HEALTH SURVEILLANCE OF WORKERS EXPOSED TO LEAD

W.C.M. ZWENNIS

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GEZONDHEIDSBEWAKING VAN BEROEPSMATIG AAN LOOD BLOOTGESTELDE PERSONEN

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de Rector Magnificus
Prof. Dr. P.W.C. Akkermans M. Lit.
en volgens besluit van het college van dekanen.
De openbare verdediging zal plaatsvinden op
donderdag 8 september 1994 om 13.30 uur

door

WILLEM CORNELIS MARIE ZWENNIS

geboren te Den Haag

PROMOTIECOMMISSIE:

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The studies reported in this thesis were carried out at the former TNO Medical Biological Laboratory, Rijswijk, the Netherlands and financially supported by the Directorate General of Labour of the Ministry of Social Affairs and Employment.

The co-operation of the managements of the industries involved in the studies and of many anonymous employees is gratefully memorized.

Financial support by the TNO Nutrition and Food Research Institute, Zeist, the Netherlands for the publication of this thesis is gratefully acknowledged.

CIP-GEGEVENS KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Zwennis, W.C.M.

Health surveillance of workers exposed to lead: Gezondheidsbewaking van beroepsmatig aan lood blootgestelde personen/W.C.M. Zwennis - Delft: Eburon
Proefschrift Rotterdam - Met lit. opg. - Met samenvatting in het Nederlands.
ISBN 90-5166-407-9
NUGI 735
Trefw: gezondheidsbewaking; arbeidstoxicologie.

'Where shall I begin, please your Majesty?' he asked.
'Begin at the beginning,' the King said, very gravely,
'and go on till you come to the end: then stop.'

Lewis Carroll: Alice's Adventures in Wonderland.

voor Boukje

CONTENTS

CHAPTER 1

INTRODUCTION

1.1 EVOLUTION OF OCCUPATIONAL LEAD EXPOSURE

Lead is one of the longest-known metals. Archeological findings have been made of leaden objects that are about 7,000 years old [Woolley (1984)]. Nowadays, lead still is one of the most extensively applied non-ferrous metals. The total world production in the 1980's was estimated to be about 25.10^6 tons, making it the fifth used metal. Not surprisingly, in many professions workers come into contact with lead or lead-containing materials. The Occupational Safety and Health Administration in the U.S.A. has identified over 120 occupations in which lead is applied, varying from the manufacturing of lead storage batteries to printing and the production of glazed pottery [OSHA (1978)].

Although during the last decades in a number of industrial branches and for certain applications the use of lead has undoubtedly decreased, there still are a great number of workers potentially exposed. From 1981 to 1983 the National Institute for Occupational Safety and Health conducted a survey in the U.S.A. and estimated that almost 1.4 million workers were exposed to inorganic lead or lead compounds [NIOSH (1988)]. In 1992 the TNO Medical Biological Laboratory* (MBL) carried out a survey amongst Occupational Health Services in the Netherlands with regard to the number of workers possibly exposed to various heavy metals. Lead scored highest with 5,000-10,000 workers. The large majority were males [MBL, internal report 92-15, in Dutch]. In the period 1973-1983 the Institute of Occupational Health in Helsinki performed over 55,000 analyses of lead in blood samples in order to screen male workers for exposure to lead. Also about 7500 analyses were conducted in samples obtained from women [Taskinen (1988)].

In the 19th century serious health effects, such as wrist drop, colics and anaemia, often occurred in workers occupationally exposed to lead. A 'lead line' on the gums was frequently observed and was considered evidence of extremely high exposure. However, in this century improvement of hygiene and working conditions, as well as adaptations of industrial processes have resulted in gradually declining levels of exposure and in decreasing numbers of cases of occupational lead poisoning [Davies (1984)].

Nowadays, lead poisoning mainly occurs in a limited number of occupations. Industrial activities with traditionally high levels of exposure are primary and

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* As from January 1st, 1994 the laboratory has merged in TNO Nutrition and Food Research Institute, Zeist, the Netherlands.

secondary lead smelting, manufacture of storage batteries and production of lead-containing pigments. Also welding, thermal cutting and sand-blasting of lead-coated constructions may result in lead toxicity [NIOSH (1992)]. However, Froines et al. (1990) identified altogether 52 types of industry which during inspection showed more than 1/3 of the levels of lead-in-air samples greater than the permissible exposure limit.

In the last decades substantial progress has been made with regard to the knowledge of adverse health effects induced by lead in man. Formerly, investigations were mainly directed to overt clinical aspects of lead exposure such as anaemia. But the application of molecular-biological tests and the advancement in screening methods has focused attention on more subtle adverse effects to systems and organs as on reproduction and kidney function. It has become clear that these effects in man may occur at much lower levels of exposure than initially supposed.

To avoid adverse effects in the general population, various measures have been taken to terminate or decrease the application of lead in several widely used products such as petrol and paints, and to reduce the concentration of lead in food and drinking water. These improved conditions may be illustrated by figures for the intake of lead by the population in the Netherlands. The 'Commission for the Co-ordination of Measurements of Radioactivity and Xenobiotic Compounds' calculated that the average total intake of lead from food, water and air decreased from about 107 µg/day in 1976/78 to about 37 µg/day in 1984/86 [CCRX (1990)].

Health surveillance of lead workers, aimed at preventing the occurrence of adverse health effects, is traditionally based on monitoring the concentration of lead in environmental air (PbA) at the workplace (stationary air sampling, SAS) or in the breathing zone of the workers (personal air sampling, PAS) and/or on biological monitoring (BM) of lead-in-blood (PbB). Sometimes additional analyses are carried out in order to detect possible early effects induced by lead *(Chapter 2)*. However, it has become gradually evident that such programmes do not always meet requirements as to the prevention of health impairment in occupationally exposed workers. Therefore, existing programmes for health surveillance of lead workers have been reconsidered in the light of the results of the present study.

The MBL, where this study was carried out, initiated and performed research in the field of occupational health for the purpose of protecting workers in industry and agriculture from adverse health effects caused by exposure to chemical substances. In this context, health surveys have been performed in which data was collected on the exposure to lead of workers in Dutch lead-processing industries in order to get insight into the prevalence of health risks and the need of preventive measures. The surveys were restricted to metallic lead and inorganic lead compounds. The toxicology of organic lead compounds and the health risks of exposure to these are completely different. Moreover, industrial application of

organic lead compounds is limited. Lead salts that ionize and are soluble in water (e.g. lead acetate) are generally considered as inorganic compounds.

1.2 OBJECTIVE AND OUTLINE OF THE PRESENT STUDY

The objective of the present study was improvement of the quality of health surveillance of lead workers. To that end, the following themes were given attention:

A. The quality of the assay of lead in blood.
B. The significance of the measurement of blood pressure and of haemoglobin in the health surveillance of lead workers.
C. The meaning of BM of lead in blood of workers exposed to insoluble lead chromate.
D. The use of zinc protoporphyrin as a pre-screening method to identify persons for exposure to lead.
E. The usefulness of PbB as an indicator of changes in exposure.
F. The haematological and biochemical effects of chronic exposure to lead acetate according to a study in rabbits.

Ad A. In daily practice, decisions to minimize or even stop further exposure of lead workers, generally are taken on the basis of PbB. Erroneous analytical data may lead to unjustified decisions, which may have radical implications for the employees, as well as for the employer. Therefore, the insufficient quality of the analyses of PbB performed in many laboratories has given reasons for concern and has contributed to the organization of quality-control programmes in several countries. This has enabled participants to improve the performance of their analysis. In order to investigate the quality of the analysis of PbB in the Netherlands, two 'round robin' exercises were carried out. The results are discussed in *Chapter 3.*

Ad B. Effects of lead on blood pressure have frequently been reported. However, they mainly occurred at higher levels of exposure. It was investigated whether the presently observed levels of exposure in the Netherlands make it appropriate to check blood pressure as part of health surveillance of lead workers regularly. The conclusions are presented in *Chapter 4.*

Anaemia is one of the earliest noticed adverse health effects induced by exposure to lead. In order to establish whether the determination of haemoglobin should be part of the protocol for health surveillance, the relation between haemoglobin and PbB in three groups of male Dutch and foreign lead workers was assessed *(Chapter 5).*

Ad C. The role of the solubility of lead chromate, zinc chromate, and potassium chromate on the rate of absorption of lead in the lungs of rats is discussed in *Chapter 6.* In the respiratory tract, metallic lead and many of its salts behave as soluble matter and are readily absorbed into the blood. However, absorption of inhaled lead chromate, which is almost completely insoluble in aqueous solution at neutral pH and in the alveoli, is slow and incomplete. This results in a deposit in lung tissue. The level of PbB remains low, and thus reflects an underestimation of exposure. This and the presence of the deposit may have consequences for the health surveillance of the workers concerned.

Ad D. The assay of PbB is time-consuming and demands qualified personnel and sophisticated equipment. In contrast, the analysis of zinc protoporphyrin (ZPP) in blood is easily performed, might even be done at the workplace and the results are available immediately. For this reason the determination of ZPP has found wide application not only as a useful effect parameter, but also as a pre-screening method to establish whether an increased PbB can be expected in people. In this way unnecessary work and costs are avoided. In *Chapter 7* a method is presented to use ZPP as an alternative for PbB in screening lead workers.

Ad E. In *Chapters 8 and 9* the level of exposure to lead and the results of technical and hygienic measures in two different types of working conditions, aimed to reduce the intake, are reported. It is proposed to apply other measurements, in addition to PbB, to estimate the internal exposure of workers who are exposed to extremely high levels of lead.

The rate of decline of PbB after termination of exposure *(Chapter 8)* or after improvement of working conditions *(Chapter 9)* depends on the duration of previous exposure and on the half-time of Pb in the deposits in the body. This implies that a reduced intake of lead by prolongedly exposed workers is not necessarily reflected at short notice in a decrease of PbB. Therefore, in estimating the effect of hygienic or technical measures in the intake of lead in these workers, other measurements are required.

Ad F. In health surveillance of lead workers, PbB is almost exclusively applied as the indicator of exposure. Additionally, responses of the haematopoietic system to lead, such as an increased level of ZPP in erythrocytes or of δ-aminolaevulinic acid in urine, may be used. In *Chapter 10* the results of a study of chronic exposure of rabbits to lead acetate are presented. The aim of this study was to identify haematological or clinical parameters which could be used as biological-effect parameters for health surveillance of lead workers, in addition to those already available.

A general discussion on the data in *Chapters 3-10* and concluding remarks are presented in *Chapter 11.* As the *Chapters*, are based on separate articles, an

overlap is sometimes inevitable. In the *Annex* a possible protocol for health surveillance of lead workers is outlined. It is based on available protocols into which the relevant results of the studies presented in the *Chapters 3-10* and some additional data from reports in literature are incorporated.

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CHAPTER 2

REVIEW OF THE LITERATURE

2.1 TOXICOKINETICS OF INORGANIC LEAD

Absorption

Lead is a toxic metal that accumulates in the human body. It may be absorbed via the lungs and the gastrointestinal tract. Lead and inorganic lead compounds are not absorbed through the skin, although a slight absortion of lead soaps and of lead naphtenate has been reported [Van Peteghem and De Vos (1974)].

The deposition of lead in the respiratory tract of man exposed to lead dust for two tidal volumes was studied by Nozaki (1966). The concentration of the inhaled fumes was 10 mg/m^3. *Figure 2.1* shows that a smaller air tidal, e.g. during exertion

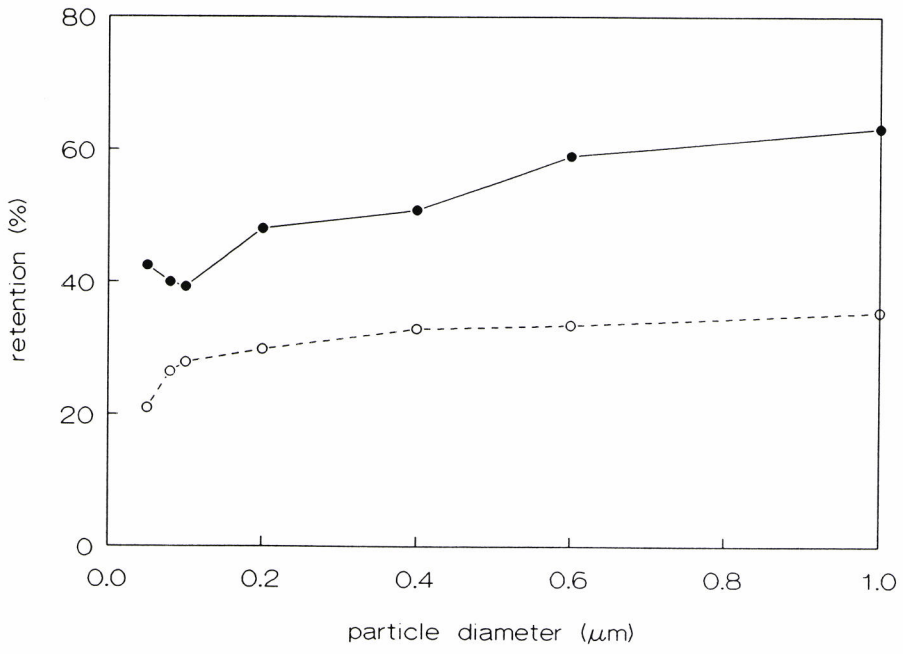

Figure 2.1 Retention of lead in the human respiratory tract.
Lead fumes were inhaled at a concentration of 10 mg/m^3.
——●——: 10 respirations/min, air tidal: 1350 ml.
-----O-----: 30 respirations/min, air tidal: 450 ml.
Data adopted from Nozaki (1966).

as in work, results in a decreased retention. The results were similar to those of Kehoe (1961) who exposed volunteers to levels of 10-150 μg lead oxide/m^3. The extensive balance studies of Kehoe, which were carried out between 1937 and 1972, have been summarized by Gross (1981). Under an average alveolar ventilation of 15 m^3/day for the general population, the retention of inhaled lead particles <5 μm was about 30%. Under occupational conditions, deposition may differ appreciably because depth and frequency of ventilation as well as particle size differ strongly between workers and the general population.

Particles with an aerodynamic diameter <0.1 μm are deposited mainly by diffusion in the gas exchange region of the lung [Heyder et al. (1986)]; these particles are transported to the blood with an efficiency of almost 100% [Hodgkins et al. (1991)]. Deposition of particles >5 μm occurs mainly in the upper respiratory tract, where they are removed to the gastrointestinal tract by mucociliary clearing. This may cause further absorption in the gut. Kehoe (1961) reported that exposure of a volunteer to aerosols of large particles of lead oxide resulted in a substantial increase in faecal excretion of lead. There is no evidence of accumulation of lead in the alveolar part of the lung. In experiments with humans, lead isotopes were found to be transferred almost completely to the blood within 24 h [Chamberlain (1985)]. Thus, lead deposited in the lungs is further absorbed into the blood or is transferred to the gastrointestinal tract.

Gastrointestinal absorption of ingested lead and soluble lead salts is usually less than 10%. The absorption is influenced by factors such as age and the physiological and nutritional status of the subject. In young children it may be as high as 50% [Alexander et al. (1973)].

Absorption by the body does not solely depend on the amount of lead presented to the routes of entry per unit of time, but also on composition and particle size. However, the solubility of lead compounds is by far the most important source of the observed variability in absorption. Therefore, this must in any case be taken into account when considering uptake, distribution and excretion. In *Chapter 6* this aspect is discussed for soluble and insoluble chromates. However, it is also important to note that the solubility of lead compounds in water or buffers is not always representative of the solubility in biological fluids with their abundance of chelating ligands. In many types of industry, exposure to lead is mainly in the form of lead oxide dust.

Absorption of lead is greatly reduced by simultaneous intake of calcium and phosphate [Heard and Chamberlain (1982)]. This may explain the empirical practice for over a century of drinking milk to prevent the symptoms of lead poisoning. However, Stephens and Waldron (1975) concluded that the overall effect of drinking milk is rather promoting absorption of lead from the gut.

Distribution

Lead that is deposited in the alveoli and in the gastrointestinal tract is absorbed into the blood plasma. Within minutes about 94% is taken up by the red blood cells. About 80% is bound to haemoglobin, and about 14% is associated with the erythrocyte membranes [Moore (1988)]. Because of its greater bioavailability, the remaining 6% exerts greater influence on the concentration in other compartments and, thus, influences effects in various organs more than lead bound to haemoglobin. Therefore, the level of lead in plasma should be considered a better estimate of the 'biologically active' fraction than the level in whole blood [Cavalleri et al. (1978)].

From the blood plasma lead is transported to other organs. Although on theoretical grounds between two and seven compartments have been argued, from a practical point of view a two-compartment model may suffice to describe the kinetics of PbB [Schütz et al. (1987)]. The skeleton contains more than 90% of the body burden of lead; in workers occupationally exposed to lead this fraction may even be higher. In this compartment lead is not evenly distributed. There is a pool contained in cortical bone with about 70% of the deposit and another one in trabecular bone [Barry (1975)]. The turn-over of lead in trabecular bone is more rapid than that in cortical bone. The half-time of lead in finger bone, which was considered representative of the whole skeleton, was estimated to be a decade or more. But also a half-time of seven years has been reported. The turn-over in the skeleton causes a continuous release of endogenous lead. Depending on the extent and duration of earlier exposure, it may have an impact on PbB as large as 1 μmol/L [Schütz et al. (1987[a])].

