COMMISSION OF THE EUROPEAN COMMUNITIES

industrial health and safety

Human biological monitoring of industrial chemicals

4. Inorganic lead

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P R E F A C E

The Council of the European Communities has adopted in 1979 the First Action Programme on Safety and Health at Work to be comprehensive at Community level. This Action Programme sets out a number of priorities many of which emphasize the need to increase protection against dangerous substances.

The need to promote new monitoring and measuring methods for the assessment of individual exposure, in particular through the application of sensitive biological indicators is stressed.

These monographs are aimed at providing the practicing occupational doctor with up-to-date information on the possibilities and limitations of biological monitoring for a number of agents.

The advent of modern micro-analytic techniques, the increased automatization of methods and our better understanding of biochemical processes have allowed the development and multiplication of biological tests for the early detection of "excessive" exposure to industrial chemicals and hence for preventing occupational diseases.

Human biological monitoring, in the context of these monographs, is the evaluation of occupational exposure to chemical agents using the exposed worker himself. The analytical determination will be carried out on representative biological materials (indicator media) of the exposed organism, it will either consist of the determination of the toxic agent itself or its metabolites or some early reversible biological effects which are considered useful for the prevention of excessive exposure.

The biological monitoring approaches described in these monographs are not indicated as health screening procedures aimed at detecting clinical effects. Biological monitoring may be complementary to monitoring of the working environment and in several situations may be the most relevant approach.

Biological monitoring provides an indirect insight into what happens inside the body of exposed workers, and some more relevant parameters for estimating the risk of health impairment, by

- taking into account total exposure (via the respiratory tract, the skin and the gastro-intestinal tract).
- taking into account various host factors affecting pharmacokinetics and pharmacodynamics.

Some biological monitoring tests are best used for individual screening while some are best suited for group exposure conditions.

Biological indicators are at present available for only a small number of toxic agents and furthermore the procedures for biological monitoring are limited also by the availability of excreta and readily accessible body fluids and tissues for analysis.

Regarding these biological materials emphasis must be placed on the precautions which must be taken in handling these materials before and during analysis. Care must also be taken with the interpretation of the results, taking into account the always possible analytical errors, and the biological variability from individual to individual. Finally, it should be kept in mind that excessive exposure does not necessarily mean health impairment.

It must be recognized that the biological monitoring approach has not yet reached a very advanced stage of development and that considerable research is still necessary. This is reflected by the monographs which are published in this series and which reflect the current state of knowledge in this field taking only into consideration relevant human data.

Dr. P. Recht
Director.

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ABBREVIATIONS

1) ALAD = δ-aminolevulinic acid dehydratase activity of erythrocytes

2) ALAU = δ-aminolevulinic acid in urine

3) CPU = Urinary coproporphyrin

4) EP = Erythrocyte protoporphyrin

5) PbA = Atmospheric lead levels

6) PbB = Lead in blood

7) PbU = Urinary Lead

8) PbUEDTA = Amount of chelatable lead excreted with 24-h urine after administration of $CaNa_2$ EDTA (1 g intravenously)

9) ZPP = Erythrocyte Zinc protoporphyrin

1.0 SUMMARY

This document reviews inorganic lead as related to occupational exposure and the possibilities of the biological monitoring of exposure.

The main route of absorption in occupational exposure is the respiratory apparatus. Derangement in heme synthesis is currently considered the first adverse effect associated with increasing concentration of lead in the soft tissues.

A vast number of tests which permit an evaluation of the degree of exposure, body burden, and toxic effect are available for monitoring lead workers.

For periodic monitoring of workers exposed to lead it is recommended that two tests be used simultaneously; one test should be designed to indicate internal dose and another to indicate effect. In general it is advisable to use blood lead levels as a measure of internal dose, and erythrocyte protoporphyrin as an indicator of effect.

For screening studies an inexpensive test which is easy to perform, sensitive, specific and precise should be used to identify subjects with high exposure. Both protoporphyrin and delta-aminolevulinic acid dehydratase comply with these requirements.

For assessment on a group analysis basis of the environmental condition of a work place, the urinary tests may be used; blood tests, however, provide more accurate information.

Individual blood lead levels in male workers should not exceed 60 µg/100 ml, and in women workers of child-bearing age they should not be higher than 40 µg/100 ml because of the potential adverse effect of lead on the foetus.

Further investigations are required on the relationship between external and internal dose and standardization of the various biological tests.

2.0 INTRODUCTION

2.1 Chemical and physical properties

Lead is a chemical element represented by the symbol Pb and with an atomic number of 82, atomic weight 207.21, specific weight 11.342. Melting point: 327°C; boiling point: about 1740°C. Starting from temperatures of 550-600°C, there is considerable production of vapours which combine with oxygen in the air to form lead oxide. Lead is found in the natural state in mineral deposits. The most common and most widely used mineral for extraction is galena (PbS). The lead content in directly mined mineral varies from 3 to 10%.

2.2 Effects on Humans

Derangement in heme synthesis is currently considered the first adverse effect (critical effect) associated with increasing concentration of lead in the soft tissues; in fact, lead can inhibit some enzymatic activities of heme biosynthesis (Chisolm, 1971; De Bruin, 1971; Baloh, 1974; Waldron and Stoefen, 1974). See Figure 1 (Chisolm, 1971).

The inhibition by lead of ALAD and heme synthetase, which are enzymes containing SH groups, is well documented. Due to ALAD inhibition, an accumulation of ALA occurs in the serum and consequently in the urine; inhibition of heme synthetase (iron chelatase) produces an accumulation of protoporphyrin IX in the erythrocytes. An increase in urinary coproporphyrins is an indirect evidence of coprogenase inhibition by lead.

The combination of decreased delta-aminolevulinic acid dehydratase activity in red blood cells, increased urinary delta-aminolevulinic acid, increased urinary coproporphyrin, and increased erythrocyte protoporphyrin is pathognomonic for lead, distinguishing it from all other disorders of pyrrole metabolism in man (Chisolm, 1975).

Also changes in nerve conduction velocity should be regarded as a critical effect (Zielhuis, 1977). These changes will not, however, be considered here because the investigation methods are rather time-consuming, difficult to perform in working

environments, and are not yet standardized; furthermore, the individual results are not always reliable since the alterations are unspecific. Such investigations can nevertheless be very useful for studies on groups of workers.

3.0 METABOLISM

In working environments the main route absorption is the respiratory apparatus. It is generally considered that 35-50% of the lead that reaches the lower respiratory tract is absorbed into the blood stream.

The potential increase in the body burden of lead can be expressed as:

$$BB = L \times V \times R \times D \times 10^{-3}$$

where BB=potential increase in body burden in mg; L=air lead concentration in mg/m^3; V=pulmonary ventilation in m^3/day; R=fraction of inhaled lead retained; D=duration of exposure in days. R values vary according to the solubility and particle size of individual lead compounds.

The uptake of lead by the gastro-intestinal tract is less complete than by the lung. Not more than 5-10% of ingested lead is generally absorbed, the balance being excreted in the feces.

A potential gastro-intestinal absorption in industry should not be underestimated. Both because it can increase due to particular personal habits, e.g. smoking, eating in the work-place and because as much as 40% of inhaled lead of large diameter trapped in the upper respiratory tract may be swallowed (Kehoe, 1961; Knelson et al., 1973; Hamilton and Hardy, 1974; Waldron and Stoefen, 1974).

In a steady-state situation, lead intake equals output and the skeletal system contains about 80-90% of the total body burden of lead.

Figure 1

Biosynthesis of heme is inhibited by lead, resulting in accumulation of intermediates in the synthetic pathway. Lead inhibits two steps (solid arrows) and may inhibit two others (broken arrows).

