. Clin. Pathol. 25: 80-85.

World Health Organization. (1977) Lead: Environmental Health Criteria 3. United Nations Environment Programme. Geneva, Switzerland; pp. 59-65.