Blood, tissue fluids and some organs, particularly the kidneys and the liver, contain about 8% of the body burden. The half-time of these deposits amounts to 20-40 days.

Lead passes the placental membrane and to some extent the blood-brain barrier. It is also transported to the male reproduction system [Barry (1975)].

Excretion

Lead that enters the blood stream and is not deposited, is excreted almost exclusively through the renal system and the gastrointestinal tract. Balance studies have shown that 54-78% of the daily excreted lead leaves the body with urine [Rabinowitz et al. (1976)]. Excretion into urine occurs by glomerular filtration, followed by partial tubular reabsorption [Araki and Aono (1989)]. During exposure to lead the level of Pb in urine (PbU) increases more than in blood, possibly because PbU depends more strongly on the level of lead in plasma. The latter increases more than PbB at higher level of exposure.

Excretion of lead in faeces occurs via hepatic bile and pancreatic juice and amounts to about 16% of the absorbed quantity [Ishihara et al. (1987)].

There are some other routes of elimination of lead from the body, such as via nails, hair and sweat. However, these are without practical relevance for the assessment of exposure [WHO (1977)].

In *Figure 2.2* a scheme is presented of the uptake, distribution and excretion of inorganic lead in man. The toxicokinetics of lead has recently been reviewed by Skerfving (1993).

2.2 (ADVERSE) HEALTH EFFECTS OF LEAD IN MAN

Lead has found extensive use since classical times. Its high malleability and low melting point, which makes processing relatively easy, and the high resistance to corrosion contributed much to its application. It was used for water pipes, containers, coins, sieves, cosmetics, etc. Nowadays, lead is mainly used for battery grids, tank linings, soldering, roofing material and as sound and vibration damping material.

The consequences of the ignorance of the toxic properties of the metal have been dramatic. Although health hazards associated with the usage of lead were already recognized in the Roman period, widespread and frequently fatal epidemics, usually caused by lead-contaminated drinking water and foods, and by lead-adulterated wines, have occurred repeatedly throughout history [Woolley (1984)].

This potentially toxic metal probably has no useful physiological function [Nolan and Shaikh (1992)]. Organs and systems that are affected by lead are the haematopoietic system, the central and peripheral nervous systems, and the kidneys. Also the male reproduction organ and the foetus are very sensitive to impairment. The effects may range from relatively mild and reversible harm to overt damage and chronic diseases.

The objective of the present *Section* is to provide an overview of the (adverse) effects induced by lead in man. Since the literature on this subject is voluminous, the review will be limited to the more important data with respect to health surveillance of lead workers. It aims also to support the recommendations in the protocol presented in the *Annex*.

Effects on the gastrointestinal tract

Gastrointestinal symptoms caused by lead are constipation or diarrhoea, epigastric pain and indigestion. In a study of Hänninen et al. (1979), 45 lead workers whose mean PbB at the time of the survey was 1.6 ± 0.6 μmol/L, experienced more subjective symptoms than a control group. PbB of none of the former had ever been higher than 3.4 μmol/L.

Colic is considered an early warning of potentially more serious effects likely to occur during continuous industrial exposure. Its occurrence at relatively low exposure levels of PbB is well known. Beritic (1971) reported that in a cohort of

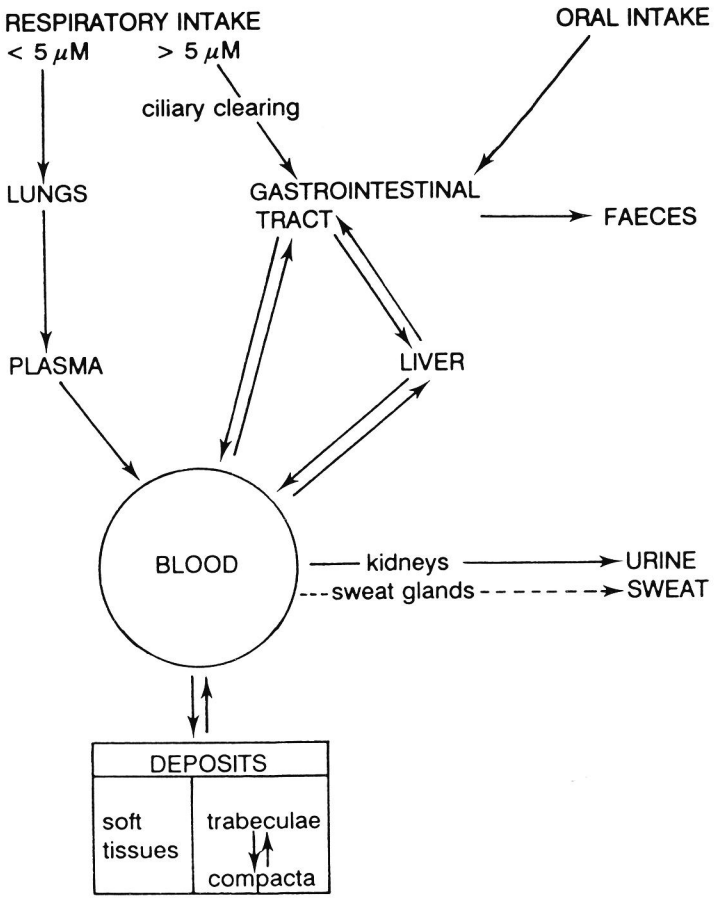

Figure 2.2 Scheme of intake, distribution and excretion of lead in humans. Based on a scheme by Zielhuis (1959).

64 lead workers suffering from colics, 13 had PbB levels between 1.9 and 3.9 μmol/L.

Effects on the central and peripheral nervous systems

Inorganic lead may cause severe effects on the central and peripheral nervous systems. Symptoms such as dullness, restlessness, irritability, muscular tremor, and loss of memory may result from chronic high lead exposure. However, these early changes in the nervous system do not produce clinical effects.

Hänninen et al. (1978) reported for 49 otherwise asymptomatic workers with a mean PbB of 1.5 ± 0.5 μmol/L a diminished neuropsychological performance, as compared with 24 controls. PbB of none of the workers ever exceeded 3.4 μmol/L. The functions impaired most were those dependent on visual intelligence

and visual motor co-ordination. Effects on the central nervous system of 90 secondary lead smelters (average PbB levels about 2.5 μmol/L) were reported by Valciukas et al. (1978). In a 2-years follow-up study Seppäläinen et al. (1983) showed that effects of lead in the peripheral nerves start at levels of PbB of 1.4 μmol/L. They recommend to monitor these effects in prospective screenings. Rosén et al. (1983) established significant sub-clinical effects on conduction velocities in motor and sensory nerves of the lower limbs of lead workers with mean PbB levels in the range 1.8-2.4 μmol/L.

In 133 workers at a battery plant, Triebig et al. (1984) found a negative relation between PbB and the conduction velocity of the sensory nerves in the forearm only in workers with PbB levels exceeding 3.4 μmol/L. Baker et al. (1984) showed that in workers with PbB levels between 1.9 and 2.9 μmol/L effects on the central nervous system appeared well before effects on the peripheral nervous system became manifest. Compared with a control group, Jeyaratnam et al. (1986) observed a poorer performance in several neuropsychological tests for 49 lead workers with a mean PbB level of 2.35 ± 0.7 μmol/L.

Muijser et al. (1987) tentatively concluded that exposure to high levels of lead (mean PbB was 4.0 μmol/L) for a few months resulted in a partly reversible decrease in motor conduction velocity, whereas sensory conduction velocities were not depressed (details of exposure levels of the workers are described in *Chapter 8*). Exposure for more than six months may result in a decrease in motor conduction velocity, which recovers only several years after termination of the exposure [Corsi et al. (1984)].

Conclusion. Although in several studies no effects of lead on the central and peripheral nervous system were established, the results of many other studies show that impairment may already occur at levels of PbB of about 1.5-2 μmol/L.

The studies on effect of lead on the nervous system have been reviewed by Seppäläinen (1988).

Nephropathy and effect on blood pressure

Nephropathy, ultimately leading to kidney failure, has been recognized as an effect of relatively high exposure to lead since long [Landrigan (1989)].

Effects of lead on renal function have been assessed by applying various parameters as indices of tubular or glomerular function. However, clinical signs, such as the level of urea in blood or creatinine in serum, or a decreased basal glomerular filtration rate, are only suitable for detecting effects on the kidneys when the greater part of the renal function has already been impaired [Bernard and Becker (1988)].

Frequently, proteinuria is considered an early and sensitive indicator of renal damage, but the measurement of excreted specific proteins is more indicative of kidney damage than measurement of total protein. Examples are the increased

excretion of retinol-binding protein and of β2-microglobulin in urine as early indications of tubular or glomerular function impairment, respectively [Lauwerys and Bernard (1987)].

In 25 lead workers Buchet et al. (1980) found no relationship between renal function and level of PbB in the range 1.6-3 μmol/L. Verschoor et al. (1987) examined in 244 workers the influence of lead exposure on the urinary excretion of several proteins. They concluded that some changes in tubular parameters may exist at levels of PbB below 3 μmol/L and that at these levels tubular parameters are more affected than glomerular parameters.

In a study of renal function in the general population, Staessen et al. (1992) found that in males creatinine clearance was inversely correlated with PbB (geometric mean 0.55 μmol/L) and ZPP levels. There were also positive correlations between creatinine in serum and the levels of PbB and ZPP. Compared with control groups, Gerhardsson et al. (1992) found no signs of tubular or glomerular malfunction in 70 active (PbB between 0.24 and 2.3 μmol/L) and 30 retired lead workers who experienced long-term exposure.

Conclusion. The extent to which low to moderate exposure to lead contributes to impairment of renal function is not clear, as available data are conflicting and not sufficient in number to allow a proper judgement.

It has long been hypothesized that hypertension is a consequence of nephropathy [Bernard and Becker (1988)]. Until recently, epidemiological and experimental results were inconsistent. However, there is now evidence that lead has direct effects on kidney arterioles and processes related to calcium metabolism, resulting in hypertension.

Harlan (1985) analysed data from a National Health and Nutrition Examination Survey in the U.S.A. A significant relationship was found between PbB (levels <1.4 μmol/L) and blood pressure in the general population. After correction for several confounding factors, Weiss et al. (1986) established a relationship between systolic blood pressure and PbB in three groups of males with mean PbB levels of <1, 1-1.4 and >1.4 μmol/L, respectively. In a study carried out in the Netherlands, De Kort et al. (1987) found an increased prevalence of potential hypertension in 53 lead workers with PbB levels between 2.1 and 2.5 μmol/L. PbB of the control group was about 0.4 μmol/L.

The studies on nephrotoxicity and hypertension have recently been reviewed by Bernard and Becker (1988) and by Nolan and Shaikh (1992).

Reproduction toxicity

Evidence of the deleterious effects of lead on human reproduction dates back to ancient Rome. It has been suggested that lead in wine and food even caused the

declining of populations. Lead has long been known as a spermicidal agent and as an abortifacient.

Compared with a control group, Wildt et al. (1983) observed subtle adverse effects on the sperm quality in 14 workers with an average PbB level of 2.2 µmol/L (range 1.6-3.2 µmol/L), indicating a decreased function of the genital glands.

Assennato et al. (1986) compared the sperm production or sperm transport in 18 battery workers (mean PbB: 3.0 µmol/L; SD: 1 µmol/L) and 18 cement workers (mean PbB: <1 µmol/L). Possibly confounding factors (smoking, alcohol and coffee consumption, frequency of intercourse) were not significantly different between the two groups. The levels of the various sex hormones in both groups were similar. However, compared with the control group, a significantly different sperm count or sperm transport was found in the exposed group.

Coste et al. (1991) performed a cohort study on 229 workers at a battery factory (mean PbB: 2.2 µmol/L; 19% of the workers had a PbB >2.9 µmol/L) and a control group of 125 non-occupationally exposed males. In a follow-up of about five years lead exposure did not appear to be significantly associated with a reduction in fertility as expressed in live births for couples. The negative results were explained by the moderate levels of exposure, but also by the low predictive value of several semen abnormalities for pregnancy outcome. In the study no confounding factors with regard to the women were considered.

The effect of lead on the fertility of 74 workers at a battery plant (mean PbB: 2.3 µmol/L; range 1.1-3.6 µmol/L) was assessed by Gennart et al. (1992). Compared with a matched control group, in the study a tendency toward decreased fertility with increasing period of exposure, evaluated as birth experience of their wives was observed. However, a significant relation between PbB levels and fertility could not be identified.

Conclusion. Koëter et al. (1989) reviewed the literature on effects of lead on human reproduction capacity. They concluded that there was sufficient causal evidence that lead negatively affects spermatogenesis. Aberrations may occur in sperm volume, sperm count and morphology. These effects appear to be reversible. The average no-adverse-effect level of PbB is about 1.7 µmol/L. Studies on effects of lead on the endocrine system related to spermatogenesis were considered inadequate.

As lead passes unimpededly through the placental membrane from mother to child, the lead burden of the foetus is determined by the lead burden of the mother [OSHA (1978)].

In several longitudinal epidemiological studies it has been established that foetal neurological damage may occur at levels of PbB only slightly higher than those in the general population, in particular during the first weeks of pregnancy. Bellinger et al. (1984) studied the relationship between lead levels in umbilical cord blood

and both the Mental Development Index (MDI) and Psychomotor Development Index in six month old infants ('The Boston Study'). At birth PbB levels were between 0.5 and 1.4 μmol/L. PbB in cord blood is about 80% of that in maternal blood, implying that PbB of the mothers was between 0.6 and 1.8 μmol/L. Higher levels of PbB in cord blood were associated with lower MDI. In follow-up studies of the same children at the age of 6, 12, 18 and 24 months, the differences in MDI appeared to persist. This suggests that the impairment had already occurred in the early stages of pregnancy.

Dietrich et al. (1986) studied the behavioural development and PbB levels in 185 new-born children. As in the Boston study, adverse effects in the MDI test were observed at the age of 6 and 12 months at mean levels of PbB of 0.4 ± 0.2 μmol/L. It was concluded that pre-natal exposure had a greater effect than exposure after birth and that the effects in male children were much greater than in female children.

Conclusion. There was convincing causal evidence of subtle damage to the central nervous system in offspring of women with levels of PbB, that were only slightly higher than those in non-occupationally exposed women [Koëter et al. (1989)]. Therefore, pregnant women and women in reproductive age should be considered as a group at risk.

Genotoxic effects/carcinogenicity

Thomas and Brogan (1983) reviewed studies on the effects of lead on the prevalence of chromosomal damage. In ten studies an increased incidence of chromosomal damage was found. However, six other studies failed to establish such an association. Therefore, the question as to whether chromosomal abnormalities are induced by lead cannot be answered yet [WHO (1987)].

There is only limited information available with regard to carcinogenicity of lead and lead compounds in humans. The WHO classifies lead and lead compounds in group 2B ('inadequate evidence of carcinogenicity for humans, but sufficient evidence in experimental animals') [WHO (1987)]. However, in a review article, Magos (1991) suggested that the small increased risk observed in some human studies gives rise to concern about human carcinogenicity.

The adverse effects of lead which have been discussed in this *Section* so far, often reveal themselves as a result of past exposure at levels of intensity and duration which nowadays do not occur any more. This implies that the data of present exposure need not merely be applicable to establish valid response/effect relationships with PbB.

Effects on the haematopoietic system

The parameters involved in the interference of lead with haemoglobin synthesis are discussed in *Section 2.3 and in Chapter 5.*

2.3 MONITORING OF LEAD-EXPOSED WORKERS

Monitoring of lead in environmental air

Berlin et al. (1984) defined environmental air monitoring (EM) as 'the measurement and assessment of agents in air at the workplace and evaluation of environmental exposure and health risks to an appropriate reference'.

EM of lead aims at estimating the exposure by measuring levels of lead dust or aerosols in air at the workplace. These measurements may be performed in two ways.

Firstly, air is sampled at fixed spots at the workplace. This is called 'stationary air sampling'. The method is mainly applied to identify sources of emission and to investigate trends in concentrations at particular places and in time. However, it gives little information on the actual exposure of a worker.

Secondly, air is sampled in the breathing zone of a worker. This method (personal air sampling, PAS) estimates the actual intake of lead but takes also into account the mobility and the working style of the subject. These largely affect the concentration in the breathing zone and thus the intake of lead. Therefore, PAS has the advantage that it provides an estimate of the exposure of the individual worker.

Biological monitoring of lead in blood

Biological monitoring (BM) has been defined as 'the measurement and assessment of workplace agents or their metabolites either in tissues, secreta, expired air or any combination of these to evaluate exposure and health risk compared to an appropriate reference' [Berlin et al. (1984)].

It is the biologically active fraction of the body burden, i.e. the lead in plasma, which is considered to cause most of the (adverse) health effects. However, practical reasons do not allow the reliable determination of Pb in plasma. Therefore, the level of PbB is generally applied as an indicator for the assessment of the exposure and estimation of health risks. PbB reflects both recent and past external exposure. In a steady state exposure a relationship exists between external exposure, and thus intake, and the body burden or internal dose.

Relations between PbB levels and the occurrence of several types of adverse effect, particularly on the haematopoietic system, have been established [Zielhuis and Wibowo (1978); Alessio and Foà (1980)]. Consequently, biologically permissible levels of exposure are generally expressed in PbB.