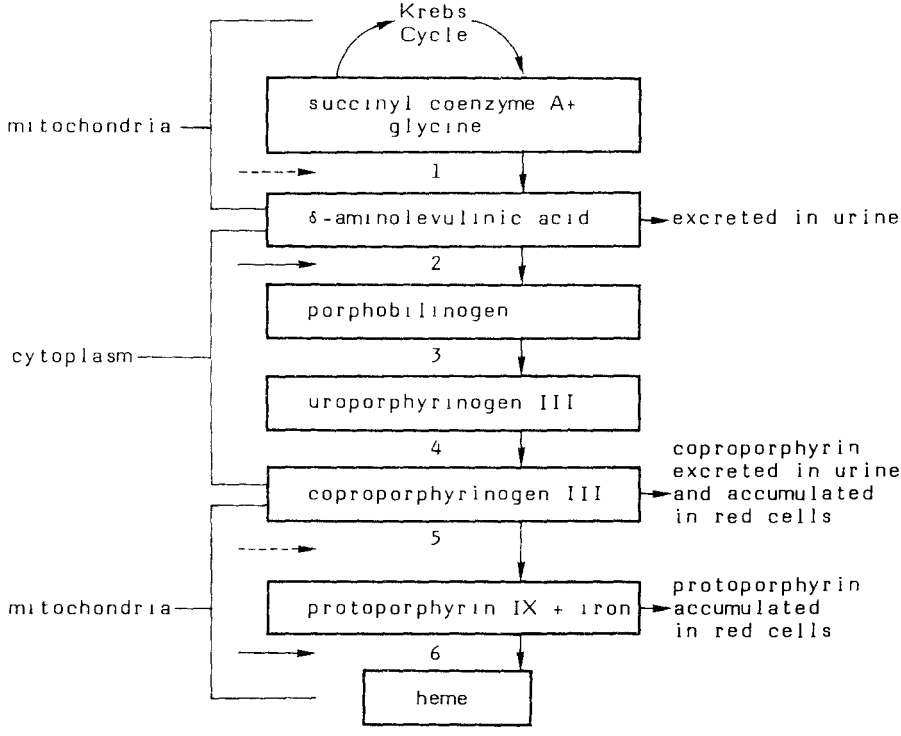


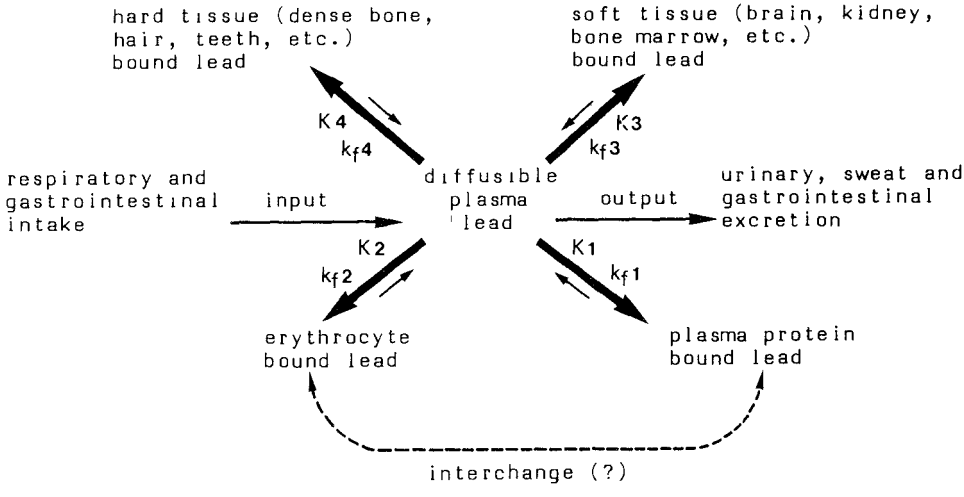
Figure 2 (Baloh, 1974) gives a schematic representation of the dynamic interchange of the body lead pool. Blood is the major factor in determining the steady state distribution of lead in body tissues. There is a dynamic equilibrium between red cell lead and plasma lead on the one hand and between extracellular lead and intracellular lead on the other. It is likely to be the ionic fraction of the plasma lead which is transferred to the other body compartments. The equilibrium constants of the reactions probably rank in the following order: $K_4 > K_3 > K_2 > K_1$. However, since a state of equilibrium is rarely reached, the rate constants K_f also become important. The rate constants indicate the speed with which the state of equilibrium can be reached in any given reaction.

The rate constants in Figure 2 are probably in the reverse order of the equilibrium constants, i.e., $K_{f1} > K_{f2} > K_{f3} > K_{f4}$, indicating that the bone takes more time to reach its final lead concentration than erythrocytes or proteins (Baloh, 1974; Waldron and Stoefen, 1974).

According to Pietrovsky (1970) the total body burden of lead can be roughly divided into: 1) rapid exchange pool in blood and soft tissues; 2) intermediate exchange pool in skin and muscles; 3) exchange pool in bone (intermediate exchange in bone marrow, trabeculae, and slow exchange in dense bone and teeth).

Figure 2

The dynamic interchange of the body lead pool



4.0 BIOLOGICAL INDICATORS

4.1 Indicators of Internal Lead Dose

Dose should, ideally, be defined as "the amount or concentration of a given chemical at the site of its action", i.e., where its presence leads to a given effect. Since the determination of this amount is often impossible in practice, the dose may have to be estimated by various means and in most cases one can speak only in terms of these dosage estimates. Metal concentration in biological media can often be used as indicators (or indices) of exposure and of concentration in the critical organ (Nordberg, 1976).

Below are considered the biological tests which may be used as indicators of an internal lead dose.

4.1.1 Concentrations in blood

The level of lead in blood (PbB) is a function of the quantity of lead absorbed from the environment minus the lead deposited in the bone cortex and soft tissues and the lead excreted with urine and feces (Waldron, 1971). PbB is about 2% of the total lead burden. Approximately 90% of blood lead is bound to erythrocytes and is not readily diffusible; plasma lead (0.2% of the total burden) is made up of two fractions: the plasma protein bound fraction and the diffusible fraction, the latter being probably the metabolically active center of the body lead pool (Baloh, 1974). See Figure 2. Diffusible plasma lead probably gives the best approximation of the biologically effective lead burden, although at present it is not possible to measure it. It should be noted, however, that the plasmatic fraction of lead is not a constant function of the total blood lead concentration and therefore cannot be predicted by PbB (Waldron, 1974). However, for groups of subjects, PbB is probably a reasonable indication of plasmatic levels (Zielhuis, 1975a).

In practice PbB is the most reliable means of measuring the extent of exposure: it allows distinctions to be made between "normal" subjects, subjects with "permissible" absorption levels, and subjects with "non-permissible" absorption levels. It is, moreover, particularly useful in epidemiological studies. In fact, a good correlation exists between PbB and lead levels in the atmosphere (Williams et al., 1969).

Interpretation of blood lead levels must take into account the fact that they reflect only one point in time, and a dose which is steady, increasing or decreasing (Nordberg, 1976). However, although these levels allow a satisfactory evaluation of current exposure, they are not necessarily always correlated with the lead body burden. In fact, after cessation of exposure, PbB may reach "normal" values while a body burden persists. This is demonstrated by a high urinary lead excretion after chelating therapy (Prerovska and Teisinger, 1970), or when disorders of heme synthesis are still evident (Selander and Cramer, 1970; Alessio et al.,1976c). On the other hand, cases are known in literature of adults and children who showed clinical symptoms of intoxication but who had relatively low PbB values (Beritic, 1971; Moncrieff et al., 1964). This apparent "paradox" could be due to the fact that measurement occurred some time after cessation of exposure (Kehoe, 1972).

Studies on volunteers who received different quantities of lead also showed that the PbB levels reach a given ceiling even when the body lead burden increases continuously during exposure (Kehoe, 1961).This was also observed in occupational exposure (Benson et al., 1976).

Factors exist which can influence PbB levels independently of exposure and body burden. For example, blood lead levels are greatly affected by the red blood cell mass (anemia, polycythaemia). There has been much discussion of whether these levels should be corrected according to the haematocrit values, but there is disagreement on the biological validity of such correction (Lauwerys, 1975).

Further, the measurement of lead concentration in blood also presents difficulties (Berlin et al., 1974; WHO, 1977; NIOSH, 1978). According to Baloh (1974), there is a 15% error in the measured value on multiple runs of the same blood sample even in the most competent laboratories. A number of interlaboratory control programs have revealed high rates of variation in the results, which are probably due to the fact that this parameter is measured with methods and instruments that differ considerably one from the other. In January 1978, an interlaboratory control program for PbB was sponsored by the EEC (within the frame of the activities provided for in the EEC guidelines of 29.3.77). This program is still under way and preliminary results indicate that the extractive methods followed by flame AAS always gives lower results than the other techniques with atomic absorption. The

flameless methods or the "Delves Cup" give similar results but it should be noted that the "Delves Cup" tends to overestimate the values.

4.1.2 Concentrations in urine

The kidney is presumed to excrete lead by two routes: glomerular filtration and transtubular flow or excretion (Vostal and Heller, 1968)

The relative importance of the two routes is uncertain, but the formation of lead containing inclusion bodies suggests that in subjects with heavy lead exposure, transtubular flow may assume a greater importance (Cramer et al., 1974).

Since the analysis of lead in urine (PbU) does not require blood withdrawal, it is sometimes preferred to PbB determination (Lauwerys, 1975). The "normal" PbU concentration in adults usually oscillates between 10 and 80µg/l, lower than 50µg/g creatinine (Baloh, 1974; Lauwerys, 1975). In subjects under continuous exposure, a satisfactory correlation was found between atmospheric lead levels and PbU and between PbB and PbU (Williams et al., 1969). In the case of new lead exposure there is also a good correlation between PbB and PbU, but while PbB increases without any demonstrable time lag, the increase in PbU requires a latency period of about 2 weeks (Tola et al., 1973).

Many factors other than lead absorption such as fluid intake and specific gravity of the urine may influence the excretion of lead (Ellis, 1966). Patients with chronic nephritis frequently have PbU levels within "normal" limits in spite of the existence of high lead stores (Lilis et al., 1968). Prerovska and Teisinger (1970) have demonstrated that subjects with heavy lead exposure in the past can have normal urinary lead excretion even when excretion of chelatable lead remains high.

4.1.3 Concentration in feces

In the non-occupationally exposed general population the quantity of lead eliminated with the feces is clearly higher than that eliminated with the urine. In fact, the greater part of the metal present in the feces consists of ingested lead that has not been absorbed by the intestines (Kehoe, 1961).

The levels of lead in feces of "normal" subjects varies between 240 and 400µg/24h (Kehoe, 1961; Barry, 1975). During occupational exposure the values increase to 760-3800µg/24h, according to the data of Saita and Moreo (1958).

Measurement of lead in feces can be used to determine absorption by ingestion (accidental or intentional). Fecal lead excretion above 4mg/100g, 4 weeks after occupational exposure has ceased, is a sure indication of ingestion (Zielhuis, 1972). The analysis is valid only when performed during the period of ingestion or in the days immediately following (Vigliani and Debernardi, 1934).

4.1.4 Chelatable lead

Chelatable lead is strictly dependent on the active deposit of the metal in the soft tissues of the body, including the trabecular bone (Teisinger et al., 1969), and as a result it provides a more direct measurement of the rapid exchange pool.

Chelatable lead can be measured by injections of $CaNa_2$ EDTA or by penicillamine per os. The levels of PbU EDTA (mean + 2 SD) in 26 inhabitants of Milan who were not occupationally exposed to lead, were 630µg/24h (Alessio et al., 1976a).

Limited data on humans strongly suggest that the $CaNa_2$ EDTA mobilization test may be a better indication of the concentration of lead in affected organs of man (Nordberg, 1976).

Since $CaNa_2$ EDTA is capable of binding only with extracellular lead, (Teisinger et al., 1958; Castellino e Aloj, 1965), it is likely that measurement of metal in urine after administration of this drug permits an indirect, though rough, evaluation of the levels of diffusible lead.

After administration of $CaNa_2$ EDTA, the reduction in levels of lead in the plasma creates a cells/plasma gradient which slowly disappears. After the first few days of treatment in fact, urinary lead is greatly reduced and it is necessary to interrupt administration for a few days so that an equilibrium may be established in the distribution of lead in the cellular and extracellular compartments and so that a high excretion of the metal may once again be obtained (Saita, 1962).

In studies on children and adolescents (Chisolm et al., 1976), a statistically significant linear relationship was found between blood lead concentration and the logarithm of the quantity of lead excreted in the 24-hour period immediately following administration of $CaNa_2$ EDTA. In our laboratory, studies in progress have shown that a good correlation exists between PbB and PbU EDTA in adult subjects with current occupational exposure to lead. In subjects with past occupational exposure, the correlation between the two parameters is definitely lower, although still statistically significant. Analysis of the regression curves shows that for corresponding values of chelatable lead, subjects with past exposure have lower blood lead levels than currently exposed subjects. The slopes of the regression lines are statistically different (Figure 3).

Chelatable lead cannot be used in epidemiological studies because it necessitates administering a drug and also because 24-hour urine samples are difficult to obtain.

The induced urinary lead test is capable of detecting and evaluating the existence of lead absorption which occurred in the past. It can therefore be used to determine whether former acute manifestations or current chronic manifestations are attributable to lead intoxication, even when the other indicators of internal dose have returned to normal (Saita, 1962; Prerovska and Teisinger, 1970).

Teisinger (1971) maintains that in subjects with past exposure, a urinary lead excretion above 1mg/24h after administration of $CaNa_2$ EDTA (2g i.v.) is indicative of a potentially dangerous body burden of the metal; for subjects still exposed, however, the author sets this critical level at 2mg/24h.

4.1.5 Relationship between external lead exposure and indicators of internal dose

Many studies have demonstrated the existence of a correlation between PbB, PbU and the atmospheric lead levels in the working environment (PbA).

The relationship between PbA and PbB has a similar profile both when the atmospheric lead levels are low and when levels of $0.2mg/m^3$ are reached (Harada, 1976). See Figure 4. Williams et al. (1969) found a close correlation between PbA and PbB ($r=0.90$) and between PbA and PbU ($r=0.82$) with high statistical significance ($p<0.01$). Table 1 (Williams et al., 1969) gives the mean values and

Figure 3

Relationship between chelatable lead (PbU EDTA) and PbB in male subjects with current (a) and past exposure (b) to lead.

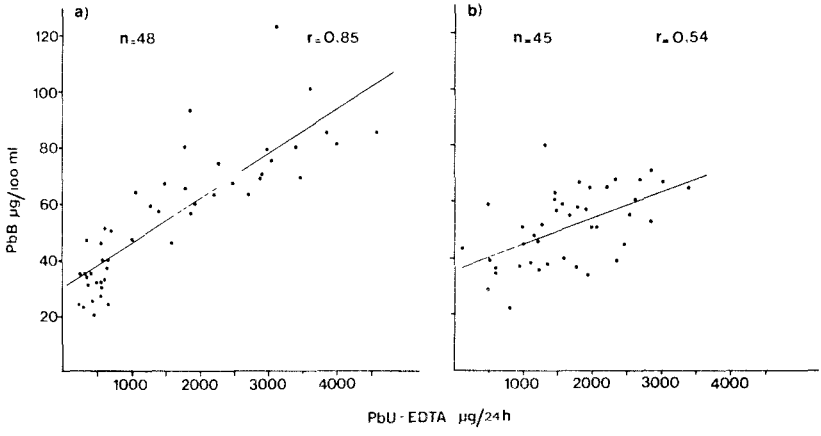


Figure 4

Relationships between lead concentration in air and lead concentration in blood of lead workers. The curves are those obtained from a study of the PbA/PbB relationship in newspaper industries (Harada, 1976), extended to plot also the values of Elkins (1959), Harada et al. (1960) and Tsuchiya and Harashima (1965).

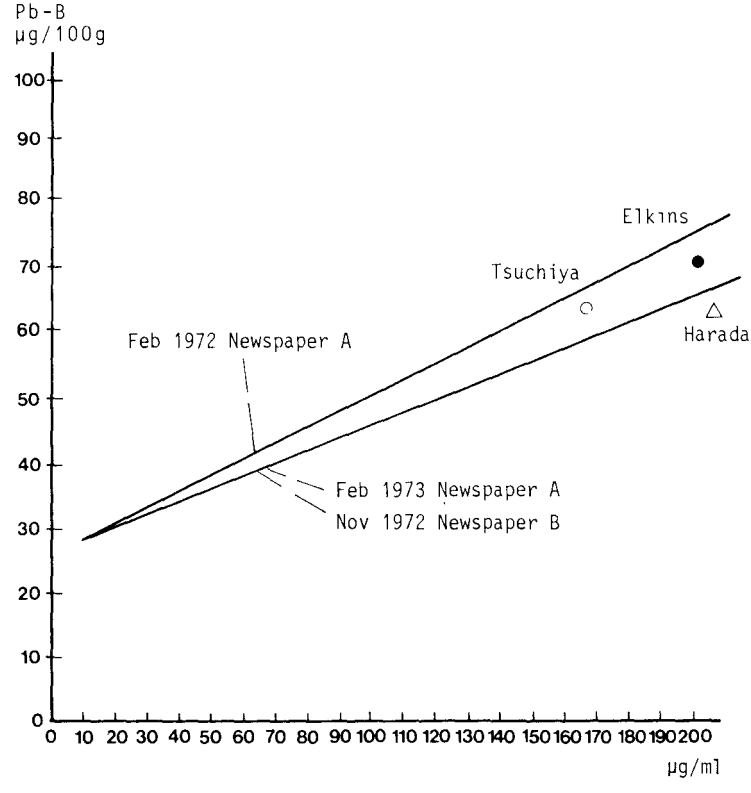


Table 1

Mean values and 95% confidence limits of single determinations of lead in blood and urine which correspond to two lead-in-air concentrations.

PbA ($mg\ Pb/m^3$)		PbB ($\mu g/100\ ml$)	PbU ($\mu g/l$)
0.20	Mean	70	143
	95% C.L.	48-92	56-230
0.15	Mean	60	118
	95% C.L.	38-82	31-205

the 95% confidence limits of single determinations of PbB and PbU which correspond to 0.2 and 0.15mg/m^3 lead in air. The wide range of the confidence limits is evident from these data.

On the basis of Williams' data, Zielhuis and Verberk (1974) examined the validity of various PbB and PbU levels as indicators of "unacceptable" exposure; they assumed PbA=0.12mg/m^3 to be the "acceptable" level. See Table 2. In this sample, for the cut-offs considered, PbB levels have a higher validity than PbU levels as indicators of unacceptable exposure. PbB > 40 has maximum sensitivity (no false negatives: all individuals with PbA > 0.12 have PbB > 40); however, specificity is moderate (also PbB > 40 in subjects with PbA < 0.12: 44% of false positives). PbB > 80 is highly specific (no individual with PbA < 0.12 has PbB > 80, i.e., no false positives); but sensitivity is moderate (46% false negatives). On the basis of these results, Zielhuis and Verberk (1974) conclude: "If one wants to be certain that all subjects with PbA > 0.12 are selected out of a universe of exposed workers, PbB > 40 will serve this objective, however, at the cost of a number of false positives. If, on the other hand, one wants to select only individuals with PbA > 0.12, then PbB > 80 will serve this objective, however with many false negatives."

The number of subjects in whom validity has been studied is limited (about 30 cases) but it is likely that even with a larger number of subjects the validity values will be similar since air sampling involves many limitations; i.e.:

- effect of particle size and solubility of particle;
- representation of only a small fraction of total volume of air inhaled;
- ingestion remains unmeasured;
- effect of contamination and position of sampling head;
- effect of the entry of particulates into sampling heads;
- failure to evaluate individual differences in pharmacokinetics according to age, type of respiration, congenital or acquired diseases, etc. (Lyman, 1975).

Table 2

Validity of different PbB and PbU levels for predicting an unacceptable lead exposure (PbAir 0.12mg/m^3)

		Se	Sp	Validity
PbB	>40	1.00	0.66	1.66
µg/100 ml	>60	0.72	0.80	1.52
	>80	0.56	1.00	1.56
PbU	> 60	0.88	0.53	1.41
µg/l	>120	0.56	0.95	1.51
	>160	0.12	0.95	1.07

Se = sensitivity

Sp = specificity

Sp + Se = Validity

At the 2nd International Workshop on Permissible Limits for Occupational Exposure to Lead (Zielhuis, 1977), the conclusion was reached that a "standard" for lead in air based upon the relationship between PbB and PbA could not be established. Such a standard, it was felt, would best be based on PbB alone.

To conclude this section, it seems appropriate to make the following points:

a) Lead in the blood and lead in the urine are indicators of exposure since the levels of these parameters are closely influenced by the environmental concentration of lead.

b) Chelatable lead may be considered a "true" indicator of dose, the levels of which reflect the active lead deposit.

c) In currently exposed subjects the indicators of exposure permit prediction of the quantity of chelated lead.

d) In subjects no longer exposed the indicators of exposure do not permit a reliable evaluation to be made of chelatable lead.

4.2 Indicators of Effects in Adult Males

Biological tests which may be used as indicators of a biological lead effect are separated according to sex since in recent years it has been shown that some indicators of effect behave differently in males and females.

4.2.1 Erythrocyte delta-aminolevulinic acid dehydratase (ALAD)

The ALAD activity of circulating erythrocytes is highly sensitive to inhibition by lead; inhibition of ALAD in red blood cells (RBC's) parallels inhibition in other tissues, e.g., liver (Secchi et al., 1974).

A very close negative correlation exists between erythrocyte ALAD and lead blood levels (PbB). The enzyme undergoes distinct inhibition in the range of PbB values

below 40µg/100ml (Hernberg et al., 1970 Haeger-Aronsen et al., 1971; Zielhuis, 1972; Lauwerys et al., 1974). There is suggestive evidence that the no-effect level is about 10µg PbB/100ml (Granick et al., 1973).

Up to 1974, studies on the relationship between ALAD and PbB have generally used the method of Bonsignore et al. (1965) or methods derived from this for the determination of ALAD. At present the European standardized method (Berlin and Schaller, 1974) is widely used. Determination of ALAD using the method of Bonsignore is of little use in monitoring occupationally exposed subjects (Alessio et al., 1976b). See Figure 5. In fact, when PbB increases beyond 40µg/100ml, the enzymatic activity is reduced to a level too low to allow identification of different blood lead levels (de Bruin, 1968; Basecqz et al., 1971; Hernberg et al, 1972; Secchi and Alessio, 1974). However, ALAD can have a wider application in monitoring lead workers when it is measured with the CEC method, since a marked inhibition of the enzymatic levels occurs only when PbB values exceed 50-60µg/100ml. See Figure 5.