An advantage of applying PbB, instead of other parameters to estimate intake of lead, is that its level depends on all sources, including leisure, smoking and hand-to-mouth contact. Sources outside work, like food, traffic and hobbies, are generally negligible compared with occupational exposure. People living in urban communities show only slightly higher levels than those living in rural areas. The level of PbB in non-occupationally exposed populations (the reference value) is generally lower than 0.75 μmol/L.

A limitation of BM of lead in blood is that the sampling requires an invasive intervention. This may form an ethical problem and an inconvenience and, albeit very small, a health risk to the subject.

When a lead worker is no longer exposed, his PbB level decreases with a rate which depends on the kinetics of lead in the deposits. O'Flaherty et al. (1982) concluded that the decay depends on the length of exposure and on the value of PbB at the time of suspension of exposure. Schütz et al. (1987) estimated that newly employed workers may display a decay of PbB from 3.0 to 2.0 μmol/L in less than a month, whereas in workers with much longer times of employment, and thus larger deposits of lead in the body, it will take more than a year to reach such a level.

Biological monitoring of lead in urine

As has been mentioned before, part of the absorbed lead is excreted in urine. Thus, levels of lead in urine (PbU) may be used in biological monitoring, particularly to detect recently absorbed lead. It is surprising, however, that in health surveillance of lead workers PbU is hardly used as an index of lead intake.

During prolonged exposure, PbU rises to reach a steady state after about two weeks [Tola et al. (1973)]. A short heavy exposure of volunteers to lead dust results in an exponential correlationship between PbB and log PbU [Schütz and Skerfving (1976)]. PbU remains increased for a long time after termination of exposure.

There is a considerable interindividual variation in PbU at a certain PbB level. The PbU level seems inversely related to the urine flow. But also a circadian rhythm has been established in the excretion [Aono and Araki (1988)]. To adjust for these effects on the urinary concentration of Pb, the analysis should preferably be carried out in samples collected over a period of 24 h. Also normalization with regard to specific gravity or the concentration of creatinine* may be required.

In practice, BM of PbU should preferably be used as a screening method in addition to the analysis of PbB. The latter being the first choice [Lerner (no year)]. A problem is that urine samples may be easily contaminated by hands and clothing, particularly when voiding occurs at the workplace.

Monitoring of lead, whether environmental air monitoring or biological monitoring, plays a crucial role in health surveillance and health maintenance of man at the workplace. It is also important for the assessment of personal hygiene and the efficacy of healthy work practices.

An important goal of EM is the determination of the levels of emission near sources. Also the compliance to permissible limit values may be monitored. The

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* The quantity of creatinine which is excreted in urine per unit of time is grosso modo constant and largely independent of the volume of the urine.

method yields the actual concentration present at the time of measurement. BM is more directly related to adverse health effects because it indicates the bioavailability of the metal in the body, instead of the potential external exposure which is estimated by EM. It is clear that EM and BM should not be regarded as alternatives. They are in fact complementary and should preferably be carried out in parallel to assess both exposure and potential health effects.

Biological-effect monitoring

In addition to monitoring of lead, particular parameters may be measured to assess biological effects on organs and systems. This approach is known as biological-effect monitoring (BEM).

Zielhuis and Henderson (1986) defined BEM as 'the measurement and assessment of early biological effects, of which the relationship to health impairment has not yet been established, in exposed workers, to evaluate exposure and/or health risk compared to an appropriate reference'.

A number of tests are available to measure various haematological and biochemical effects induced by lead. In establishing the relationship between PbB and these effects several definitions may be appropriate [Zielhuis (1977)].

'Effect' refers to a particular biological phenomenon in an *individual*, induced at a specified PbB level. The *'no-detected-effect level'* is the threshold level below which such an effect has not been reported. *'Response'* refers to the relative frequency of occurrence of a specified reaction in a *group of subjects* at a given dose of PbB. At increasing exposure the intensity of the effect increases and also the percentage of people in a population at risk showing such an effect.

The term *'critical effect'* has frequently been used to denote the earliest adverse or undesirable effect in an organ. A *'critical organ'* is defined as that organ which first shows critical effects under specified circumstances of exposure. The critical organ for effects of lead in the haematopoietic system is bone marrow [TGMT (1976)]. At exposure levels lower than those resulting in critical effects, other effects may appear that do not impair functions and are not considered as adverse but which are still evident in biochemical or other tests.

Individuals differ in dose-effect/response relationships with regard to threshold level and slope, i.e. there is a considerable interindividual variation in effects at certain PbB levels [Gibson et al. (1968)]. In *Figure 7.1 (Chapter 7)* this is illustrated for the effect parameter zinc protoporphyrin. Thus, subjects may show symptoms or even signs of toxicity at relatively low PbB, while others show hardly any symptoms at relatively high PbB. It has been suggested that these differences are caused by variations in the binding of lead to a particular protein in the red blood cell and in the capacity for synthesizing this protein [Raghavan et al. (1980, 1981)]. Also, in hereditary porphyrias an increased sensitivity to lead, reflected in a decreased level of δ-aminolaevulinic acid dehydratase (see below), has been observed [Batlle et al. (1987)].

Because exact dose-response relationships and no-response levels cannot be determined, one has to rely on practical approaches [Zielhuis (1975)]. In practice dose-effect/response curves are only applicable when they meet requirements as to sensitivity, i.e. the slope of the curves should not be too small. In this respect, it should also be mentioned that these relationships should not be used in incidental or short-term exposure but only after an equilibrium between intake and uptake has been attained.

In the following, the characteristics and dose-response/effect relationships are discussed of the parameters related to effects of lead on the haematopoietic system. These relationships have been studied extensively.

Most studies on effects of lead on the haematopoietic system date from the 1970's and earlier. More recent publications on this subject are scarce, however, its data are not in defiance of older results. Extensive reviews have been published by Zielhuis (1959); WHO (1977) and Moore (1988).

Already in the 19th century haematoporphyrinuria was observed in leadpoisoned rabbits [Stokvis (1895)]. Following absorption, a small part of the lead is transported to bone marrow where it interferes with several enzymatic steps of the biosynthesis of haem and globin in young erythrocytes. In particular, cytoplasmatic enzymes with functional sulphydryl (-SH) groups are highly sensitive to inhibition by lead. These effects are currently considered as the first signals in man associated with an increased body burden of lead. Several of the effects can be detected readily, prior to the onset of other, more serious symptoms. *Figure 5.1 in Chapter 5* shows a rough scheme of the interference of lead with haemoglobin synthesis.

Inhibition of δ-aminolaevulinic acid dehydratase (ALAD)

The inhibition of ALAD in erythrocytes is currently considered the most sensitive indicator of human exposure to lead [Bernard and Lauwerys (1987)]. Only excessive consumption of alcohol and smoking of cigarettes gives a comparable effect. The logarithm of the ALAD activity is negatively and linearly related to the PbB level. Under good analytical conditions correlation coefficients are between –0.8 and –0.9 [Tola (1973)]. In *Table 2.1* dose-response relationships are presented of a cohort of 221 male lead workers.

TABLE 2.1 PERCENTAGE OF ADULTS WITH >40% AND >70% INHIBITION OF THE AVERAGE ALAD ACTIVITY IN CONTROLS (PbB ≤0.70 μmol/L)[a]

PbB (μmol/L)	n	>40%	>70%
≤0.70	-	-	-
0.71-1.20	30	13	3
1.21-1.65	26	62	12
1.66-2.15	32	97	22
2.16-2.60	53	100	92
2.61-3.10	37	100	92
3.11-3.57	43	100	95

a. From Zielhuis (1975).

Berlin and Schaller (1974) standardized the method of Bonsignore et al. (1965) for the assay of ALAD in blood. In contrast with the original method of Bonsignore, this 'European Standardized Method' is applicable to levels of PbB as high as 3 μmol/L. Despaux-Pagès et al. (1986) developed another method for the analysis of ALAD which also may be used in screening lead workers. Both modifications allow measurement of ALAD inhibition by lead over a wider range of PbB than the original method. A disadvantage of the analysis of ALAD is that it has to be carried out within hours after blood sampling [Berlin et al. (1977)]. Haeger-Aronsen et al. (1974) showed that in subjects who have been exposed to lead to a moderate degree, ALAD rises only slowly to normal levels after termination of exposure.

Increased excretion of δ-aminolaevulinic acid in urine (ALAU)

Inhibition of ALAD leads to a metabolic block and accumulation of ALA in blood plasma. As a result the substrate is excreted in urine. The concentration of ALAU has been used as an effect parameter in occupational lead exposure for a long time, because the increased concentration of ALAU correlates with the level of lead in blood. However, there is a threshold for the increase of ALAU at a level of PbB of about 2 μmol/L [WHO (1977)]. Therefore, measurement of this parameter is only appropriate at higher levels of exposure. The correlation coefficient between PbB and ALAU is low, about 0.4 [Tola et al. (1973)].

In a cohort of 207 male workers, the percentage with ALAU >5 mg/L urine (the upper limit of the reference value) and with ALAU >10 mg/L (considered the acceptable value for lead workers) was calculated. The results are shown in *Table 2.2*.

In recently exposed subjects, there is a latency period of about two weeks before ALAU increases [Benson et al. (1976)]. After cessation of exposure, the level of ALAU shows a similar decrease as PbB. Therefore, this parameter does not reflect any previous exposure that is not also detectable by means of measurement of PbB [Alessio et al. (1981)].

TABLE 2.2 DOSE-RESPONSE RELATIONSHIP BETWEEN PbB AND ALAU IN ADULT MALES[a]

PbB (μmol/L)		ALAU (mg/L)	
	n	>5	>10
0.53-1.0	17	0	0
1.01-1.49	27	0	0
1.50-1.95	36	14	3
1.96-2.45	55	33	11
2.46-2.93	38	74	37
2.94-3.38	34	88	50

a. From Zielhuis (1975).

Accumulation of zinc protoporphyrin in erythrocytes (ZPP)

As a result of the interference of lead with iron transport in the bone marrow, accumulation of the substrate protoporphyrin IX takes place in erythrocytes, as the complex zinc protoporphyrin. This, and the application of ZPP as an effect parameter to screen people for an increased level of PbB, is discussed in more detail in *Chapter 7* [see also Labbé and Rettmer (1987)].

In males the level of ZPP starts to increase clearly at levels of PbB around 1.5-2 μmol/L. Females show an increase already at levels of PbB of about 1.3-1.5 μmol/L, and thus are more vulnerable than males. Therefore, to observe a change in concentration of ZPP at these low levels, the determination should preferably be carried out in a longitudinal programme. Also one has to reckon with the lower sensitivity and specificity of ZPP at lower PbB levels *(see Chapter 7)*.

Lilis et al. (1977) postulated that ZPP reflects the average exposure to lead over several months, whereas PbB reflects also short-term exposure. In workers with a long history of lead exposure, ZPP may remain high for many years. It has been suggested that this is caused by a continued negative effect on haem synthesis by lead released from deposits into the blood. Therefore, ZPP is considered a better estimate of the 'active' body deposit of lead than PbB. In fact, when there is no anaemia induced by iron shortage or by a disturbance in the utilization of iron, high levels of ZPP in subjects with past exposure to lead are evidence of a still active lead deposit [Alessio et al. (1981)]. After cessation of exposure, ZPP returns to normal more slowly than other effect parameters.

There are indications that ZPP correlates better with particular effects on kidney function [Lilis et al. (1980)] and on the nervous system [Lilis et al. (1985)] than does the PbB level. However, the toxic effects of lead on the nervous system are unrelated or only secondarily related to alterations in haem synthesis [Moore et al. (1987)].

Increased excretion of coproporphyrin in urine (CPU)

The inhibition of coproporphyrinogen oxidase within the mitochondria by lead results in an increased excretion of the substrate coproporphyrin (CP) in urine. This excretion parallels that of ALA. But the specificity of CPU to predict the level of PbB is more modest than that of ALAU, making its application as a screening test of limited value [Alessio et al. (1976)]. So measurement of CPU does not result in additional information relative to monitoring ALAU. However, Omae et al. (1988) reported that after separation by chromotography of the CP isomers CP-I and CP-III, analysis of CP-III is sufficiently sensitive and specific for the early detection of lead exposure. The excretion of CP-III in urine induced by alcohol abuse should be taken into account.

Decreased concentration of haemoglobin (Hb)

It is well known that anaemia, which in adults is of the normocytic type, may occur in lead poisoning *(Chapter 5)*. Several mechanisms are involved in its

development. Lead induces a marked inhibition of haem and globin synthesis and also shortens the survival of red cells [Moore et al. (1980)].

In many studies a decrease of the Hb concentration was observed in workers with PbB levels >2 µmol/L. But the failure to find a dose-response relationship between the PbB and Hb levels in several investigations may also reflect the non-specificity of this parameter, as well as the great interindividual variation in its sensitivity [Moore (1988)].

Inhibition of pyrimidine 5'-nucleotidase in erythrocytes (P_5N)
Lead inhibits the activity of the enzyme P_5N which is present in erythrocytes and catalyses the dephosphorylation of pyrimidine 5'-monophosphate. This disturbance of porphyrin metabolism is considered useful for detecting exposure to lead over a wide range as the inhibition is proportional to the level of PbB. It starts at about 0.5 µmol/L and is complete at about 5 µmol/L. Ichiba and Tomokuni (1988) established a correlation coefficient of –0.82 in a study of 77 lead workers with PbB levels between 0.8 to 4.6 µmol/L. In a study on the inhibition of P_5N in 16 lead workers and a control group, Cook et al. (1986) observed a similar correlation as between PbB and ZPP.

Lead usually affects systems and organs gradually. Moore and Goldberg (1985) consider a single effect on the haematopoietic system only as an indication of a change in function. All factors together may, however, represent a more serious health impairment.

Table 2.3 presents an overview of the onset of haematological and other adverse effects which have been discussed in *this Section* and *in Section 2.2*. The wide variations in individual sensitivity and the non-specific character of these parameters render it difficult to define exact PbB levels for the appearance of these effects. Thus, if it is suspected that they are induced by lead, this must be confirmed by an increased level of PbB and/or by additional measurements. This implies also that the PbB level of a subject does not reflect his/her condition of health, but only the risk to health impairment.

TABLE 2.3 NO-EFFECT LEVELS OF LEAD IN BLOOD OF ADULTS*

PbB (μmol/L)	Effects	References
3.5	Effects on gastrointestinal tract (Colics, constipation, diarrhoea)	Hänninen et al. (1978)
3	Nephropathy	Verschoor et al. (1987)
2.5	Anaemia	Moore (1988) See also Chapter 5
	Hypertension?	De Kort et al. (1987)
2	Effects on central nervous system	Baker et al. (1984)
	Increased excretion of ALAU	WHO (1977)
	Increased excretion of CPU	WHO (1977)
	Impaired spermatogenesis	Koëter et al. (1989)
	Increased level of ZPP in blood (males)	Zielhuis (1975) See also Chapter 7
1.5	Decreased conduction velocity in peripheral nerves	Seppäläinen et al. (1983)
	Effects on central nervous system of foetus	Köeter et al. (1989)
	Increased level of ZPP (females)	Zielhuis (1975)
1	Inhibition of P_5N	Ichiba and Tomokuni (1988)
0	Inhibition of ALAD	Baloh (1974)

* The values are approximated 'no-response levels', i.e. the effects are observed in <5% of the population studied.

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CHAPTER 3

QUALITY CONTROL IN THE ASSAY OF LEAD IN BLOOD

The extent of exposure to lead and the concomitant occurrence of risks to health of people is generally based on the level of lead in blood (PbB). In this respect, the legislation in the Netherlands, as well as in other countries of the European Community, of the so-called 'Lead Decree' requires, among other things, the regular measurement of lead in blood of occupationally exposed workers. Also for the establishment of the relation between exposure and adverse responses/effects in lead workers the measurement of PbB is decisive. Therefore, there is an increased necessity for a reliable assay of this parameter. To obtain insight into the quality of the assay of PbB in the Netherlands, two interlaboratory surveys were organized. Almost all laboratories that determine PbB more or less regularly took part. The outcome of these inspections was that, on the whole, the quality of the analysis needed improvement in terms of precision and accuracy.

3.1 INTRODUCTION

In the last decades the interest in scientific studies on the relationship between (increased) exposure to metals and the associated health hazards has grown considerably. However, extensive investigations have clearly shown that the analytical equipment available today does not guarantee correct results of the determinations of metals in biological samples. These analyses are an essential part of the studies. Sometimes determinations in identical samples, within one laboratory or in different laboratories, yield widely different results [Parsons (1992)]. This fact has been recognized earlier for clinical-chemical determinations [Jansen et al. (1977)]. Possible causes are the complex and often inhomogeneous composition of the samples to be analysed as well as the application of different methods and inadequate analytical techniques.

In various countries the problem concerning has led to the setting up of round robin programmes for clinical-chemical analyses. These programmes aim at standardize and improve the quality of the results of the participating laboratories by comparing their results. In the United States, the Centers for Disease Control in Atlanta has a central role in such extensive programmes [Cooper (1975)]. In the Netherlands, quality control programmes for e.g. haematological, serological and clinical-chemical determinations are carried out by the 'Stichting Kwaliteitsbewaking Ziekenhuislaboratoria' (SKZL, Quality Control Foundation for Hospital Laboratories). Some suppliers of diagnostics also organize quality programmes aiming in principle at their own products.