Validity of ALAD is rather moderate for PbB levels lower than 40µg/100ml which therefore implies a very high percentage of false classifications. Thus when subjects with only environmental lead exposure are studied according to Ziehluis (1974),"it is not possible to base a biological quality guide on individual ALAD levels". Validity of ALAD does, however, improve markedly for higher PbB levels. For example, the validity of ALAD (measured with the CEC method) is good at a PbB cut-off of 60µg/100ml. At this PbB level, at a cut-off of 15µ/RBC the enzyme displays a sensitivity of 0.96 (i.e. 4% false negatives) and a specificity of 0.85 (i.e., 15% false positives). These data indicate that ALAD may be used as a screening test for occupationally exposed subjects (Table 3).

After a worker's first exposure to lead, ALAD activity decreases rapidly without any appreciable time lag, parallel to the increase in blood lead concentration (Hernberg et al., 1972). According to Tola (1972) and Haeger-Aronsen et al. (1974), when exposure to lead ceases, ALAD activity progressively returns to normal, parallel to PbB. Thus, according to these findings, ALAD does not indicate any former lead exposure that cannot be detected from an elevation of PbB.

Figure 5

Correlation between PbB and ALAD determined by two different methods in adult males currently exposed to lead

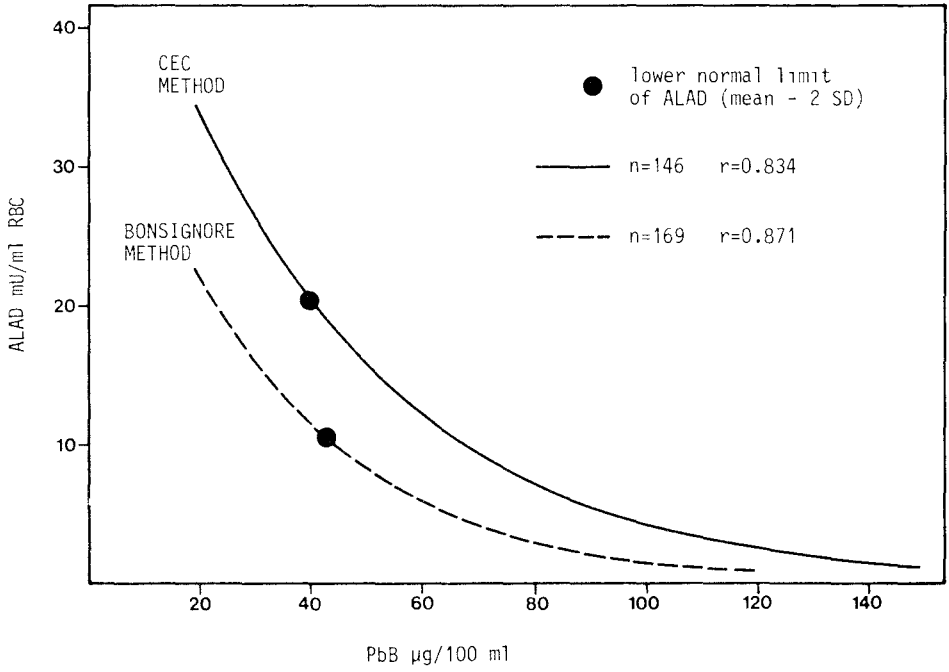


Table 3

Validity of ALAD for Predicting Different PbB Levels. Analysis made on 108 adult males currently exposed to lead

PbB µg/100ml	ALAD mU/ml RBC	Se	Sp	Validity
≥ 40	≤ 20	0.71	0.80	1.51
≥ 60	≤ 15	0.96	0.85	1.81
≥ 70	≤ 10	0.94	0.92	1.86

Se = sensitivity, Sp = specificity, Validity = Se + Sp. ALAD determined according to the European standardized method.

However, other studies (Vergnano et al., 1969; Sakurai et al., 1974) indicate to the contrary, that in cases of severe past exposure, ALAD remains inhibited out of proportion to the current PbB. It has been conjectured that in this situation enzymatic inhibition is due to an inhibitor other than lead, probably of a thermolabile proteic nature (Vergnano et al., 1969).

The significance of erythrocyte ALAD inhibition due to lead in regard to health is still open to discussion (Zielhuis, 1975a). As regards ALAD inhibition for the range of PbB up to 40µg/100ml "its biological significance is dubious because it is unaccompanied by any detectable effects on the biochemical function of man" (NAS, 1972). According to Nordberg (1976), an inhibition of ALAD in the cells of the bone marrow is a subcritical effect which precedes an increased level of delta-aminolevulinic acid in blood and urine and the occurrence of anemia (critical effects). A decrease in ALAD activity in blood is an example of an indicator of subcritical effect of lead exposure.

Until recently, it was not clear whether the inhibition of peripheral erythrocyte ALAD by lead was a phenomenon which really occurred in vivo, or a phenomenon which only occurred in vitro, i.e. a result of membrane-bound lead getting access to the intracellular enzyme as a result of haemolysis in the test tube required for determining ALAD activity. Roels et al. (1974a) have shown that the decrease in the erythrocyte enzyme is a true reflection of the enzyme activity in vivo when PbB is 120µg/100ml. ALAD inhibition is highly specific for increased lead absorption: e.g. no reduction of ALAD activity has been observed in workers occupationally exposed to cadmium and mercury (Lauwerys et al., 1974; Lauwerys and Buchet, 1973). A transitory inhibition of ALAD occurs after acute ingestion of high quantities of alcohol, and returns to normal with the normalization of blood alcohol (Moore et al., 1971). In chronic alcoholism high PbB values may be found, but ALAD appears more depressed than might be expected from blood lead levels, and these levels remain low for a number of days after suspension of alcohol consumption (Krasner et al., 1974: Secchi and Alessio, 1974a).

In lead-exposed subjects, false negative results of ALAD may be obtained when hyper-regenerative erythropoietic disorders exist, e.g. bleeding anaemia, haemolytic anaemia (Bonsignore et al., 1970; Battistini et al., 1971).

The European standardized method for determination of ALAD proved to be accurate and with good reproducibility. The interlaboratory coefficient of variation in the

intercomparison programme sponsored by the CEC in 1974 was 10% (Berlin et al., 1974).

In the view of Berlin and Schaller (1974), the routine use of ALAD is limited by technical problems, particularly the conservation of the blood sample at 0°C for a limited time interval. In our experience, when the sample is stored at 4°C, no loss of enzyme occurs after 24 hours . See Table 4.

Table 4

Erythrocyte ALAD activity (m U/ml) before and after storage at 4°C

samples	before storage	after storage	
		24 hours	48 hours
1	38.0	37.6	33.0
2	21.4	20.8	17.2
3	19.8	19.9	16.2
4	16.0	16.0	13.0
5	9.8	9.7	9.0

4.2.2 Erythrocyte Protoporphyrin IX

Heme synthetase is extremely sensitive to the action of lead and the inhibition of this enzymatic activity causes an accumulation of EP in erythrocytes. This is related to the fact that the mitochondrial enzyme regulates the incorporation of iron in the porphyrin molecule. In occupationally exposed subjects, the concentration of this erythrocyte metabolite rises and can reach levels from 10 to 50 times higher than the values found in subjects not occupationally exposed to lead (Vigliani and Angeleri, 1935; Rubino et al., 1958, de Bruin, 1971).

The methods of erythrocyte protoporphyrin determination are numerous. Some permit selective measurement of different porphyrins (Schwatz and Wikoff, 1952; Sassa et al., 1973), others measure the total concentration of erytrocyte porphyrins (Piomelli et al., 1973). All these methods use extractive techniques.

The discovery that erythrocyte protoporhyrin that rises following an abnormal lead absorption (or following sideropenia) is not "free" but bound to zinc, revolutionized the determination methods. In fact, zinc protoporphirin can be determined on capillary blood diluted with water or alcohol by direct fluorimetric reading (Lamola, 1974). Since 1976 instruments have been developed - hematofluorimeters - for the immediate determination of zinc protoporphyrin on undiluted blood (Blumberg et al., 1977).

Henceforth, protoporphyrin determined with extractive methods will be shown as EP and zinc protoporphyrin as ZPP.

For greater clarity, EP and ZPP are dealt with separately.

4.2.2.1 Erythrocyte protoporphyrin determined with extractive methods

EP measurement has made considerable advances in paediatrics as a result of studies carried out using microanalytical methods (Kammholz et al., 1972; Sassa et al., 1973; Piomelli et al., 1973; Chisolm et al., 1974). This test had not been used for monitoring occupationally exposed subjects until recently. A highly significant correlation was found between EP and PbB in adult males under stable lead exposure. In this situation EP is also closely correlated with urinary lead and chelatable lead (Roels et al., 1975; Tomokuni et al., 1975; Alessio et al., 1976a).

The behaviour of EP is uniform with respect to the three indicators of dose. EP first undergoes a modest increase with the elevation of the internal load within the normal values. Beyond such normal values a net increase occurs, which continues up to an asymptotic value which is not further altered by the increase in dose (Alessio et al., 1976a).

In adult males the increase in EP in the 40 to 80µg/100ml blood lead range appears very marked, so that the difference between "normal" subjects, subjects with "permissible" and subjects with "not permissible" absorption appears more distinct than that which can be revealed by blood lead. (Fig. 6) It should be pointed out that at PbB levels which do not cause an elevation in EP, a reduction in ALAD levels is already in operation. On the other hand, the dose-response relationship calculation has shown that in adult males there is a no-response PbB level for an increase in EP of 25-35µg/100ml (Roels et al., 1975; Zielhuis, 1975a). The no-response PbB level for ALAD appeared to be 15-20µg/100ml (Zielhuis, 1975a).

Moreover, EP permits a fairly accurate prediction of the amount of chelatable lead (Alessio et al., 1976a). This seems particularly interesting since it is very likely that, as an indicator of biologically effective internal dose, chelatable lead is more relevant than lead in blood.

EP can be reliably used as a screening test for monitoring occupationally exposed groups since it has good predictive validity in the 40-70µg/100ml range for PbB and in the 500-2000µg/24h range for PbU EDTA. See Table 5, Alessio et al. (1976a).

For example, at a blood lead level of 60µg/100ml, EP at a cut-off of 75µg/100ml correctly classified 97% of positive subjects and 99% of negative subjects. Thus only 3% false negatives and 1% false positives were obtained.