It is estimated that in the Netherlands each of 15-20 laboratories performs on the average 250-300 analyses of PbB per year. However, only a few laboratories participate in continuously running external quality control programmes. Incidentally, samples are exchanged between laboratories, or concise programmes organized. In most laboratories, however, the necessary continuity in external quality control is lacking.

It is desirable that this situation is improved. In particular for the assay of lead in blood, because in the Netherlands, as well as in the other countries of the European Community, an EC-directive has become legally binding [EC (1982); Labour (1988)]. This so-called 'Lead Decree' indicates which measures should be taken in lead-processing industries in order to prevent health hazards to employees by exposure to lead and its inorganic compounds. One of the measures comprises the determination of the concentration of lead in blood. Depending on the particular lead concentration found in a group of workers or in an *individual* worker, additional measures should be taken, i.e. repetition of the determinations, or reduction (or where appropriate suspension) of the exposure.

The introduction of the Lead Decree implies that the results of assays of lead in blood may sometimes have radical and far-reaching financial and social consequences for employer and employee. If too high PbB values are reported, it may lead to drastic measures, such as technical changes or adaptations of processes and ultimately to unnecessary removal of the worker from his job. In the reverse case that values are reported too low, measures to decrease exposure will probably be omitted, possibly leading to hazardous situations. Although generally valid, the Lead Decree makes it even more imperative that the results of the determinations meet standards with regard to reproducibility and accuracy. In the United States the Occupational Safety and Health Administration has issued criteria for approving laboratories for PbB analysis. These include the requirement that the result of a PbB control analysis is performed within 0.3 μmol of the mean of all results (under the condition that the mean is less than 1.9 μmol/L) [OSHA (1991)].

In this perspective, two interlaboratory surveys into the quality of the determination of lead in blood as it was carried out in the Netherlands were organized in 1986/87. Including the MBL, almost all Dutch laboratories performing the analysis more or less regularly took part.

3.2 QUALITY CONTROL OF METAL DETERMINATIONS

The purpose of internal control of metal determinations in a laboratory is to spot erroneous results and trends in good time. It is not strictly necessary that the concentration of the element to be determined in the samples is known accurately.

External quality control is carried out on samples in which the concentration of the element to be determined is not known (in advance) in the laboratory. This method allows a retrospective check of the results. Abroad, external quality inspections (so-called interlaboratory surveys or intercomparison programmes) of

procedures for metal determinations are carried out regularly. Some of these surveys run continuously.

Paulev et al. (1978) reported the results of an interlaboratory comparison of lead and cadmium in blood, urine and aqueous solutions in which five laboratories participated. The coefficient of variation (CV) of the results of lead measured in two samples were 12.3 and 24.7%.

Saltzman (1985) collated the results of three programmes for lead determinations carried out in the United States between 1973 and 1983. At a mean lead concentration of about 2.5 µmol/L the results of 28, 54 and 237 laboratories fell within a CV of 10, 13 and 19%, respectively. In another round robin programme with approximately 30 participants, the mean interlaboratory CV of the analysis of PbB decreased between 1975 and 1985 from 19.2 to 5.5% [Taylor and Briggs (1986)].

In an international interlaboratory survey two bovine blood samples were analyzed by 25 participants. The average results (0.27 and 1.2 µmol/L, respectively) deviated about 10% of the reference values obtained by mass spectrometric isotope dilution analysis [Subramanian and Stoeppler (1986)].

Sugita et al. (1991) reported results of a quality control programme with 153 participants. Between 1980 and 1987 the standard deviation of the mean 'evaluation score' (a measure of the technical level of the analysis of PbB) decreased from about 25 to 17%. In an internal quality control programme of the analysis of PbB, running over a period of five years, Schaller et al. (1990) obtained a CV of 6.7%.

Parsons (1992) reported that the overall performance of the assay of PbB has improved steadily since the 1970's. Among other things, this is attributed to participation in external quality control programmes.

At present continuously running quality control programmes for heavy metals are organized in several countries. In Europe these programmes are available from:

- Danish National Institute of Occupational Health. Copenhagen, Denmark.
- German Society of Occupational and Environmental Medicine. Erlangen, Germany.
- Queen Elizabeth Hospital. Birmingham, England.
- Robens Institute of Industrial and Environmental Health and Safety, University of Surrey. Guildford, England.

Since 1973, the Clinical Chemistry Department of the Queen Elizabeth Hospital jointly with the Health and Safety Executive in London, carries out the 'U.K. External Quality Assessment Scheme' (UK EQAS), among others for the determination of lead in blood. Between 1979 and 1985 the number of participants in the programme increased from 50 to 120; in this period the mean CV of the results of the participating laboratories decreased from about 12% to about 6% [Bullock et al. (1986)].

3.3 DESIGN OF THE SURVEYS

The fourteen Dutch participants in the first survey received six lead-enriched human blood samples, at intervals of several weeks. The samples had been put at our disposal by dr. D.G. Bullock (Queen Elizabeth Hospital, Birmingham), who used identical samples in the UK EQAS. Therefore, it was possible to compare the results of the MBL survey with those of the 66 to 95 English participating laboratories. The Dutch participants were requested to carry out the determinations according to their usual procedures and to process the samples in routine fashion with own standard solutions. The majority used the technique of flameless atomic absorption spectrophotometry.

The second survey was carried out with samples prepared by the MBL. The sixteen participants received eight times, with intervals of several weeks, a sample of lead-enriched human blood and once a solution of lead in 1% nitric acid. Half a litre of blood from a single person, lysed with EDTA (1.5 g/L), was divided into two portions (A and B). To portion A, a solution of lead in 1% nitric acid was added up to 4.83 μmol/L, after which samples of both portions were mixed in varying proportions and subjected to ultrasonic vibrations. The blood was then sterilized with X rays (7 hours, 60 Gray). In this way samples with the desired lead concentration were obtained. These mixtures were distributed over lead-free tubes in 2-ml portions under sterile conditions. As in the first survey, the receivers of the samples were asked to carry out the determinations in routine fashion according to their usual method of analysis and with their own standard solutions.

It cannot be excluded that determination of lead in blood to which lead has been added (so-called 'spiked' blood) yields different results from those obtained with blood into which the same concentration of lead has come by 'natural', i.e. occupational exposure. In order to test this, a mixture of blood from persons occupationally exposed to lead was sent twice to the participants in the first survey. The samples consisted of lysed blood distributed over lead-free tubes without any further treatment.

3.4 RESULTS AND DISCUSSION

In the first survey, two laboratories did not send in any results. In all, determinations in 75% of the samples distributed were received. In *Table 3.1* the results obtained by both the English and Dutch participants are reported.

According to the two-tailed Welch test the means of the English and Dutch participants are not significantly different. Although for sample 5 $P_2 = 0.019$ (if for a single result $P_2 < 0.05$, there is a statistically significant difference with a confidence limit of 95%), the chance that all six P_2's > 0.05 is only 73.5%. It is only at a $P_2 < 0.008$ that the difference may be considered significant in this experimental design.

TABLE 3.1 RESULTS OF LEAD DETERMINATIONS (μMOL/L) BY DUTCH[a] AND ENGLISH[b] LABORATORIES IN THE FIRST SURVEY

	Dutch laboratories, sample number					
Lab.[c]	1	2	3	4	5	6
2	1.77	3.18	1.97	2.51	3.92	2.19
3	1.53	2.88	2.02	2.92	2.86	2.42
4				2.13	3.83	3.22
5	2.10	3.60			4.60	1.80
7	1.72	2.70	1.93	2.53	3.54	2.43
8	1.94				3.27	2.60
9	1.28	2.05		2.75	5.17	2.22
11	1.93	3.43	2.25	2.48	4.25	2.93
12	2.40	3.10	2.30	2.40	4.30	2.90
14	3.04	2.64	2.23	3.19	4.25	2.43
15	1.85	2.90	1.80	3.62	3.60	3.62
16	1.74	1.77	1.82	2.51	3.41	2.31
Arithm. mean	1.94	2.83	2.04	2.70	3.92	2.59
Coeff. of variation (%)	24	20	10	16	16	19
	English laboratories					
Number	95	66	78	71	85	66
Arithm. mean	1.72	2.84	1.98	2.57	3.41	2.48
Coeff. of variation (%)	9.7	8.4	7.4	7.1	9.1	7.5
P_2[d]	0.15	0.96	0.43	0.37	0.019	0.47

a. Among the Dutch results there were none that deviated from the mean by more than three times the standard deviation (SD).
b. After elimination of results that deviated from the mean by more than three times the SD.
c. In the text and the tables the various laboratories are referred to by the same code numbers.
d. According to the two-tailed Welch test of the difference between the Dutch and the English mean.

Except for sample 3 the coefficient of variation (CV), and therefore the range of the Dutch results, is far wider than that of the English. This points to a smaller accuracy of the Dutch lead determinations, as compared with the English results. This appears also from the results plotted in *Figures 3.1a-b.*

Most of the values measured by laboratories 11 and 12, minus the mean of all laboratories, are significantly >0 (P_2 <0.05), which means that these results are systematically too high (t-test).

It is clear from this survey that the quality of the results of the lead determination by the Dutch participants is on the average far poorer than that of the English participants. For four out of six samples the CV is more than twice that of the corresponding English results *(Table 3.1).* The individual results in *Table 3.1* also show this unacceptable variation around the mean. In three out of six samples the highest concentration measured is more than double the lowest one.

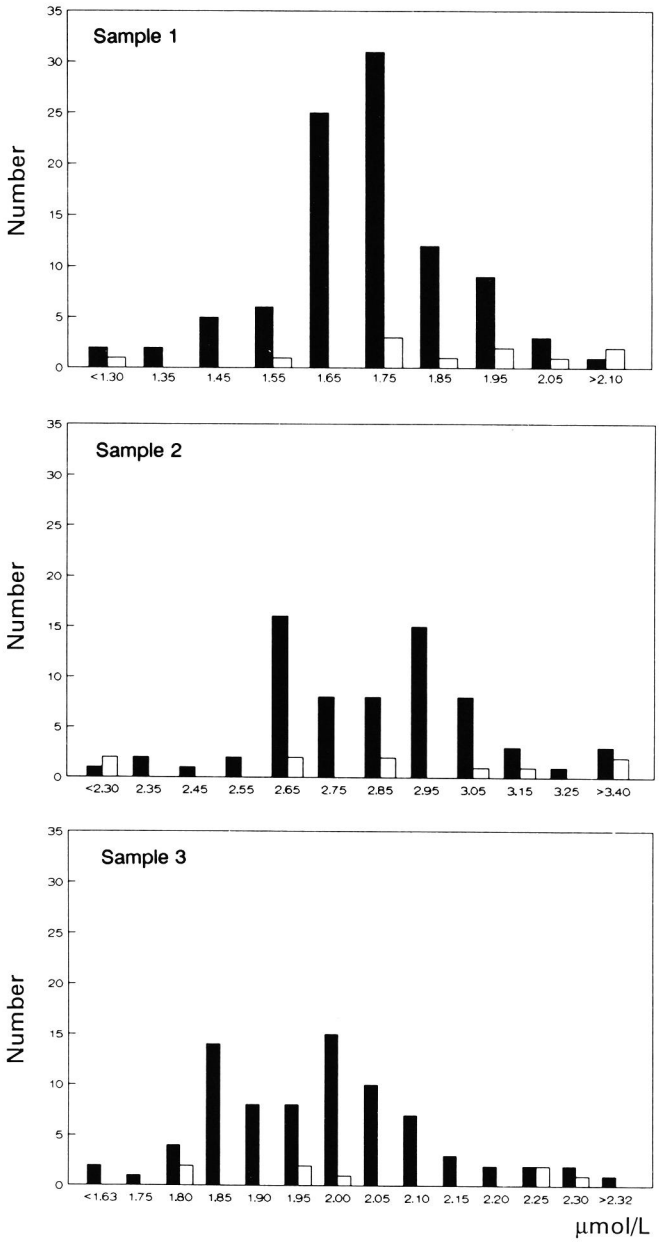

Figure 3.1a Results of lead determinations in samples 1-3 by English (closed bars) and Dutch (open bars) laboratories in the first interlaboratory survey.

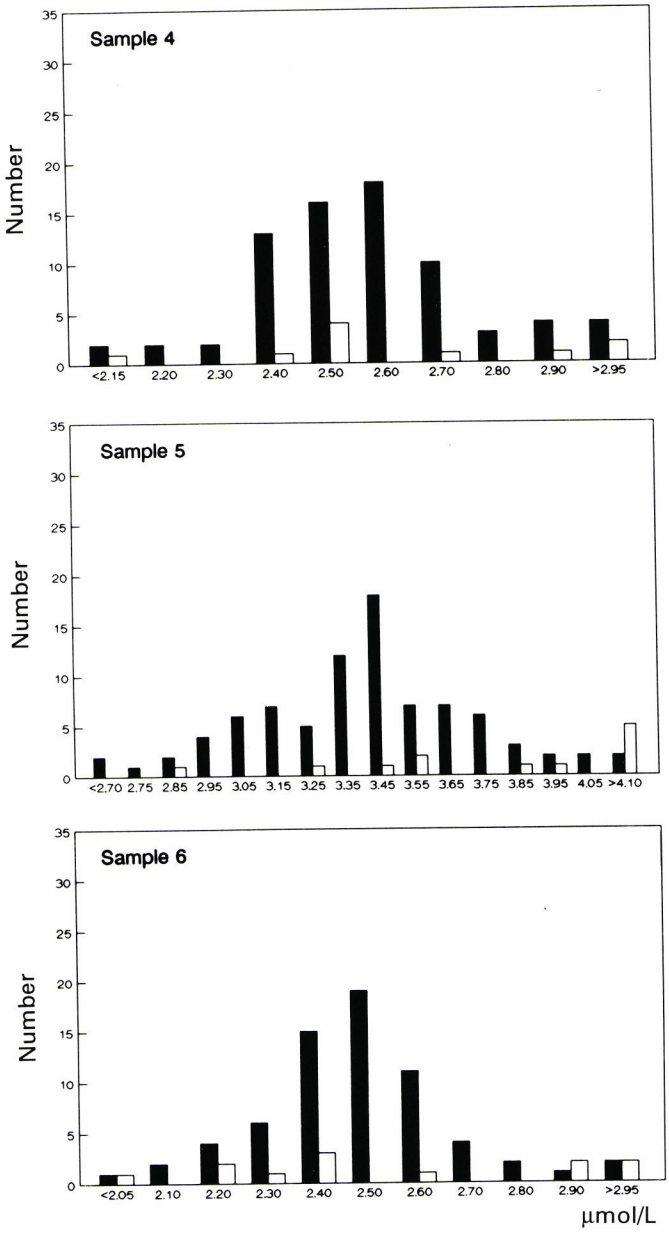

Figure 3.1b Results of lead determinations in samples 4-6 by English (closed bars) and Dutch (open bars) laboratories in the first interlaboratory survey.

In the second survey, results of 99% of the samples sent were received. In *Table 3.2* the results are reported. Sample 10 was the blood to which no lead had been added. The mean value therefore represents the 'natural' concentration. Sample 11 consisted of the solution of lead in nitric acid that had been used to enrich the lead concentration in samples 12 through 18. The concentration of lead in these samples therefore amounts approximately to the mean found in sample 10, increased by the added quantity, also indicated in *Table 3.2*.

TABLE 3.2 RESULTS OF LEAD DETERMINATIONS (μMOL/L) BY DUTCH LABORATORIES IN THE SECOND SURVEY

	Sample number								
Lab.	10	11[a]	12	13	14	15	16	17	18
1	1.11	2.03	1.51	1.30	1.70	1.23	2.89	3.07	3.50
2	0.98	2.28	1.15	1.06	1.21	1.25	2.62	3.87	4.01
3	1.32	1.82	1.54	1.87	1.36	2.27	2.63	2.90	3.89
4	1.16	2.64	1.11	0.84	1.88	0.73	2.23	3.60	3.25
5	1.50	2.80	1.60	1.40	1.70	0.80	1.20	2.20	4.20
6	1.45	1.81	1.74	0.82	1.75	1.79	4.64	8.50[b]	4.73
7	2.86	0.94	1.00	0.89	1.23	2.42	3.36	3.61	
8	1.06	0.06	1.15	1.05	0.96	1.12	2.23	3.66	3.31
9	1.01	1.45	1.06	0.97	0.68	1.09	2.61	2.95	
10	1.26	0.94	1.35	1.40	1.40	1.45	2.27	2.61	2.56
11	1.13	2.16	1.45	1.30	1.26	1.25	3.42	3.79	3.63
12	0.61	2.39	0.80	1.10	1.50	1.26	1.97	2.70	3.00
13	1.32	2.51	1.32	1.33	1.38	1.49	3.01	5.62	3.91
14	0.76	2.48	1.01	1.00	0.98	1.02	2.51	3.43	3.99
15	0.65	1.98	1.30	1.14	1.40	1.30	3.04	3.77	3.38
16	1.01	2.48	1.14	1.00	1.22	1.37	2.45	3.34	4.01
Arithm. mean	1.09	2.04	1.26	1.16	1.33	1.29	2.64	3.39	3.67
Coeff. of var. (%)	25	36	21	22	26	28	28	23	14
Lead added	0	2.41	0.25	0.10	0.15	0.25	1.69	2.66	2.90

a. Solution of lead in 1% nitric acid.
b. X > mean + 3 SD, not included in calculation of the mean.