Moreover, EP was found to correlate with other indicators of effect, suggesting that it could be used to predict both the internal lead load and the modifications of the other indicators of effect (Tomokuni, 1975; Alessio et al., 1976b).

When examining recently exposed subjects, account must be taken of the fact that between the beginning of lead absorption and the increase in EP there is a time lag evaluated by Sassa et al. (1973) as 2 months, and by Stuik (1974) as 2 to 3 weeks.

Figure 6

Relationship between PbB and EP, in 201 adult males currently exposed to lead - r = 0.94.

Upper frame: linear scale on ordinate. Lower frame: logarithmic scale on ordinate. EP determined according to the Schwartz and Wikoff Method.

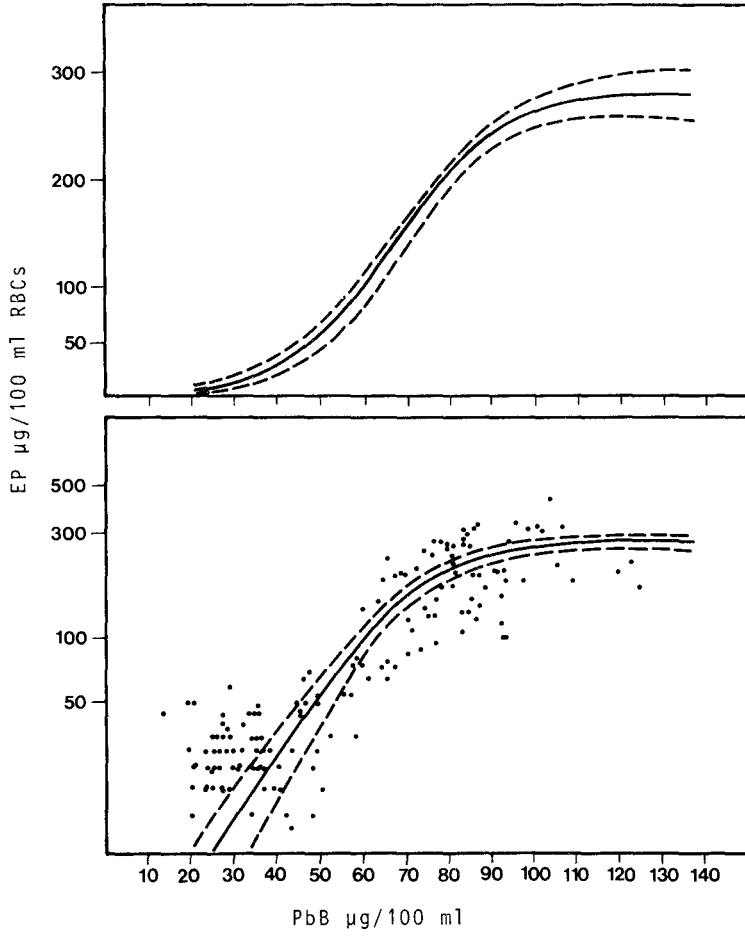


Table 5

A) Validity of EP for predicting different PbB levels. Analysis made on 201 adult males currently exposed to lead.

PbB (µg/100ml)	EP (µg/100ml RBC)	Se	Sp	Validity
≥ 40	≥ 50	0.83	0.98	1.81
≥ 60	≥ 75	0.97	0.99	1.96
≥ 70	≥ 100	0.98	0.90	1.88

B) Validity of EP for predicting different PbU EDTA levels. Analysis made on 92 adult males currently exposed to lead.

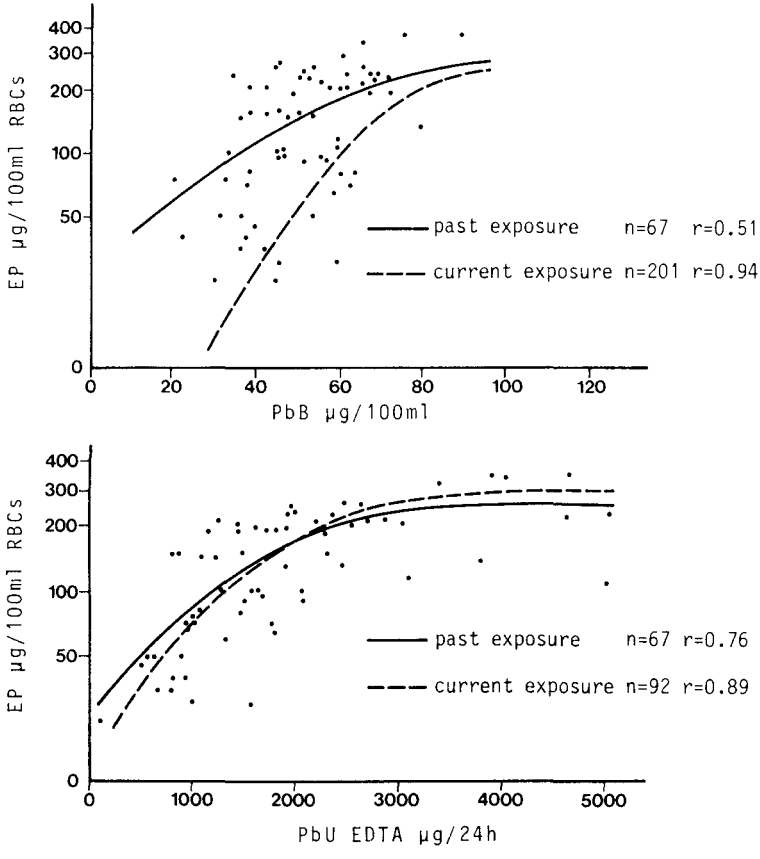
PbU-EDTA (µg/24ore)	EP (µg/100ml RBC)	Se	Sp	Validity
≥ 500	≥ 50	0.84	1.00	1.84
≥ 1000	≥ 75	0.92	1.00	1.92
≥ 1500	≥ 100	0.96	0.97	1.93
≥ 2000	≥ 150	0.93	0.83	1.78

Se = sensitivity, Sp = specificity, Validity = Se + Sp.
EP determined according to the Schwartz and Wikoff Method.

Figure 7

Relationships between EP and PbB (upper frame) and EP and PbU-EDTA (lower frame) in adult males with past lead exposure.
Scatter diagram: individual date of past-exposed subjects.
Logarithmic scale on ordinate.

EP determined according to the Schwartz and Wikoff method.



Normalization of EP after cessation of exposure is slower than that of PbB, ALAU and CP. In fact, in erythrocytes of subjects who have been exposed to lead there is a surplus of EP which persists until the red blood cells are destroyed (Albahary, 1972). However, in severely exposed subjects, EP stays at high levels even for many years after cessation of exposure (Saita et al., 1954; Gajdos, 1957; Rubino et al., 1958). Alessio et al. (1976c) recently demonstrated that the correlation existing between EP and PbB is decidedly lower in male subjects no longer exposed to lead than in currently exposed subjects, and that for the same PbB values, the EP levels are markedly higher in subjects who are no longer exposed.

EP and chelatable lead are closely correlated both in currently exposed subjects and in subjects with past exposure, and the regression curve in both groups takes on an almost identical profile. See Figure 7, Alessio et al., (1976c). These data seem to indicate that EP remains at high levels for a long period of time due to a direct inhibition of heme synthetase by the lead released from the deposits.

The erythrocyte metabolite can therefore be used to detect the existence of past exposure and to determine whether a patient who has had past exposure should resume work with lead.

EP levels as high as those occurring in severe lead poisoning might be found in erythropoietic protoporphyria, a rare congenital disorder, and in thalassemia major. Moderate increases have been found in cases of iron deficiency, serious liver diseases, and tumours (Baloh, 1974; Saita et al., 1966).

4.2.2.2 Zinc protoporphyrin

Determination of zinc protoporphyrin with portable hematofluorimeters is a very practical test which is easier to perform and lower in cost than the extractive methods.

There is a very close correlation between ZPP and PbB in adult males: The regression curve between the indicator of exposure and effect takes on the same profile as already observed for EP (Fig. 7); in fact, at PbB levels below 35-40

µg/100 ml, ZPP undergoes only a moderate increase, but subsequently the increase is very marked (Schaller and Schiele, 1977; Alessio et al., 1978) (Fig.8).

Without cases having PbB levels above 90 µg/ml it is not possible to check whether the regression curve takes on the asymptotic slope observed for EP determined with the Schwartz and Wikoff method.

Research in progress in our laboratory has shown a high predictive validity of ZPP and PbB levels $\geq$ 60 µg/100 ml: using a cut-off of ZPP $\geq$ 80 µg/100 ml validity was 1.77, with very high sensitivity (0.98), signifying 2% false negatives. The test may therefore be used to advantage in screening studies of occupational exposed subjects. Such studies are facilitated by the fact that the instrument is portable, gives immediate results and allows a large number of subjects to be examined in a short time.

It should however be noted that this test has been in routine use for a short time only so that certain features must be studied more closely.

As the hematofluorimeter takes account of the absorption spectrum of oxyhemoglobin, the ZPP levels determined on capillary blood are decidely higher than those determined on venous blood; whereas they are identical to those determined on venous blood after oxygenation (Alessio et al., 1978).

A close correlation exists between ZPP and EP; but it should be noted that while Alessio et al.,(1978) found that EP levels, determined according the Piomelli method, were higher than ZPP levels, Blumberg et al.,(1977) found that ZPP levels were higher than EP levels, and Schaller and Schiele (1977) found that ZPP levels were practically identical to erythrozyte protoporphyrin levels. A tentative explanation of the discrepancy in results might be the use of a different standard, the analytical values of erythrozyte protoporphyrin are lower (about 50%) than those obtained using a protoporphyrin standard.

It should moreover be noted that the research in progress in our laboratory has shown that by using three hematofluorimeters of different make, significantly different values are obtained (Fig. 9).

Figure 8

Relationship between PbB and ZPP in 211 adult males currently exposed to lead. ZPP determined with an ESA 4000 apparatus.

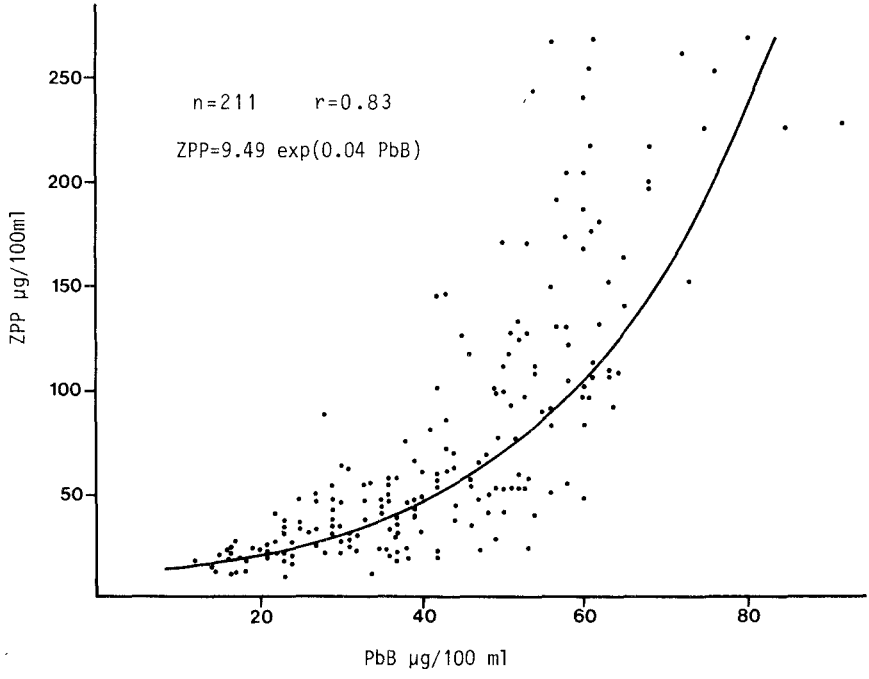


Figure 9

Relationship between ZPP values obtained using different makes of hematofluorimeters.

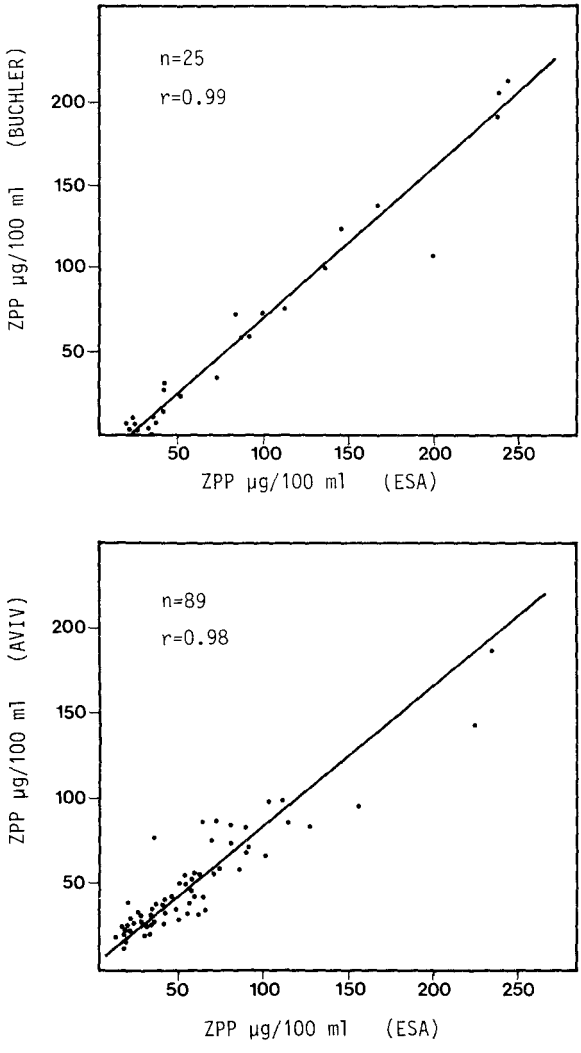
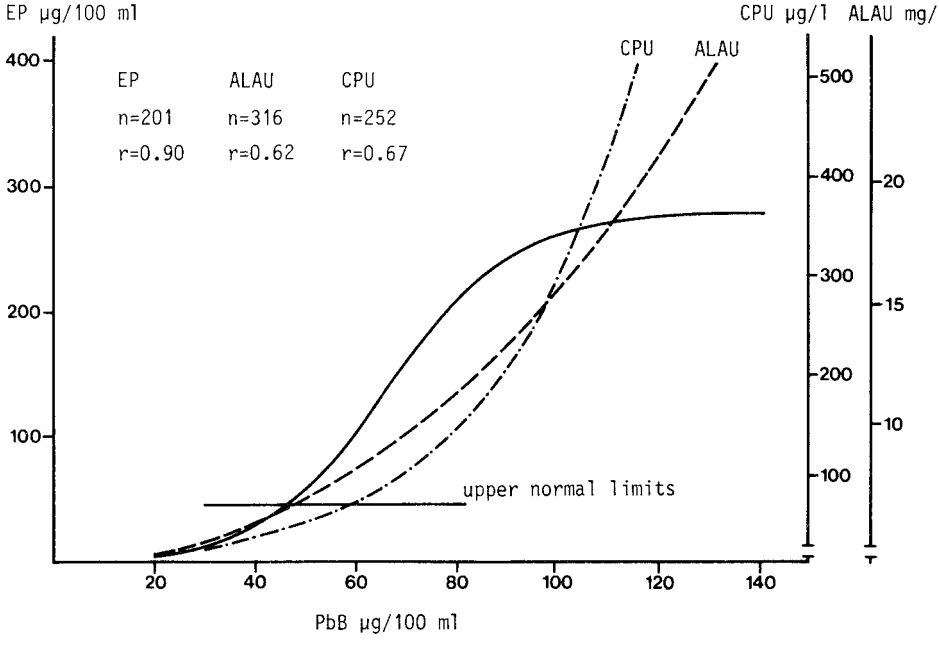


Figure 10

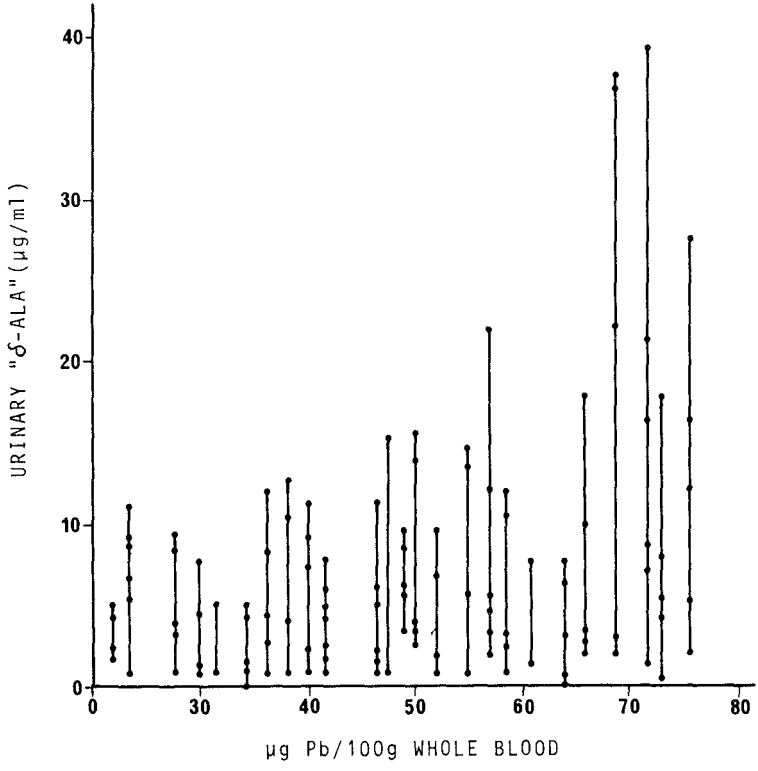
Relationship between PbB and indicators of effect in adult males currently exposed to lead.



EP determined according to the Schwartz and Wikoff method. ALAU determined according to Grisler and Griffini method CPU determined according to Grisler et al. method.

Figure 11

Range of ALA concentrations in individual urine samples passed by the same children in a single 24 hour period. Data are shown for 25 children with blood lead concentrations ranging between 23 and 75 µg PbB. Each vertical bar represents data from a single child and each solid circle on the bar represents the concentration of ALA (µg/ml) found in separate specimens from that child during a single 24 hour period. The wide range of concentration of ALA in single voidings of urine is apparent.



It is to be hoped that hematofluorimeter manufacturers carry out a joint study as soon as possible to standardize the calibration of the instrument, so that ZPP values may be readily compared in all laboratories.

4.2.3 Delta-aminolevulinic acid in urine

Due to the inhibition of the ALAD of the maturing RBC's by lead, the transformation of ALA into prophobilinogen is obstructed, resulting in an increase in ALA in the serum and in the urine. On the subject of behaviour of ALA in the serum, a few studies of children with acute encephalopathy are available. However, at present it does not appear that the test can be used routinely, since detection of only moderately increased levels of ALA requires more than 10ml of plasma (Chisolm, 1975).

Many studies are, however, available on ALAU. Researchers have found a good correlation between the urinary metabolite, PbB and PbU (Williams et al., 1969; Selander and Cramer, 1970; Haeger-Aronsen, 1971; Soliman et al., 1972; Lauwerys et al., 1974). The coefficient of correlation between PbB and ALAU is usually between 0.5 and 0.7, and therefore is not as close as the correlation which generally exists between PbB and the blood tests (ALAD and erythrocyte protoporphyrin). A significant increase in ALAU can be seen at PbB levels slightly higher than those at which there is an increase in erythrocyte protoporphyrin values. See Figure 10, Alessio et al. (1976b). This phenomenon is clearly seen from examination of the dose-response relationship. In fact, the approximate no-response PbB level for ALAU is 35-45µg/100ml, while for FEP it is 25-35µg/100ml (Roels et al., 1975; Zielhuis, 1975b).

Erythrocyte protoporphyrin permits better discrimination between exposed workers with "permissible" absorption and those with "potentially dangerous" absorption than ALAU. In fact, at a PbB concentration below the currently accepted TLVs, the progressive elevation of erythrocyte protoporphyrin is more marked than that of ALAU. See Figure 10. It should be noted, however, that the levels of ALAU, like the levels of CPU, also undergo increasing elevation when PbB values exceed 80-90µg/100ml, while erythrocyte protoporphyrin values do not undergo any further

increase. (This phenomenon has not yet been verified for ZPP). Therefore, urinary tests may have an important application when metabolic damage such as that which can occur in lead intoxication must be evaluated (Alessio et al., 1976b).