The expected values and the corresponding means found do not differ significantly (t-test). The range of the results, however, is unacceptably wide, as appears from the coefficient of variation. In most samples the highest and lowest values measured differ by a factor of 2 or more. The results from laboratory 9 are systematically too low (t-test).

In imitation of the Queen Elizabeth Hospital the results of the assays were calculated for each sample with the indices of variation (IV) according to the method of Whitehead (1977).

$$IV = \frac{X - \overline{X}}{\overline{X}} \cdot 100 \cdot \frac{100}{C}$$

in which: IV= index of variation
X = individual result
$\overline{X}$ = mean of all results (in the first survey the mean of the English results)
C = selected coefficient of variation of 15%

The CV of 15% in the formula implies that deviation of a result of that size of the mean of all results gives an IV of 100. By means of the IV both the quality of the laboratories' results for one sample and the variation in quality with different samples in time within one laboratory can be compared.

Table 3.3 shows the results of the first survey after analysis according to the method of Whitehead. For this analysis not the mean of the Dutch results but that of the English ones was used. This mean has been obtained from far more results and is, therefore, more likely to represent the correct lead concentration in the samples than the Dutch mean.

TABLE 3.3 INDEX OF VARIATION (IV) AND ARITHMETICAL MEAN OF THE LEAD DETERMINATIONS BY DUTCH LABORATORIES IN THE FIRST SURVEY

Lab.	1	2	3	4	5	6	Mean
2	19	80	3	16	100	78	49
3	74	9	13	91	108	16	52
4				114	82	199	132
5	147	178			233	183	185
7	0	33	17	10	25	13	16
8	85				27	32	48
9	171	185		47	344	70	163
11	81	138	91	23	164	121	103
12	264	61	108	44	174	113	127
14	400[a]	47	84	101	164	13	145
15	50	14	61	272	37	306	123
16	8	251	54	16	0	46	63

a. When IV >400 a value of 400 is used.

In *Table 3.4* the IV values and the mean IV of the results obtained in the second survey are presented, calculated according to the method of Whitehead.

The English authorities accept only results of lead determinations when these have been carried out by a laboratory that takes part in the UK EQAS. Furthermore, they demand that the CV, as calculated from the last ten results, is 12% or less (this means a IV of 80 or less). If this norm is applied to the Dutch results, in each survey five laboratories meet this demand *(Tables 3.3 and 3.4).*

TABLE 3.4 INDEX OF VARIATION (IV) AND ARITHMETICAL MEAN OF THE LEAD DETERMINATIONS BY DUTCH LABORATORIES IN THE SECOND SURVEY

Lab.	10	11	12	13	14	15	16	17	18	Mean
1	12	3	132	80	185	31	63	63	31	67
2	67	78	58	57	60	21	5	94	62	56
3	141	72	148	400[a]	15	400[a]	3	96	40	146
4	43	196	79	184	276	289	104	41	76	143
5	251	248	180	138	185	253	364	234	96	217
6	220	75	254	195	211	258	400[a]	400[a]	193	245
7		268	169	92	221	31	56	6	11	107
8	18	400[a]	58	63	185	88	78	53	65	112
9	49	193	106	109	326	103	8	87		123
10	104	359	48	138	35	83	93	153	202	135
11	24	39	101	80	35	21	197	79	7	65
12	294	114	243	34	85	16	169	136	122	135
13	141	154	32	98	25	103	93	400[a]	44	121
14	202	144	132	92	175	140	33	8	58	109
15	269	20	21	11	35	5	101	75	53	66
16	49	144	63	92	55	41	48	10	61	63

a. When IV >400 a value of 400 is used.

However, it should be noted that in the surveys the mean IV has been calculated from nine or fewer values.

According to the Bartlett test there is no difference between the variances of the lead determinations in blood to which lead has been added and those in blood into which the lead has come by exposure ($P = 0.099$) (Data not shown). This means that the ranges of the results of the two series of samples do not differ significantly. So there is no reason to suppose any influence from the way in which the lead came into the blood on the accuracy of the results.

In the first survey five laboratories achieved an IV lower than 80 and in the second survey again five. Of the latter, four had also participated in the first survey. Only two laboratories showed a mean IV lower than 80 in both surveys. Out of twelve laboratories participating in both surveys, four had a higher mean IV in the second survey. Thus, in the second survey their results deviate more from the mean values, so the quality of their determinations has deteriorated. Another four laboratories had a smaller mean IV, in these the quality of the determinations has improved. Four laboratories achieved an approximately equal IV in both surveys.

3.5 CONCLUSIONS AND RECOMMENDATIONS

Almost all Dutch laboratories that carry out lead determinations in blood took part in the two interlaboratory surveys. In the first survey with six samples the mean deviation of the arithmetical mean was less than 10% in five out of twelve participants and in the second survey with nine samples in five out of sixteen

participants. Only two participants out of twelve that took part in both surveys deviated in both surveys on average less than 10% from the mean.

The small number of participants in the surveys did not allow judgement of the results with respect to the analytical method used. However, based on the results of the UK EQAS, Bullock et al. (1986) concluded that owing to the improved overall performance, small differences between the methods of analysis had disappeared since 1983. In the present surveys there were no indications that the accuracy of the results was influenced by the manner in which the lead had come into the blood.

On the whole the performance of the determination of lead in blood in respect to precision and accuracy is in need of improvement. In order to improve the data it is suggested that the analyses should be carried out in qualified laboratories by technicians who have comprehensive experience in measurements of low-level trace elements in biological samples, and which carry out these analyses regularly. Also a detailed, validated protocol can contribute to the quality. Furthermore, in daily routine reproducibility must be checked by analysis of so-called 'internal control samples' along with each series of actual determinations. The results help in identifying any trends in the intralaboratory performance which occur in course of time and aim also to keep precision within acceptable limits. Such (certified) samples are available commercially, among others from the Community Bureau of Reference of the European Community in Brussels, Belgium, and from the National Institute for Standards and Technology, Gaithersbury, U.S.A. Finally, participation of laboratories in external quality control programmes, preferably with a large number of participants, is strongly recommended. This allows the analysis of samples with unknown concentrations of lead, together with many other laboratories and thus estimation of the deviation of obtained results from the 'true' value. In these quality control programmes, the true value of Pb in a sample is usually considered the mean of the results of all participants, after the removal of extreme deviant results.

These recommendations may result in a coefficient of variation of the PbB analysis of about 6% [Bullock et al. (1986)], instead of the values obtained in the present surveys, which ranged between 10 and 36%.

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CHAPTER 4

BLOOD LEAD AND BLOOD PRESSURE: SOME IMPLICATIONS FOR THE SITUATION IN THE NETHERLANDS*

W.L.A.M. de Kort and W.C.M. Zwennis

Studies performed earlier have shown a positive relation between blood lead (a parameter for lead body burden) and blood pressure, whereas such a relation between urine cadmium (a parameter for cadmium body burden) and blood pressure could not be shown. Median (i.e. 50^{th} percentile, P_{50}) blood lead levels in the general population in the Netherlands are in the range of 80 to 150 μg/L**. Persons occupationally exposed to lead show median blood levels that may exceed 400 μg/L. To study causality, a prospective study among lead workers is desired.

4.1 INTRODUCTION

In the literature, a positive, rather than a negative relation between lead and blood pressure is more often cited. Furthermore, experimental (animal) studies render some support to this relation, although a satisfactory explanation by some underlying mechanism is not yet at hand. Recently, the U.S. Environmental Protection Agency published an extensive report on lead, which contained a review on this topic [EPA (1986)]. Apart from the fact that positive results are more easily reported than negative results, the actual existence of a positive relation should be seriously considered.

The relation between occupational lead exposure and blood pressure was studied in the Netherlands [De Kort et al. (1987)]. After a brief review of this study, some results are presented of studies on the blood lead values in the general population in the Netherlands. An attempt will be made to evaluate the consequences of these results.

4.2 LEAD WORKER STUDY

A group of 53 occupationally exposed workers (from a plant processing lead and cadmium compounds) was compared with a group of 52 workers not occupa-

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* Published in Environmental Health Perspectives 78:67-70 (1988).

** Note added: 100 μg lead corresponds to 0.48 μmol.

tionally exposed (from a plant where insulation materials are produced). For the results to be included in the analysis, the worker had to have been employed for more than 1 year and not be under treatment for hypertension. Further details concerning the methods used in this study have been described previously [De Kort et al. (1987)]. The groups were comparable with regard to their socio-economic background, physical exertion, workplace conditions (apart from exposure), place of residence, and some lifestyle characteristics. A statistically significant difference existed concerning their age and their duration of employment *(Table 4.1)*.

The average values of systolic and diastolic blood pressure were found to be higher in the exposed group *(Table 4.2)*. Because the workers were exposed to both lead and cadmium and because the exposed group was on the average older

TABLE 4.1 AGES AND DURATION OF EMPLOYMENT[a]

	Pb-exposed	Controls	Significance
n	53	52	
Age	42.1 ± 1.2[b]	38.2 ± 1.1	$p < 0.05$
Years at work	12.5 ± 1.2	8.6 ± 0.8	$p < 0.01$

a. From de Kort et al. (1987).
b. Values are means ± SEM.

TABLE 4.2 BLOOD PRESSURE AND HEART RATE[a]

	Pb-exposed	Controls	Significance
n	53	52	
Systolic blood pressure, mm Hg	140 ± 3[b]	131 ± 3	$p = 0.01$
Diastolic blood pressure, mm Hg	86 ± 2	80 ± 2	$p = 0.01$
Heart rate, beats/min	78 ± 2	81 ± 2	NS[c]

a. From de Kort et al. (1987).
b. Values are means ± SEM.
c. NS, non-significant.

than the control group, further statistical analyses were performed. Calculation of the correlation coefficients revealed significant results for the correlation between blood lead and blood pressure while controlling for age and heart rate; additional adjusting for urine cadmium (only feasible for a smaller number of complete data sets) revealed a non-significant result at the 0.05 level. On the other hand, no significant result was obtained for the correlation between cadmium and blood pressure if age effects were controlled for *(Tables 4.3 and 4.4)*.

The following regression equation was obtained for systolic blood pressure using multiple linear regression analysis:

$$P_{syst\ (mm\ Hg)} = 73.8 + 0.018 \times PbB\ (\mu g/L) + 0.52 \times age\ (years) + + 0.45 \times heart\ rate/min$$

The regression equation for diastolic blood pressure contained no significant contribution from blood lead (the coefficient in the equation being 0.010, $p > 0.10$).

Kidney function parameters (serum creatinine, blood urea nitrogen, retinol binding protein in urine, relative uric acid clearance, and total protein) revealed no significant differences between the groups. From the study, which had its limitations (small groups, insufficient data on body mass index), it was concluded that a positive relationship may exist between blood lead level and blood pressure (notably systolic blood pressure). A relationship between urine cadmium and blood pressure appeared to be linked with age.

TABLE 4.3 ZERO-ORDER CORRELATION COEFFICIENTS FOR SYSTOLIC BLOOD PRESSURE[a]

Parameter	r	p
Blood lead	0.29	0.003
Urine cadmium	0.22	0.04
Age	0.34	0.001
Heart rate	0.34	0.001

a. From de Kort et al. (1987).

TABLE 4.4 PARTIAL CORRELATION COEFFICIENTS FOR SYSTOLIC BLOOD PRESSURE[a]

Parameter	r	p
Blood lead, adjusted for age, heart rate	0.22	0.02
Urine cadmium, adjusted for age, heart rate	0.13	0.22
Blood lead, adjusted for age, heart rate, urine cadmium	0.20	0.07

a. From de Kort et al. (1987).

4.3 BLOOD LEAD LEVELS IN THE NETHERLANDS

Several studies have been performed to gain a clearer insight into the average blood lead levels in the Dutch population [PHC (1984)]. Some studies were particularly aimed at assessing blood lead levels in children [Zielhuis et al. (1979); Ligeon et al. (1981)]. In other studies samples from draftees, which are being gathered at regular intervals, were examined for heavy metal contents such as lead and cadmium [Ellen et al. (1984)]. Another study reported on blood lead levels and other trace metal levels in elderly men [Kromhout et al. (1985)]. Occupationally exposed persons frequently have been the subject of epidemiological studies. Some of the results are presented here.

Blood lead levels in children

In 1984, a committee of the Public Health Council in the Netherlands has published recommendations for air quality criteria for lead [PHC (1984)]. The committee has also taken into consideration accepted limits for blood lead levels in children as recommended by Zielhuis et al. (1979) *(Table 4.5)*.

In co-operation with the European Community, blood lead levels were assessed in children under the age of 6 years, in several small and large cities, in 1979 and in 1981 [Ligeon et al. (1981)]. The results showed that the limit values were generally not exceeded, with median (50^{th} percentile, P_{50}) values declining over the years, being at a level of 130 µg/L in 1981 *(Table 4.6)*.

TABLE 4.5 DUTCH PUBLIC HEALTH STANDARDS FOR CHILDREN[a]

Percentile	Blood lead (µg/L)
P50	200
P90	250
P98	300

a. From Zielhuis et al. (1979).

TABLE 4.6 BLOOD LEAD LEVELS IN CHILDREN, AGE 6 YEARS[a]

	Blood lead (µg/L, P50/P98)	
City	1979	1981
Rotterdam-centre (n ± 99)	160/330	130/220
The Hague (n ± 88)	180/320	130/230

a. From Ligeon et al. (1981).

Blood lead levels in draftees

The National Institute of Public Health and Environmental Protection in the Netherlands has reported on blood lead levels in draftees [Ellen et al. (1984)]. In random samples (of at least 1% of all draftees in one year) blood lead levels of draftees in several years have been assessed *(Table 4.7)*. The results in 1980 and 1982 showed markedly lower values when compared with those of earlier years (1976). Furthermore, trend analyses showed a significant downward trend over time within the 1980/1982 groups. The investigators suggested that reducing the gasoline lead content in 1978 might have played a role.

TABLE 4.7 BLOOD LEAD LEVELS IN DRAFTEES[a]

	1976	1980	1982
n	1170	175	150
Blood lead (µg/L, P50/P98)	140/350	90/210	80/200

a. From Ellen et al. (1984).

Blood lead levels in lead workers

It is estimated that in the Netherlands (14.5 million inhabitants) less than 10,000 and approximately 5,000 workers are occupationally exposed to lead, a figure that is expected to become smaller. In the near future, a directive from the European Community will be implemented in Dutch legislation, including action levels and threshold values for blood lead levels of occupationally exposed workers *(Table 4.8)*[*].

TABLE 4.8 PROPOSED ACTION LEVELS AND THRESHOLD LEVELS OF PbB FOR OCCUPATIONALLY EXPOSED PERSONS

Blood lead level (µg/L)	Requirements
>300	Information to workers Hygienic measures Opportunity to undergo medical examination
>500	Medical surveillance Information to workers Hygienic measures Opportunity to undergo medical examination
>600	Intensive medical examination[a]
700 (threshold) or 700 - 800, provided ZPP[b] <20 µg/g Hb	Medical surveillance Information to workers Hygienic measures If occurring twice within 3 months, withdrawal from exposure

a. Applies only to the worker concerned.
b. ZPP, blood zinc protoporphyrin content.

Supported by the Directorate General of Labour, the TNO Medical Biological Laboratory performed a health and health hazard survey in branches of industry, where occupational exposure to lead is an important factor. The blood lead levels obtained in this survey were pooled, and the results *(Table 4.9 and Fig. 4.1)* show that median (P_{50}) values vary between 288 µg/L and 339 µg/L for Dutch workers and between 381 µg/L and 451 µg/L for workers of mediterranean origin. However, it must be emphasized that the workers examined in this survey probably do not constitute a group that is representative for all lead workers. More likely they represent those who are exposed to relatively high levels of lead, as the survey was preferentially performed in workplaces with levels of exposure expected to be higher than average.

[*] Note added: The Directive is legally binding from 1988.

TABLE 4.9 BLOOD LEAD LEVELS IN MALE LEAD WORKERS IN THE NETHERLANDS[a]

	Dutch workers				Mediterranean workers			
Age	n	P50	P90	P98	n	P50	P90	P98
<30	149	302	725	929	81	381	662	762
30-39	136	288	588	795	75	451	689	772
40-49	128	339	635	774	65	389	704	863
≥50	138	292	675	886	24	424	710	927

a. Blood lead levels in μg/L.