Like the validity of CPU, the validity of ALAU for predicting PbB appears to be distinctly lower than that of erythrocyte protoporphyrin.

To predict a PbB level $\geq$ 60 ug/100 ml using a cut-off of ALAU $\geq$ 10 mg/l, validity was 1.67, with sensitivity = 0.75, and specificity = 0.52; the number of false negatives is therefore very high (25%) (Alessio et al., 1976b).

In recently exposed subjects, there is a latency period of about two weeks before the urinary metabolite increases (Tola et al., 1973; Benson et al., 1976).

After cessation of lead exposure, the excretion of ALA in the urine becomes "normal" relatively quickly. This parameter is therefore not suitable for detecting past lead exposure (Haeger-Aronsen et al., 1974).

For the determination of ALAU, as for all the other urinary tests, it is difficult to obtain 24-hour urine samples or urine samples for precise periods of time, e.g. 4-8 hours. Generally the determination is therefore performed on spot samples. Owing to the different density of daily samples, widely varying levels of the metabolite e.g. from "normal" to "pathologic" can be obtained from the same subject. For an example, see Figure 11, Chisolm et al. (1976). To overcome this difficulty, the sample is currently corrected according to its specific gravity or creatinine. This correction will probably be useful in studies on groups of subjects, but in single subjects it does not permit approximation of the value obtained with respect to the ALA present in 24-hour urine samples (whether expressed in mg/l or mg/24h).

The ALAU values reported in the literature for the subjects not occupationally exposed to lead are below 6mg/l or 4.5mg/g of creatinine. High values of ALAU can also be found in subjects with acute intermittent porphyria.

Various chromatographic and non-chromatographic methods are available for the determination of ALAU. A critical evaluation of some of these techniques has been made by Koels et al. (1974b).

4.2.4 Coproporphyrin in Urine

In subjects under continuous exposure, there is a good correlation between PbB and CPU (Williams et al., 1969; Soliman, 1972; Alessio et al., 1976b).

An excretion of coproporphyrins in the urine (mainly isomer III) beyond the upper normal limits occurs when the PbB levels are slightly higher then those at which an increase in ALAU values occurs. See Figure 10. This phenomenon is also evident in the examination of the dose-response relationship between the two urinary metabolites and PbB (Wada, 1976).

From commencement of exposure to increase in CPU there is a time lag of about 2 weeks in recently exposed subjects (Tola et al., 1973a; Benson et al., 1976). With cessation of exposure, the urinary coproporphyrins return to normal within a few weeks and sometimes within a few days (Saita, 1962).

Urinary coproporphyrin is not a specific test of lead exposure. Increases in the urinary metabolite may occur also in porphyria cutanea tarda, cirrhosis, liver disease, haemolytic anaemia, malignant blood diseases, infectious diseases, and also after consumption of alcohol. However, subjects with severe lead exposure may in some rare cases show normal levels of coproporphyrin in the urine (Saita et al., 1966; Lauwerys, 1975).

The same limitations given for ALAU apply for this test as well.

The validity of CPU (determined on spot samples) to predict different PbB levels is rather modest, so its use as a screening test is limited (Alessio et al., 1976b).

Other porphyrins are not as common in urine, although increased uroporphyrin levels may occasionally be detected, especially in severe cases of lead poisoning (Stankovic et al., 1973).

4.2.5 Haemoglobin and stippled cells

These two tests are only marginally important for the routine monitoring of lead exposure. Haemoglobin and PbB are generally poorly correlated; a reduction in Hb occurs when the PbB level exceeds 100-110µg/100ml (Williams, 1966; Cooper et al., 1973).

In the past, stippled cell count was "an early indicator of abnormal lead absorption", since the appearance of stippled cells precedes the onset of anaemia (Saita, 1962). This test is not used today because it does not accurately reflect the amount of lead absorbed and because the number of stippled cells increases with a much greater time lag than the other biological changes discussed above (Lauwerys, 1975). Furthermore, the test is not specific for lead intoxication since stippled cells may be present in thalassemia, pernicious anaemia and anaemia due to renal insufficiency (Saita, 1962).

4.3 Indicators of Effects in Adult Females

Because of its relatively recent interest, the number of studies of female exposures is rather limited. They generally involve a small sample of subjects with a moderate degree of exposure.

4.3.1 Erythrocyte delta-aminolevulinic acid dehydrase (ALAD)

Studies made on groups of subjects not occupationally exposed have shown that adult females living in the same place and of the same age as a group of male controls had a higher mean value of erythrocyte ALAD activity and a lower mean value of lead in blood (Haeger-Aronsen et al., 1971; Secchi et al., 1973).

In the women it was also observed that the reduction in ALAD activity with age is less marked than in men (Secchi and Alessio, 1974b). The differences found between the two sexes was attributed to a different lead intake with food, wine, and

smoking. Toia (1973), who examined 171 women and 1199 men with PbB levels between 9 and 90µg/100ml, found no consistent differences between the ALAD values of men and women at the same blood levels. Similar results have been obtained in a study of 93 women and 95 men with PbB levels ranging from 8 to 80 µg/100ml (Alessio et al., 1977). From these data it therefore appears that there are no differences between males and females with the same level of internal lead load.

4.3.2 Erythrocyte protoporphyrin

Stuik (1974) has shown that increase in EP occurs in adult females at a lower concentration of PbB than in adult males (for females at a PbB level of 25-35µg/100ml; for males at 35-45µg/100ml), and that the increase in EP was steeper in females with the increase in PbB values.

EP was observed to behave similarly by Roels et al. (1975) in 40 male and 24 female adults with moderate occupational exposure (PbB 50µg/100ml). EP and PbB were closely correlated in the two groups; EP was markedly higher in the women at the same internal lead dose, i.e. PbB. This phenomenon can also be clearly observed in the groups considered in a study by Alessio et al. (1977), see Figure 12, which consisted of subjects with more severe exposure. Similar results are obtained when erythrocyte protoporphyrin is determined with hematofluorimeters, like ZPP.

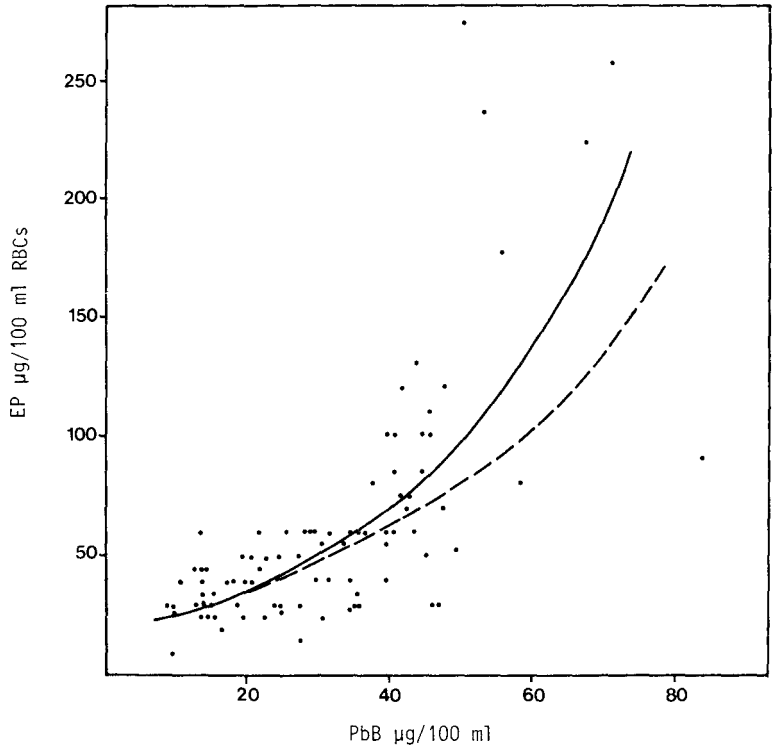
Study of the dose-response relationship does however show that the no-response PbB levels for an increase in EP are 25-35µg/100ml for males and 20-30 µg/100ml for females (Roels et al., 1975).

In women, EP is also well correlated with chelatable lead (PbU EDTA). The regression curve between the erythrocyte metabolite and PbU EDTA, shows that for the same level of internal dose, EP is higher in females than in males, even when the internal dose is measured by chelatable lead (Alessio and Foa, 1976).

In non-occupationally exposed women, the EP levels are higher than in males (Roels et al., 1975; Wibowo et al., 1977).

Figure 12

Relationship between PbB and EP in adult males and females.



_____ females No. 93 r = 0.75

- - - - - - - - males No. 95 r = 0.74

Scatter diagram shows the single females values.

EP determined according to the Schwartz and Wikoff Method.

4.3.3 Delta-aminolevulinic acid in urine (ALAU)

In occupationally exposed women, ALAU and PbB are well correlated. Levels of the urinary metabolite in the women seem slightly higher than in men, at the same PbB level (Roels et al., 1975).

Study of the dose-response relationship shows that the no-response levels for an increase in ALAU are 35-45µg/100ml for males and 30-40µg/100ml for females (Roels et al., 1975). It does not, however, appear that there is a significant difference for ALAU values in non-occupationally exposed subjects in the two sexes.

4.3.4 Urinary Coproporphyrin

Results by Alessio et al. (1977) show that CPU and PbB are significantly correlated. The relationship between the two parameters does not seem to indicate the existence of a difference in behaviour of the urinary metabolite in the two sexes.

From the available data on adult women, the following conclusions can be drawn:

- in adult women a significant correlation exists between the indicators of internal lead dose and indicators of effect, as has already been confirmed in adult men.

- the relationship between indicators of dose and indicators of effect, evaluated with the regression curve and/or the dose-response curve, shows that in the female, the "qualitative" behaviour of the indicators of effect is identical to that observed in males. In males the erythrocyte ALAU undergoes a distinct inhibition in the range of PbB values below 40µg/100ml. The erythrocyte protoporphyrin initially increases rather moderately; then, beyond a PbB level of 40µg/100ml, the increase is very marked. ALAU and CPU increase above normal for PbB values higher than those at which an increase in protoporphyrin occurs. The increase in the two urinary metabolites in relation to the increase in internal lead load is not as steep as the increase in the erythrocyte metabolite.