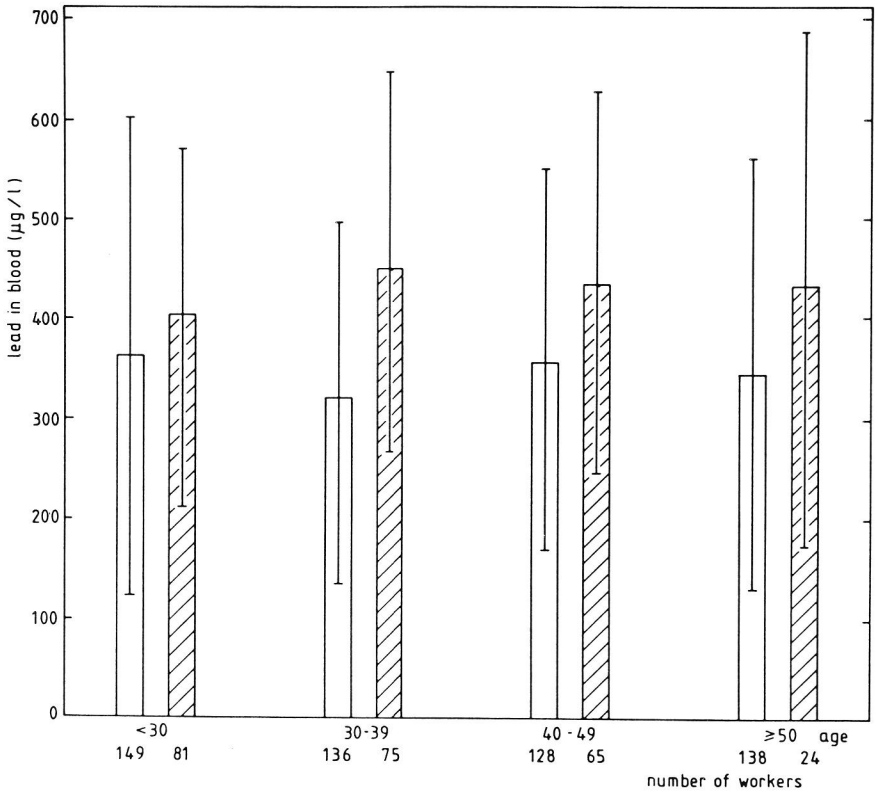

Figure 4.1 Average lead-in-blood values ± SD of Dutch lead workers (open bars) and mediterranean lead workers (hatched bars) in the Netherlands.

4.4 DISCUSSION

The study of lead workers indicates the possibility of a positive relation between blood lead and blood pressure. The effect on systolic blood pressure is in the order of magnitude of an 1.8 mm Hg increase per 100 μg/L increase in blood lead level when a linear relation is assumed. If a relationship between blood lead level and

diastolic blood pressure exists, then here the increase per 100 μg/L increase in blood lead level would amount to approximately 1.0 mm Hg. These figures are lower than those found in the NHANES II study [Pirkle et al. (1985)], but well in accordance with the results found by Kirby and Gyntelberg (1985). However, it must be kept in mind that linearity of a relation is merely an assumption, provided an effect does exist. In particular, at lower blood lead levels (i.e. values <100 μg/L), deviation from linearity would not be a surprising result. Nevertheless, since median blood lead levels of persons not occupationally exposed in general do not exceed 200 μg/L, reducing this level to zero could imply an average lowering of systolic blood pressure with 3.6 mm Hg and perhaps an average lowering of diastolic blood pressure of 2.0 mm Hg.

In most studies median blood levels are lower than 200 μg/L, and reducing this level to zero will probably not be possible. As a result, detecting an effect of the lowering of blood lead levels will not be an easy task: population standard deviation in blood pressure measurements can vary from 15 to 25 mm Hg (for systolic blood pressure). Thus, if a difference of 1.8 mm Hg is to be detected, using first and second type error probabilities of 0.05 (two-sided and one-sided, respectively), groups of at least 3200 persons each have to be examined. The detection of such differences will be further impaired because of the many factors (lifestyle, genetic, environmental, etc.), which may influence the outcome of blood pressure measurements. In statistical terms, the stability of blood pressure measurements might even decrease further if many factors are controlled for, demanding even larger groups to be examined.

Because the half-time of blood lead is rather long, a follow-up study will necessarily have to be performed over several years if one chooses to use a longitudinal study. Also, it is not known if a blood pressure-raising effect of lead is at all reversible. If cross-sectional studies are preferred, one might have to take into account the additional disadvantage of a cohort effect, which could hide an effect or erroneously reveal one. A prospective study of lead workers may yield more conclusive results, because the occupational level of lead exposure is higher than the level of exposure in the general population.

In conclusion, the expected reduction of environmental pollution with lead due to the reduction of the gasoline lead content and the expected reduction of occupational exposure due to hygienic measurements make a reduction of body burden in the general population, as well as in the occupationally exposed population, likely. In the Dutch situation, further epidemiologic evaluation of an effect on blood pressure will be difficult to perform. Prospective studies, e.g. in an occupational setting, are desired.

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ACKNOWLEDGEMENT

The authors thank Dr. R.L. Zielhuis, Amsterdam, for his remarks.

CHAPTER 5

OCCUPATIONAL EXPOSURE TO LEAD AND THE INCIDENCE OF ANAEMIA *

W.C.M. Zwennis and A.Ch. Franssen

In a study on the occupational exposure to lead of industrial workers in the Netherlands, the concentration of lead and haemoglobin was determined in blood of 494 Dutch, 59 Moroccan and 18 Turkish male employees. In the Dutch group a small but significant negative correlation was observed between the concentrations of lead (maximum 5 μmol/L) and haemoglobin ($\alpha = 0.05$, one-sided testing). In the Morrocan and Turkish groups no statistically significant correlation was found. In Dutch employees with a low-to-normal haemoglobin value, occupational exposure to lead may therefore cause anaemia.

Key words: lead, anaemia, haemoglobin concentration, occupational exposure

5.1 INTRODUCTION

Biological monitoring constitutes an important part of health control programmes for employees exposed to lead (compounds) during their work. The determination of the concentration of lead in blood (PbB), which is now in general use, gives an insight into the recent and total lead uptake of the individual employee.

In the near future a European Community Directive controlling the protection of employees against the health hazard of exposure to lead will become operative (Directive 82/605/EEC)**. This Directive indicates under which circumstances, and how often, the concentration of airborne lead dust at the work site, and/or the concentration of lead in blood of the exposed employees, must be measured. Furthermore, the Directive indicates that in certain circumstances clinical surveillance of specified parameters may also be required, e.g. zinc protoporphyrin in blood, δ-aminolaevulinic acid dehydratase in blood, or δ-aminolaevulinic acid in urine.

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* Published in 'Tijdschrift voor Sociale Gezondheidszorg' 65:601-604 (1987). (In Dutch, English summary available).

** See note page 47.

In the past the determination of lead in blood involved practical problems, and other parameters were used to judge the uptake of lead; parameters which are based on the effects of lead on the haematopoietic system. In this paper their biochemical base is discussed. The results of an investigation into the correlation between the lead concentration in blood and the haemoglobin concentration (Hb) are also reported.

5.2 EFFECTS OF LEAD ON THE HAEMATOPOIETIC SYSTEM

As early as the end of the last, and the beginning of this century, experiments with rabbits and observations of patients suffering from lead intoxication revealed symptoms pointing at effects of lead on haem synthesis [Stokvis (1895); Götzl (1911)]. Binnendijk [cited by Stokvis (1895)] was the first to note an increased porphyrin concentration in urine of intoxicated patients. In 1927 Liebig suggested that lead-induced anaemia is caused by the destruction of the erythrocytes. In 1957 Haeger noted an increased excretion of δ-aminolaevulinic acid in the urine of workers exposed to lead.

Figure 5.1 shows a simplified scheme of haem biosynthesis and the effects of lead on it.

In the first step the coupling of glycine and succinyl-CoA is catalysed by δ-aminolaevulinic acid synthetase, under formation of δ-aminolaevulinic acid (ALA). Then, under the influence of δ-aminolaevulinic acid dehydratase (ALAD), from two ALA molecules porphobilinogen (PBG) is formed. In the third step four PBG molecules are coupled by the enzymes uroporphyrinogen-1-synthetase and uroporphyrinogen cosynthetase to form uroporphyrinogen (URO). By subsequent decarboxylation and oxidative transformation effected by the enzymes uroporphyrinogen decarboxylase, coproporphyrinogen oxidase and protoporphyrinogen oxidase, protoporphyrin IX is formed.

In the last step of the pathway iron is incorporated into this molecule, leading to the formation of the haem group. This group is used for the formation of haemoglobin, but it is also a component of some enzymes, e.g. cytochrome P-450 and catalase [Moore et al. (1980)].

Lead may form complexes with chemical compounds containing sulphydryl-, amino-, carboxyl-, phenoxy- or imidazole-groups. When this complex formation concerns functional groups of enzymes it is often associated with inhibition of enzyme activity. A number of enzymes involved in the biosynthesis of the haem group are thus more or less inhibited by lead. Most of the research in this connection has been carried out with ALAD. The almost complete inhibition of this enzyme by an increased lead concentration blocks the further conversion of ALA, resulting in an increased excretion with the urine. There are no indications that this inhibition affects health. ALAD inhibition and ALA excretion are now, at least in the Netherlands, only used to a limited extent in connection with lead exposure.

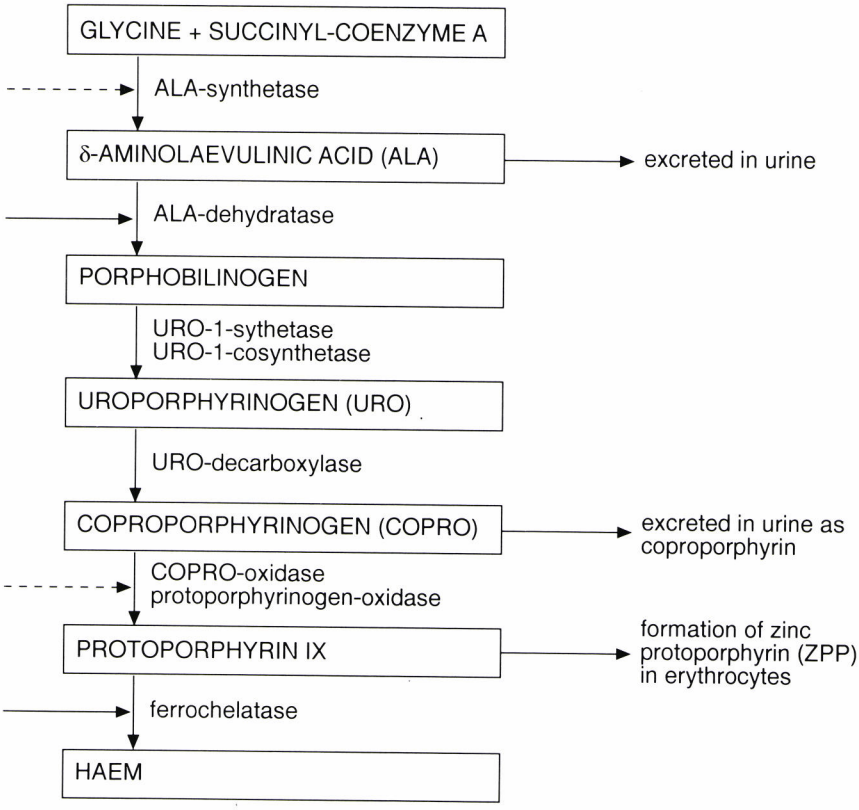

Figure 5.1 Haem biosynthesis. Lead inhibits two enzymes (horizontal drawn arrows) and possibly inhibits two more (horizontal dotted arrows).

Coproporphyrinogen oxidase is also inhibited by lead, which leads to an increased excretion of coproporphyrin with the urine. The by now obsolete determination of Donath (1956) is based on this principle. Lastly, the inhibition of ferrochelatase, the enzyme that catalyses the incorporation of iron into protoporphyrin IX, should be mentioned. The inhibition results in the incorporation of zinc instead of iron into the substrate.

Beside the above-mentioned effects on haem biosynthesis, exposure to lead also induces increased fragility and associated accelerated destruction of the erythrocytes.

5.3 ANAEMIA DUE TO LEAD EXPOSURE

A decreased haemoglobin concentration in blood due to occupational lead exposure is often observed. According to Zielhuis (1959) 13 out of 21 workers (62%) at

small accumulator factories had a Hb value lower than 8.5 μmol/L and 17 a Hb lower than 9.1 μmol/L (81%). These percentages were higher than those measured in industries with no or little lead in the atmosphere. In this study lead in blood of the workers was not measured.

Tola et al. (1973) reported that in persons exposed to lead for the first time an increased PbB up to about 2.4 μmol/L was associated with a small but significant decrease in Hb after several months (1 μmol Pb/L = 207 μg/L = 207 ppb).

Out of 46 lead workers having a PbB between 1.9 and 3.8 μmol/L, 37% had a Hb lower than 8.5 mmol/L [Lilis et al. (1977)]. Fischbein et al. (1978) established a significant difference between the mean Hb of 34 lead workers (PbB levels <3.8 μmol/L) and that of 34 controls: 47% of the workers had a Hb lower than 8.6 mmol/L against 12% of the control group.

Examination by Grandjean (1979) of 202 male workers with levels of PbB up to about 4 μmol/L, showed a statistically significant correlation between Hb and PbB, but the median of the Hb values remained within the reference range (8-11 mmol/L; 1 mmol Hb/L = 16.125 g/L, Hb/4).

In two secundary lead smelteries and in a factory producing lead-containing pigments, in 27 out of 129 persons examined a Hb concentration lower than 8.7 mmol/L was found. These 27 persons had a significantly higher PbB than the other collaborators: 20 persons having a PbB higher than 3.9 μmol/L, six a PbB between 2.9 and 3.8 μmol/L and one a PbB less than 2.9 μmol/L [Baker et al. (1979)].

In twelve secondary lead smeltery workers who were checked regularly for PbB and Hb for twelve years, no statistically significant difference was found between mean Hb at the beginning and at the end of that period. The highest PbB value found in this period was 4.2 μmol/L, while the mean PbB ranged from 1.3 to 2.7 μmol/L [Valentine et al. (1982)].

5.4 STUDY DESIGN

Within the scope of a health hazard survey in the lead-processing industry, carried out by the TNO Medical Biological Laboratory by order of the Directorate General of Labour, lead and haemoglobin concentrations were determined in blood samples of a large number of persons working in several branches of industry.

Lead was determined by means of atomic absorption spectrophotometry following the method of Schaller (1983). The coefficient of variation in time, obtained by analyzing identical control samples, was 7%. For the determination of haemoglobin the photometric method of Van Kampen and Zijlstra (1961) was used. The coefficient of variation in time, determined with identical control samples, was 2%.

Because of the extent of the survey, which involved several thousands of persons working in dozens of industries of different character, a good insight into the levels of lead in blood of occupationally exposed employees in the Netherlands was obtained. On the strength of an inventory of the use of lead and lead

compounds in the Dutch industry, it is justified to conclude that higher values than found are unlikely in the Netherlands.
In this paper the correlation between the lead and haemoglobin values found in male employees working for five years or more in the same jobs are reported. Part of the men were exposed to dust or aerosols of metallic lead, the others to dust of lead compounds like lead oxide or insoluble inorganic lead salts. The purpose of the study was to determine whether the present levels of exposure to lead in the Dutch lead-processing industry may cause a decrease in haemoglobin concentration in employees. The reference values of haemoglobin used were 8.6 - 10.9 mmol/L. These values are based on a large number of Hb values in adult Dutch men considered as healthy [Helleman et al. (1973)].

5.5 RESULTS AND DISCUSSION

The population under study consisted of employees with Dutch, Morrocan or Turkish nationality. In general, clinical laboratories in the Netherlands base the reference values of haemoglobin for ethnic minorities on those of Dutch people (personal communication, P.H. Trienekens). The influence of age, race, nutritional habits and geographical origin on the concentration of Hb of adults is small. As no data are known on possible differences in the influence of lead on Hb in Dutchmen, Morrocans and Turks, the results for the three groups of employees were calculated separately.

Figure 5.2 gives the correlation between PbB and Hb in 494 Dutchmen and *Figure 5.3* that in 59 Morrocans and 18 Turks. For the Dutchmen the slope of the regression line is –0.0586, with a standard error of 0.0307. In a one-tailed test, the null hypothesis that the Hb concentration does not decrease with increasing lead concentration is rejected at a level of significance of 5% ($P_1 = 0.032$; upper limit of slope –0.006). Tested similarly, the slope found for the Morrocans does not differ significantly from zero (upper limit slope 0.124). The observations in the 18 Turkish employees do not meet the assumptions of the linear regression model. They do not show a significant Spearman rank correlation between PbB and Hb either.

The slope found in the Dutchmen corresponds to a decrease in Hb of 0.28 mmol/L over the Pb range of 0-5 µmol/L. Using a confidence interval of 95% the maximal decrease over this range is 0.59 mmol/L. Although this decrease is small, it can lead to anaemia in those employees who have a Hb approaching the lower limit of the reference range (8.6 mmol/L) at the start of their work in the lead-processing industry. The linear regression line in the Dutchmen gives a 95% confidence interval of 9.2 to 9.5 at a PbB value of 5 µmol/L.

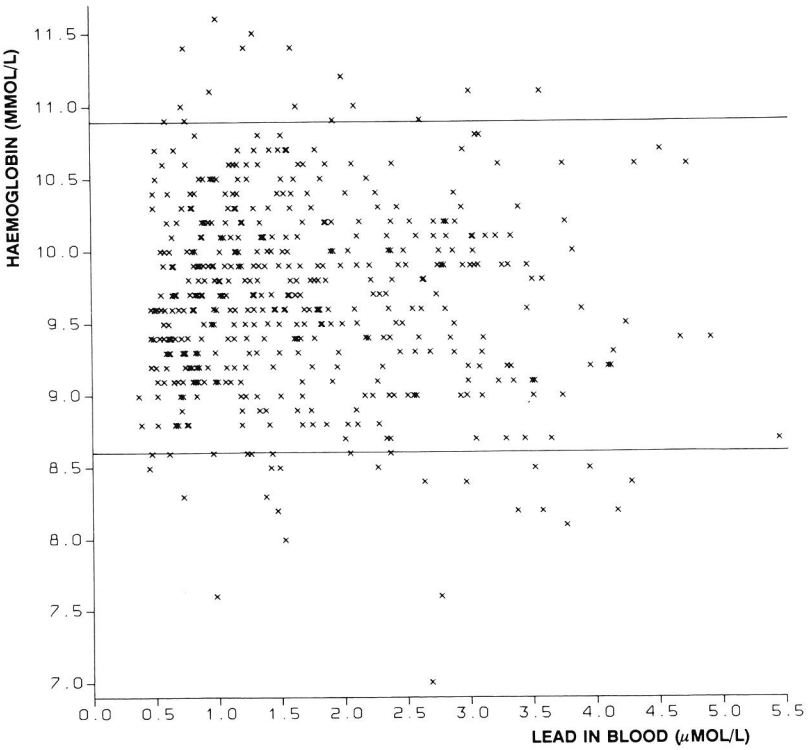

Figure 5.2 Correlation between lead concentration in blood and haemoglobin in 494 Dutchmen. The lines indicate reference values for Dutch men according to Helleman et al. (1973).

5.6 CONCLUSIONS

In a group of 494 Dutchmen who stayed in their industrial jobs for five years or longer and were exposed to metallic lead and/or inorganic lead compounds, resulting in lead concentrations in the blood between 0.3 and 5.5 μmol/L, one-sided statistical testing showed a negative correlation between the concentration of lead in blood and haemoglobin concentration. The slope of the line corresponds to a decrease in Hb of 0.28 mmol over the Pb range of 0-5 μmol/L. Using a confidence interval of 95%, this means that maximally the decrease may be 0.59 mmol/L. In persons who at the start of their work in the lead-processing industry have a Hb value around the lower limit of the reference range, this value can lead to anaemia. Therefore, it is recommended to check Hb in (future) employees in the lead-processing industry.

In a group of 59 Morrocan men and in a group of 18 Turkish men who had also been working for several years in the same industries and who had concentrations of lead in blood lower than 4.5 μmol/L and 3.5 μmol/L, respectively, no correlation between the lead and haemoglobin concentrations was found.

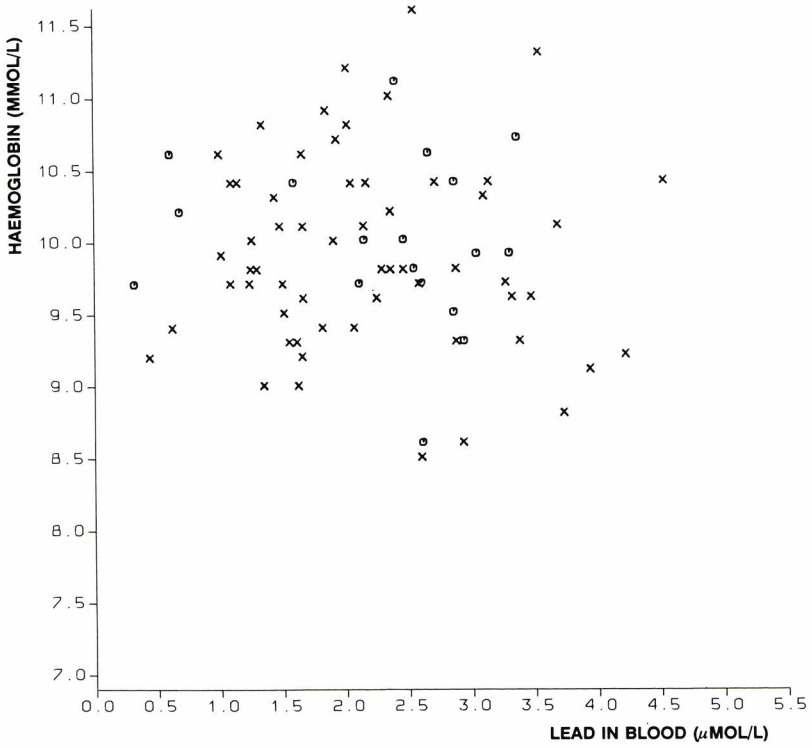

Figure 5.3 Correlation between lead concentration in blood and haemoglobin in 59 Morrocans (X) and 18 Turks (0).

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ACKNOWLEDGEMENT

This paper was written within the scope of the project 'Health hazard survey in lead-processing industries', which is subsidized by the Directorate General of Labour of the Ministry of Social Affairs and Employment. The authors thank their colleagues for carrying out the lead and haemoglobin determinations and for their critical support in the preparation of this paper. In particular they owe their gratitude to an unknown referee for a significant contribution to the discussion.

CHAPTER 6

BIOLOGICAL MONITORING OF EXPOSURE TO CHROMIUM(VI) SALTS: THE ROLE OF SOLUBILITY* **

P.C. Bragt***, W.C.M. Zwennis and A.Ch. Franssen

6.1 INTRODUCTION

Biological monitoring of chromium in urine is a useful instrument for the evaluation of possible health risks as a result of exposure to soluble chromium(VI) compounds (Cr(VI)). The Cr concentration in post-shift urine and the increase of the Cr concentration during a workday are correlated linearly with the time-weighted average exposure during the shift, especially when a correction is made for creatinine excretion ('dilution of urine') [Mutti et al. (1979)].

Allergic sensitization of the skin and lungs and renal tubular damage are the main health effects associated with long-term exposure to soluble Cr(VI) [Langard and Norseth (1986)]. A number of sparsely soluble and insoluble Cr(VI) compounds are suspected of carcinogenicity in animals and man, the most prominent compounds being chromite ore roast, calcium chromate, lead chromate, and strontium chromate [IARC (1980)]. The ferro-chromium industry, the chromate production industry, and the chrome-pigment industry have been associated with an enhanced risk of lung cancer for the workers involved [Léonard and Lauwerys (1980); Norseth (1981)].

In previous experiments in rats [Bragt and Van Dura (1983)] we have shown that intratracheally administered zinc chromate (moderately soluble in water) is absorbed via the lungs and excreted into the urine in sufficiently large amounts to allow biological monitoring. Lead chromate (practically insoluble in water) was only absorbed very slowly and incompletely. Even a high intratracheal dose did not result in appreciably enhanced blood and urinary chromium concentrations. This implies that these concentrations will not reflect exposure.

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* The study comprises lead chromate.

** Published in 'Biological monitoring of exposure to chemicals: metals'. pp. 119-126 (1991). Eds. H.K. Dillon and M.H. Ho. John Wiley & Sons, Inc., New York, U.S.A.

*** Present adress: Inspectorate for Health Protection, Ministry of Welfare, Health and Cultural Affairs, Rijswijk, the Netherlands.

The aim of this study was to investigate whether Cr concentrations in blood or urine are indicative of exposure to chromates of different solubilities. The study was performed because exposure by inhalation is a more adequate model of exposure in the work environment than intratracheal administration.

6.2 METHODS

Design of the experiment

Five groups of 10 male Wistar rats (150-200 g) were exposed to sodium chromate in a whole body exposure chamber, during 4 h per day for 1-4 days. Similar experiments were also carried out with zinc chromate and lead chromate. The exposure schedule is visualized in *Figure 6.1.*

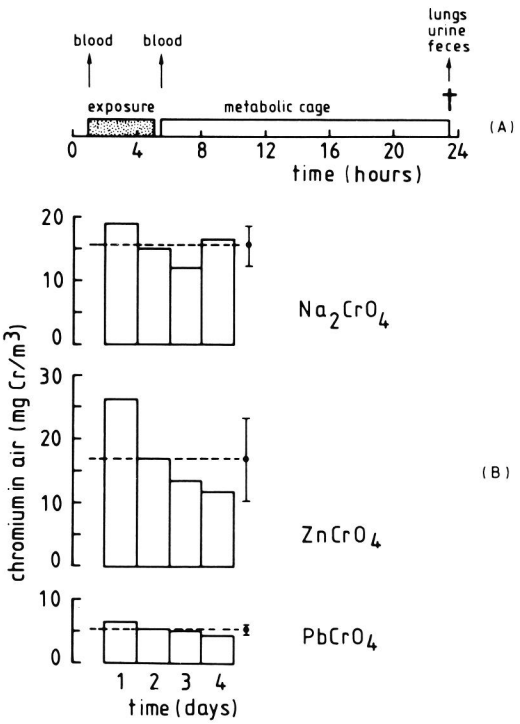

Figure 6.1 (A) Schedule of animal treatment and collection of samples during the day of exposure. (B) Exposure levels of sodium chromate, zinc chromate, and lead chromate (bars: daily exposure, dashed line: mean exposure level of 4 days with SD).

Ninety-five percent of the particles had a diameter of 1.5-4.2 μm, and all particles had a diameter less than 6 μm. The mean daily chromium concentrations (± SD)

were 15.5 ± 2.9 mg Cr/m^3, 17.0 ± 6.4 mg/m^3, and 5.3 ± 0.8 mg/m^3, for sodium chromate, zinc chromate, and lead chromate, respectively. Venous blood (tail vein) was obtained half an hour before and after each exposure period. For the separate collection of urine and faeces, the rats were placed into stainless-steel metabolic cages for 18 h after each exposure period. An unexposed control group of 30 rats was treated similarly. Subsequently, groups of rats were sacrificed after the collection of urine and faeces; arterial blood samples were taken and the lungs were dissected. The samples were frozen immediately and kept at -25°C.

Chemicals
Sodium chromate and lead chromate were purchased from E. Merck (Darmstadt, Germany) and zinc chromate ($4ZnCrO_4 \cdot K_2O \cdot 3H_2O$) was a kind gift of Mr. J. de Winter, Sikkens B.V., Sassenheim, the Netherlands.

Determination of chromium and lead
For the determination of Cr and lead (Pb), the method of Davidson and Secrest (1972) was followed in essence. Blood and urine samples were digested in nitric acid (65%). Lung tissues and faeces samples were digested in nitric acid (65%) and hydrogen peroxide (30%). After dilution with water, Cr and Pb were determined at the respective wavelenghts of 357.9 and 283.3 nm with a Perkin-Elmer AAS 4000/HGA 500 atomic absorption spectrophotometer with graphite furnace. The metals were quantified by the standard addition method. Under the conditions of this experiment, the interday coefficient of variation was less than 10%. The detection limits were 5 µg Cr/L blood or urine, 2 µg Pb/L blood, and 7 µg Pb/L urine.

6.3 RESULTS AND DISCUSSION

Chromium in the lungs
The chromium concentration in the lungs of 29 control animals was 4.6 ± 4.9 ng/g wet tissue weight (mean ± SD). No speciation was made with respect to the valence of chromium. Exposure to sodium chromate, zinc chromate, or lead chromate resulted in a dramatically enhanced chromium concentration in the lungs, as shown in *Figure 6.2*.

Sodium and lead chromate showed marked accumulation after repeated exposure, whereas zinc chromate did not, possibly as a result of the decreasing exposure level during the 4-day period *(see Figure 6.1)*. The high chromium concentrations in the lungs, even after an exposure-free period of 4 days, indicated that the half-time of clearance from the lungs is relatively long. In the experiments with intratracheal exposure, we found values of 2.4, 1.9, and 1.8 days for the respective chromates in the early phase of lung clearance [Bragt and Van Dura (1983)]. For sodium bichromate dust, a half-time of 30.5 days has been reported

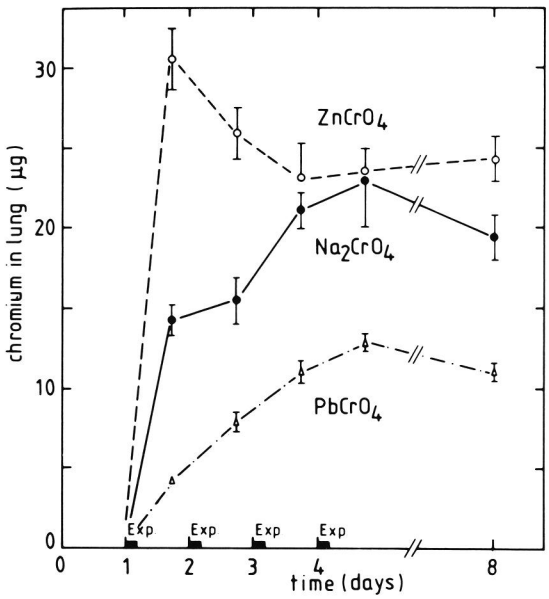

Figure 6.2 Total amount of chromium in rat lungs as a result of 1-4 exposures (exp.), or 4 exposures followed by 4 days recovery (day 8). Values are means ± SD of 10 animals.

for the second phase of lung clearance [Suzuki et al. (1984)]. At necropsy in six former chromate workers and at surgery in two workers, an average chromium concentration of 36.7 µg/g wet weight (range 0.5-130) was found in peripheral lung tissue. This indicates that chromium was retained long after the cessation of exposure, because in a formerly unexposed patient a concentration of 0.21 µg/g lung tissue was found [Tsuneta et al. (1980)].

Chromium in blood

In 30 control rats, the mean chromium concentration was lower than 9 µg/L (range <5-59). *Figure 6.3* shows that after the first exposure episode to sodium chromate and zinc chromate, the chromium concentration in blood increased rapidly.

After 2-3 exposures, an equilibrium between absorption and elimination was established. The chromium concentration in blood was only slightly elevated as a result of repeated exposure to lead chromate. From the exposure-free period a half-time of chromium in blood was roughly estimated at 5-9 days. As shown in *Figure 6.3*, the increase of the chromium concentration in the blood as a result of the second to fourth exposures is not indicative of the respective exposure levels.

Chromium in urine

The chromium concentration in the urine of 30 unexposed animals was 94 ± 134 µg Cr/L (mean ± SD, range 7-680) or 30 ± 31 µg/g creatinine (range 3.6-123.2).

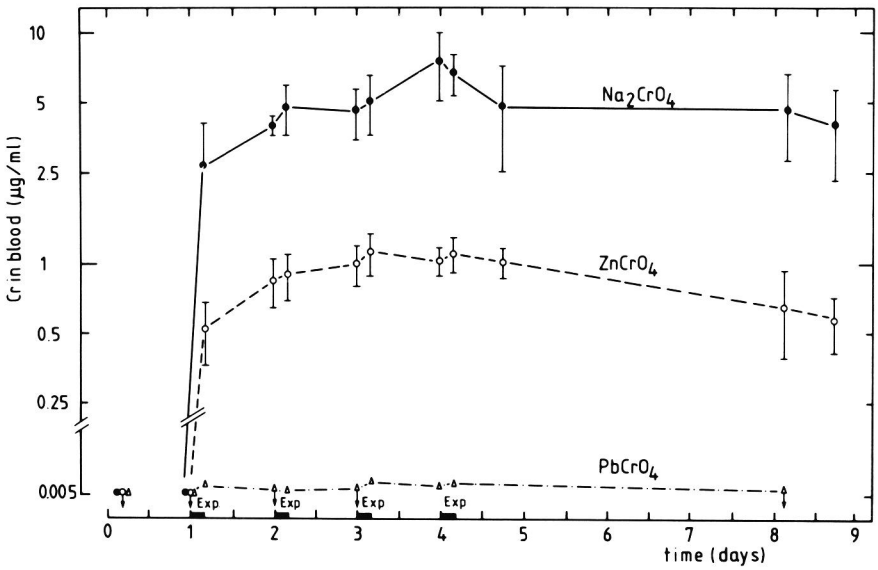

Figure 6.3 Chromium concentration in the blood of rats (log scale) as a consequence of repeated inhalatory exposure (exp.) to three chromates. The range of chromium concentrations in the blood of unexposed animals was <5-59 ng/ml (n = 30). An arrow indicates that individual values are below the limit of detection (i.e. 5 ng/ml). Each point is the mean ± SD of 10 animals.

The first exposure episode resulted in greatly enhanced chromium concentrations in the urine with either chromate *(Figure 6.4)*. An exposure-free period of 4 days resulted in a significantly decreased chromium concentration ($P < 0.001$, two-sided Wilcoxon test).

The estimated half-time of chromium in the urine was 2.4-6.2 days. The pattern of chromium excretion in the urine did not resemble the pattern of exposure *(Figure 6.1)*, possibly because an equilibrium between uptake and elimination had not yet been established. As a consequence of the high exposure levels the urinary chromium concentrations were significantly elevated ($P < 0.02$, two-sided Wilcoxon test) even with lead chromate. The present (1988) limit value for lead chromate in the Netherlands is 0.05 mg Cr/m^3 (maximum accepted concentration, 'MAC-value'),* which is more than a hundredfold lower than the lead chromate concentration inhaled by the rats in the present study. This implies that urinary chromium concentrations in workers exposed to lead chromate are expected not to be increased significantly. Relevant human data are lacking, however.

* Note added: In the 1994 MAC-list the MAC-value for lead chromate has been reduced to 0.025 mg/m^3 (TWA-15 min).