- There is a clear difference in the "quantitative" behaviour of protoporphyrin (and perhaps of ALAU) in the two sexes at identical levels of internal dose. This phenomenon appears to be due to a greater susceptibility of haemopoiesis to lead in women. The cause of such hypersensitivity might be a relative iron deficiency in women, causing increased alterations in haemopoiesis induced by lead (Stuik, 1974; Zielhuis, 1975a). Synergic action between sex hormones and lead on the enzymatic activity of heme synthesis has also been suggested (Roels et al., 1975).

5.0 CONCLUSIONS

A vast number of tests which permit a sufficiently accurate evaluation of the degree of exposure, body burden and toxic effect are available for monitoring lead workers. Given the advantages and limitations of each test, the choice of indicator or indicators will depend on the type of investigation.

Two tests should be used simultaneously for the periodic surveillance of workers exposed to lead concentrations sufficient to cause alterations in biological indicators close to the "permissible" limits. One test should be designed to indicate internal dose and another to indicate effect. In monitoring individuals, blood tests are preferable to urinary tests, the latter being subject to considerable variation due to differences in urine density. Furthermore, elevation beyond the "normal" limit values of the urinary indicators of effect, (i.e. delta-aminolevulinic acid and coproporphyrin), occurs at internal dose levels higher than those at which an alteration occurs in the blood indicators of effect, (i.e. delta-aminolevulinic acid dehydrase activity of erythrocytes (ALAD) and erythrocyte protoporphyrin).

In general, it is advisable to use blood lead levels and erythrocyte protoporphrin for periodic monitoring as these two tests integrate well. This is not only because one evaluates internal dose and the other the effect but also because blood lead evaluates a momentary situation (present exposure) while the erythrocyte metabolite permits evaluation of body burden and past exposure. These features are important in relation to the fact that industrial levels of exposure are rarely stable, so that PbB alone might give only partial information in cases of non-steady-state exposure. On the other hand, protoporphyrin does not permit assessment of current absorption.

A screening test which is inexpensive, easy to perform, sensitive, specific, precise and accurate should be used to identify subjects with the highest exposure from a group. The percentage of false negatives should be minimal, but too many false positives may give rise to excessive referrals for diagnostic evaluation, cause alarm and overcrowd busy outpatients facilities (Chisolm et al., 1974). Both erythrocyte protoporphyrin and delta-aminolevulinic acid dehydratase comply on the whole with these requirements: both tests have been shown to possess high

predictive validity of the "true situation", i.e. internal lead load measured with PbB.

Protoporphyrin offers the following advantages over ALAD: a) it also permits quantification of situations in which an internal lead load has already caused a marked inhibition of ALAD; b) it can be measured using capillary blood with micromethods which are rapid to perform. The fluorimetric zinc protoporphyrin technique appears to offer a simple, instant and repeatable measurement; c) a higher number of analyses can be performed in the course of the day; d) the sample for analysis can be stored for longer periods of time.

The urinary tests may be used for assessment of the environmental conditions of a place of work on a group basis, although blood tests provide more accurate information. If urinary tests are used, it will be appropriate to take the density of the samples into account, rejecting those with density lower than 1010, or with creatinine concentration below 0.5 g/l.

For a correct evaluation of a group investigation, it will not be sufficient to express the data solely as a mean ($\bar{x}$) and standard deviation (Δ) or range. This procedure can be applied only if the parameter follows a Gaussian distribution, and it will be appropriate to consider the percentage distribution of the data as well (Zielhuis, 1974).

The choice of biological tests must also be made on the basis of the availability of suitable equipment and trained technical staff, the possibility of easy and rapid performance, transport and cost.

The problem of "permissible" levels of biological tests for workers exposed to inorganic lead has been considered by many authors and by national and international bodies responsible for the protection of workers health, and has been discussed at numerous meetings of experts.

In September 1976 a worksnop was organized in Amsterdam under the auspices of the Permanent Commission and International Association on Occupational Health and the World Health Organization, which re-examined the problem of permissible limits for occupational exposure in inorganic lead (Zielhuis, 1977).

At the workshop the following recommended guidelines for PbB based on health criteria were drawn up: "for male workers individual PbB's should not exceed 60µg/100ml in the light of present knowledge available to this group. It is however desirable to reduce individual exposure below this level, taking into account the effects on the haematopoietic system at concentrations above 45-50µg/100ml and on nerve conduction velocity at concentrations between 50-60µg/100ml. The group could not agree on what level should be regarded as a health based permissible level for occupational exposure. So far as female workers of child-bearing age are concerned the risk of harm to the foetus at above mentioned PbB levels is not supported by factual evidence but is based on theoretical possibility. Nevertheless, because of potential effects on the foetus, a safe practice would be to avoid the employment of women of child-bearing age on lead work where blood levels might regularly exceed 40µg/100ml."

In 1978, similar recommendations were also made in the U.S.A. by the National Institute for Occupational Safety and Health (NIOSH, 1978), and by the Italian society of Occupational Medicine and Industrial Hygiene (Foa et al., 1978).

The following is a summary of the main recommendations made in the Italian document which contains many practical suggestions.

For evaluation of risk for individual workers, the following tests are proposed as being the most suitable:

- lead in blood and urinary ALA, to evaluate respectively dose and effect relative to current exposure;

- erythrocyte protoporphyrin to quantify an effect due also to past exposure.

On the basis of increasing values of the biological tests, 4 different classes should be distinguished:

-1st class:	no action
-2nd class:	surveillance
-3rd and 4th classes:	increasingly important action:

the need for action depends on consideration of the fact that subjects with values of the indicators persistently at these levels could develop a pathological condition.

Indications for the exposure of male subjects to lead are given more schematically in Table 6.

The original document gave no values for erythrocyte protoporphyrin since the authors thought it advisable to wait for further data on this. The zinc protoporphyrin (ZPP) values shown in the table are those currently used at the Clinica del Lavoro of Milano.

Table 6

Scheme of medical and environmental measures for increasing occupational exposure to inorganic lead.

Indicators	Classes of exposure			
	I	II	III	IV
PbB µg/100 ml	>40	40-60	60-70	<70
ALAU mg/l	> 6	6-10	10-18	<18
ZPP µg/100 ml	>40	10-110	110-170	<170
Individual measures	Annual check of biological indicators	3-monthly check of biological indicators	Removal from risk; examination and tests as for pre-employment	Removal from job; study and any treatment by specialists
Environmental measures	None	Check of working environment	Technological and environmental improvement necessary	

The document also states that:

- classification is to be made on the basis of a single indicator in the greatest risk class;

- persistence for at least three months of protoporphyrin values higher than those of the class corresponding to PbB and ALAU levels (when an iron deficiency can be excluded) denotes the existence of lead deposits in the organism such as to cause biological effects.

The Occupational Safety and Health Administration, U.S.A., (OSHA, 1978) has issued a standard that requires permissible PbB levels to be progressively lowered to 40 µg/100 g over the next 5 years.

6.0 RESEARCH NEEDS

In spite of the fact that lead is the most extensively studied metal from the point of view of industrial toxicology, further research is still necessary to establish safe permissible limits for exposure. The following are recommendations for further research:

- Standardization of tests. Standardization of analytical methodology would allow a comparison of the studies being carried out by the different laboratories and research workers and would facilitate discussion and application of normal values and permissible limits internationally.

  As a result of the inter-laboratory variability of lead blood levels, the relationship between these levels and other biological indicators cannot be precisely determined.

  The opinions of the various research workers on the usefulness of correcting the results of urinary tests made on spot samples are conflicting. This question should be dealt with to verify whether the corrections made for the individual subject allow a value to be obtained which is sufficiently similar to the value obtained on the same day on 24-hr urine (considering the value expressed as quantity of substance per litre and/or quantity of substance per 24 hours).

  Daily variations in the results obtained from the various biological tests should be investigated as there are very few references to this in the literature (Vigliani and Bonsembiante, 1944).

- Determination of the relationship between external and internal exposure. More extensive studies involving larger groups of subjects and taking particle size and solubility into consideration are necessary for this determination.

- Establishment of better indicators of internal lead dose. Very few studies are available on lead in plasma although diffusable plasma lead may offer the best approximation of the biologically effective body burden. It should, however, be taken into consideration that the plasma fraction is not a constant fraction of the total blood concentration.

  Chelatable lead may provide a more direct measurement of the rapid exchange pool and it may be used as a rough measure of plasma lead concentrations, since it is normally found in plasma and not in cells. It therefore appears necessary to determine the dose-effect relationships between chelatable lead and other indicators.

  The dose and rate of administrations of chelating drugs for estimating the mobile portion of the body lead burden should also be standardized.

- Research on hypersensitivity to lead. The few data available in the literature (Saita and Moreo, 1959; Girard et al., 1967; Albahary, 1972; Saita and Lussana, 1971), indicate that subjects with genetic alterations (thalassemia, haemoglobinopathy, G6PD deficiency) may be hypersensitive to the action of lead. The high incidence of these alterations in some countries of the European Community and the increasing transient population, e.g. immigrants from the Mediterranean area, point to the necessity of a re-examination of the problem.

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This document reviews inorganic lead as related to occupational exposure and the possibilities of the biological monitoring of exposure.

The main route of absorption in occupational exposure is the respiratory apparatus. Disorder in heme synthesis is currently considered the first adverse effect associated with increasing concentration of lead in the soft tissues.

levels as a measure of internal dose, and erythrocyte protoporphyrin as an indicator of effect.

For screening studies an inexpensive test which is easy to perform, sensitive, specific and precise should be used to identify subjects with high exposure. Both protoporphyrin and delta-aminolevulinic acid dehydratase comply with these requirements.

For assessment on a group analysis basis of the environmental condition of a work place, the urinary tests may be used; blood tests, however, provide more accurate information.

Individual blood lead levels in male workers should not exceed 60 µg/100 ml, and in women workers of child-bearing age they should not be higher than 40 µg/100 ml because of the potential adverse effect of lead on the fœtus.

Further investigations are required on the relationship between external and internal dose and standardization of the various biological tests.

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