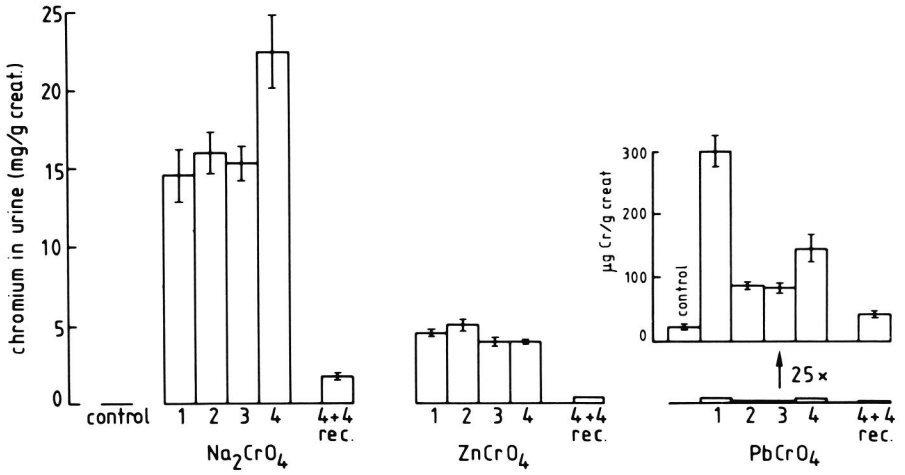

Figure 6.4 Chromium concentration in the urine of rats, corrected for creatinine excretion, after 1-4 exposure periods (days 1-4), and after 4 exposures followed by an exposure-free period of 4 days (4 + 4 rec.). Control animals had a mean concentration (± SD) of 30 ± 31 µg/g creatinine (n = 30). Each bar represents the mean ± SD of 10 rats.

Chromium in faeces

In unexposed rats the mean chromium concentration in the faeces was 33.3 ± 21.2 µg/g dry material (range 17.5-47.9, n = 30). After exposure to the three chromates, the faecal chromium concentration was strongly increased, as shown in *Figure 6.5.*

After the exposure-free period of 4 days, the concentrations decreased by more than 85% when compared with the concentration after four subsequent exposure periods. This means that the chromium concentration in the faeces is indicative of recent exposure. The faecal chromium concentrations are the result of distal deposition in the respiratory tract, transport by the mucociliary escalator, and passage through the gastrointestinal tract. However, ingested chromium caused by the rats licking their fur might have contributed to the faecal chromium concentration, because whole-body exposure took place [Langard and Nordhagen (1980)].

Lead in blood, urine, and faeces from lead chromate exposed animals

The average concentration in the blood from 10 unexposed rats was lower than 60 µg/L (range: not detectable-267 µg/L). After the first exposure episode, the lead concentration increased to 197 ± 151 µg/L (mean ± SD of five rats), and to 416 ± 133 µg/L after four exposures. The exposure-free period resulted in an average lead-in-blood concentration of <120 µg/L (range: <2-325 µg/L).

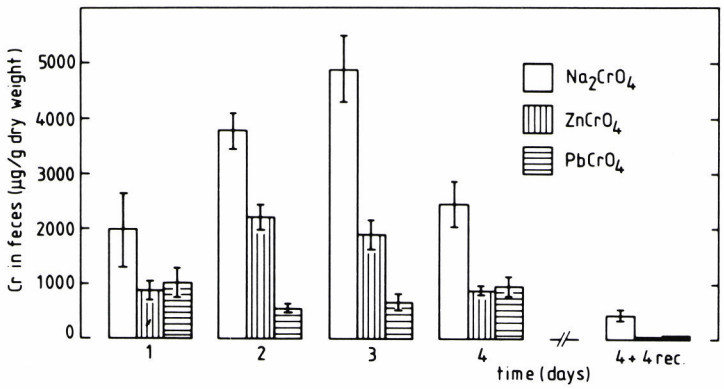

Figure 6.5 Faecal chromium concentration after 1-4 periods of exposure (days 1-4), and after 4 exposures followed by an exposure-free period of 4 days (4 + 4 rec.). Control rats had a concentration (mean ± SD) of 33.3 ± 21.2 µg Cr/g dry faeces. Each bar represents the mean ± SD of 10 animals.

In 10 unexposed rats the urinary and faecal lead concentrations were 456 ± 62 µg/L and 19.3 ± 6.2 µg/g dry material, respectively. After four exposures to lead chromate, the lead concentrations increased to 510 ± 319 µg/L urine and 4800 ± 2900 µg/g dry faeces, followed by a marked decrease as a result of the exposure-free period to 228 ± 112 µg/L urine and 719 ± 112 µg/g dry faeces (n = 5).

The atomic ratio of chromium and lead in the faeces was 7.6 in unexposed animals and 1.0 in animals with four exposure episodes with and without the recovery period of four days. This means that lead chromate in the faeces of exposed animals is most probably the result of secondarily (and primarily) ingested material and not of selective excretory mechanisms (e.g. chromium in the bile).

Furthermore, the data show that the lead concentration in the blood of repeatedly exposed rats is not dramatically increased, as might be expected from the high levels of exposure to lead chromate. This is consistent with the results of the chromium analyses.

6.4 CONCLUSIONS

Sodium chromate (solubility in water: 81 g/L) is readily absorbed after inhalation and this results in an increased chromium concentration in blood, even after one exposure. Excretion of chromium into the urine is greatly enhanced after exposure. In fact, the same is true for zinc chromate (solubility about 0.6 g/L), although the blood and urinary concentrations are lower at the same exposure level.

Lead chromate, which is practically insoluble in water (only 58 µg/L) accumulates in the lungs after repeated exposure. Dissolution in the lungs is most probably a rate-limiting factor for absorption, in view of the low chromium and lead concentrations in blood and urine even at a high level of exposure. Excretion

via the faeces was pronounced with all three chromates. An important factor may be ingestion of chromates when rats lick their fur.

If these results are extrapolated to man, the suggestion emerges that biological monitoring of chromium in urine is indicative of a recent exposure to chromates having solubilities in between that of sodium chromate and zinc chromate. The concentration of chromium in the urine decreases with decreasing solubility, at the same exposure level.

At the present (1988) occupational exposure limit for lead chromate of 0.05 mg Cr/m^3,* lead-in-blood and chromium-in-urine concentrations are too low to be indicative of exposure. However, a major advantage of biological monitoring is that it may reflect internal exposure. Thus, the low concentrations of lead in blood and chromium in urine may indicate that the risk of systemic health effects after exposure to lead chromate (e.g. nephrotoxicity), is considerably lower than after comparable exposure to the more soluble chromates.

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* See note page 63.

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ACKNOWLEDGEMENT

The authors thank Dr. Paul G.J. Reuzel, Department of Biological Toxicology, CIVO-TNO, Zeist, for performing the animal exposures and collecting biological samples, and Frans J.A. Kouwenberg for his skilled assistance.

This study was supported financially by the Dutch Directorate General of Labour, Ministry of Social Affairs and Employment, as a part of the program 'Development Biomonitoring Methods'.

CHAPTER 7

USE OF ZINC PROTOPORPHYRIN IN SCREENING INDIVIDUALS FOR EXPOSURE TO LEAD *

W.C.M. Zwennis, A.Ch. Franssen and M.J. Wijnans

We studied the relation between the concentrations of lead in blood (PbB) and zinc protoporphyrin in blood (ZPP) in a group of 801 men occupationally exposed for more than one year to lead or inorganic lead compounds. Linear regression of PbB on log ZPP provided 95% tolerance intervals for PbB values for a given ZPP value. The intervals we found are too large to warrant the estimation of PbB on the basis of ZPP measurements in health surveillance of lead workers. Instead we propose a procedure in which ZPP can be used as an indicator to decide which individuals exposed to lead need further investigation of PbB in light of existing action/limit values for PbB. The procedure is applicable only for PbB values of 2.4 μmol/L or more but may reduce considerably the costs for screening individuals or groups of people exposed to lead.

Additional keyphrases: reference values - receiver-operating characteristic curves

7.1 INTRODUCTION

Exposure to lead and its inorganic compounds leads to an increased concentration of lead in blood (PbB). It has now generally been accepted that, under conditions of more or less constant and prolonged exposure, PbB reflects the quantity of 'biologically active' lead in the body [WHO (1977); Zielhuis and Wibowo (1978); Alessio and Foà (1980); NRC (1980)]. Analysis of PbB is, therefore, the first choice for the assessment of internal exposure of lead. Moreover, assessment of health risks due to exposure to lead is generally based on the concentration of lead in blood. However, determination of PbB is expensive and time-consuming, especially if large groups of people have to be screened.

As early as the 19th century, patients suffering from lead intoxication were recognized as showing symptoms of effects by lead on the formation of haem [Stokvis (1895)]. These effects have been investigated extensively [Moore et al. (1980)]. Lead inhibits at least two enzymes that are essential for the formation of haem, namely, δ-aminolaevulinic acid dehydratase (ALAD; EC 2.6.1.43) and ferrochelatase (EC 4.99.1.1). Both the inhibition of ALAD in erythrocytes and the

* Published in Clinical Chemistry 36:1456-1459 (1990).

resulting increase in excretion of the substrate δ-aminolaevulinic acid in urine are used for early detection of the biological responses to lead, and indirectly as a measure of increased internal exposure of lead. Because of the interaction of lead with ferrochelatase in bone marrow, no iron is inserted into the substrate protoporphyrin IX, so that the concentration of the latter is increased in erythrocytes. Lamola and Yamane (1974) showed that instead of iron, zinc is incorporated into this free erythrocyte protoporphyrin to form zinc protoporphyrin (ZPP). ZPP thus reflects an effect of lead on the haematopoietic system, mainly resulting from the deposits of lead in the bone marrow. PbB, on the other hand, reflects both recent and earlier exposure. Numerous authors [see review by Wildt et al. (1987)] have shown that under steady-state conditions of exposure the logarithm of ZPP is linearly related to PbB. Iron-deficiency anaemia may also result in an accumulation of ZPP, whereas in erythropoietic protoporphyria, a rare inborn error of metabolism, the increased protoporphyrin in erythrocytes remains mainly unchelated.

The concentration of ZPP can be measured directly in a smear of blood, which can be drawn from an ear lobe or obtained by a finger prick. Because of the simplicity of the measurement, which does not require specially qualified personnel, determination of ZPP has been applied as another method to screen lead workers for a biological response to an increased exposure to lead [Grandjean and Lintrup (1978); Alessio and Foà (1980); Herber (1980); Wildt et al. (1987)]. Here we further contribute to the discussion on the validity of using the concentration of ZPP to estimate PbB. We propose using the ZPP value and the statistical correlation between PbB and ZPP to decide, on an individual basis, whether additional analysis of PbB is necessary to assess if someone has an increased health risk due to lead exposure.

7.2 SUBJECTS AND METHODS

We studied 801 men (ages 16-64 years) employed in lead-processing industries and exposed to lead or inorganic lead compounds for at least one year. Of this group, 555 were Dutch by birth, the others were of mediterranean origin. The men were involved in the production of batteries, pigments, or soldered cans or were employees of metal-recycling industries. Most of them have been checked for lead in blood several times over the last few years; for those cases, we used the first measurement in this study. In general, the individual PbB values showed relatively little variation over time. In view of the nature and time of exposure (more than a year), steady-state concentrations of lead in the bone marrow are likely.

Venous blood was collected in 5-ml lead-free tubes containing 7.5 mg of EDTA (Venoject; Terumo, Italy). We mixed 100 µl of blood with 900 µl of 1 Mol/L nitric acid solution with swirling on a shaker. The precipitate was centrifuged and the lead in the clear supernate was determined by atomic absorption spectrophotometry (Perkin-Elmer, Model 4000, equipped with a Model 500 graphite atomizer)

[Stoeppler et al. (1978)]. The quality of the analysis was checked by participation in the U.K. External Quality Assessment Scheme of the Queen Elizabeth Hospital, Birmingham, U.K. At three-week intervals, we analyzed lead-supplemented human blood samples. On the average, our results differed by 7.7% (range 0 - 22.6%) from the average results of about 80 participants, but there was no systematic deviation. We determined the concentration of ZPP with a direct-reading haematofluorometer (Environmental Sciences Associates; Bedford, MA, U.S.A., Model 4000) after oxygenation of a 100 µl blood sample by swirling on a shaker. The apparatus, which measures the ratio of the fluorescence of ZPP and the absorbance of light by oxyhaemoglobin in the sample, was calibrated at a fixed concentration of haemoglobin (Hb) of 8.4 mmol/L (for men). The coefficient of correlation (CV) for 15 analyses of the same sample carried out on five successive days was 2.6% at a ZPP content of 95 µmol/mol Hb and about 1.5% at concentrations of ZPP between 150 and 625 µmol/mol Hb.

The 95% tolerance intervals for PbB as calculated for log ZPP *(Figure 7.1)* are the simultaneous tolerance intervals of Lieberman Miller [cf. Miller (1981)], based on the regression of PbB on log ZPP. For PbB, 1 µmol/L corresponds to 207 µg/L; for ZPP, 1 µmol/mol Hb corresponds to 0.04 µg/g Hb.

7.3 RESULTS AND DISCUSSION

Figure 7.1 shows the relation between PbB and log ZPP. The highest concentration of PbB was 6.2 µmol/L and of ZPP 1253 µmol/mol Hb. Assuming a linear relation, the regression of log ZPP on PbB gives the following equation: log ZPP = 0.56 PbB + 0.86 ($r = 0.83$, $P < 10^{-4}$). This relation is in good agreement with other studies [Wildt et al. (1987)].

Table 7.1 lists 95% tolerance intervals for PbB at four different concentrations of ZPP, obtained by regressing PbB on log ZPP separately for the Dutch workers, the mediterranean workers, and the whole group. Given the considerable overlap of the intervals of the Dutch and the mediterranean workers, we decided to treat the three groups of workers as a single population. Considering the four occupations studied – production of batteries, of pigments or of soldered cans, and metal recycling – separately only slightly narrows the PbB tolerance intervals. The upper values remain more than twice the lower values.

The large ZPP-related intervals for PbB preclude an acceptable estimation of the concentration of PbB on the basis of ZPP. The width of the intervals is partly explained by the absence of a strong biological relation between PbB and ZPP. Close correlation between PbB and ZPP exists only under steady-state conditions of exposure because of the time lag in the formation of ZPP [Hernberg (1980)]. Although the exposure of most of the present lead workers meets this requirements, as shown by analysis of blood samples over several years, other factors probably also influence the width of the intervals, for example:

- The kinetics of lead absorption and formation of ZPP may differ among people.
- Iron deficiency contributes to an increase in ZPP [Lamola and Yamane (1974)].
- Interindividual variability affects the kinetics of formation of ZPP.
- Increased concentrations of bilirubin in serum have a small, positive effect on the ZPP reading [Grandjean and Lintrup (1978)].
- Analytical variation in the analysis of both analytes, especially PbB, may affect the results. The order of magnitude of these variations have already been given.

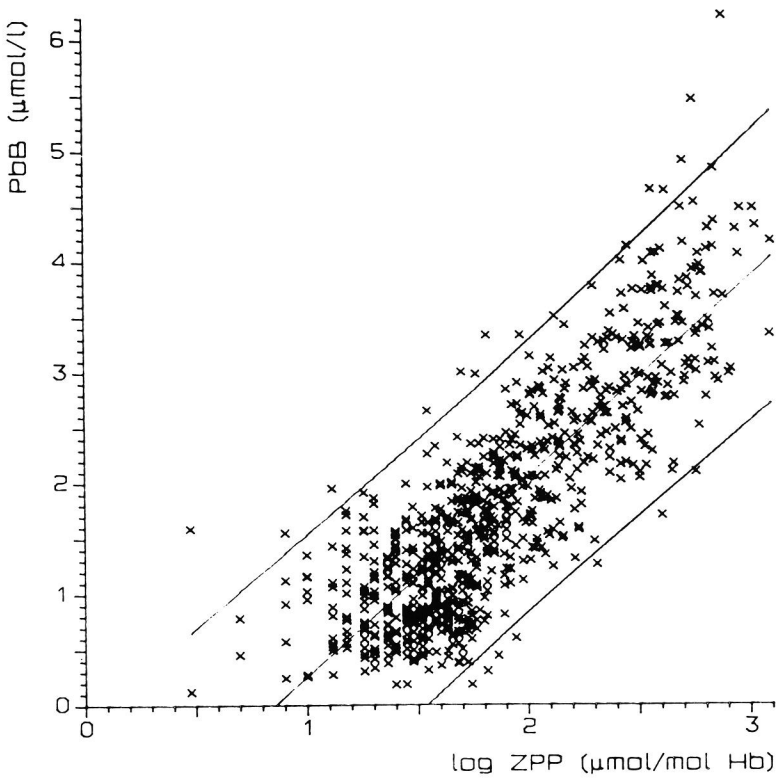

Fig. 7.1 Scatter diagram and 95% tolerance intervals of 801 PbB and ZPP pairs.

In 1982 the Council of Ministers of the European Community issued a Directive on the protection of workers from health risks due to exposure to lead [EC (1982)]. For PbB, three so-called action levels (1.45, 2.4 and 2.9 µmol/L) and a limit value (3.4 µmol/L) have been defined. Concentrations of PbB between 3.4 and 3.9 µmol/L are considered acceptable if ZPP is <500 µmol/mol Hb.

Because for many ZPP values the resulting PbB intervals include the action or limit values as given in the European Community Directive, giving rise to indecisive worker policy, we conclude that an acceptable estimate of PbB by measuring ZPP is not possible in health surveillance of individual lead workers.

TABLE 7.1 95% TOLERANCE INTERVALS FOR PbB AT DIFFERENT CONCENTRATIONS OF ZPP

	PbB intervals (μmol/L)		
	Dutch workers	Mediterranaen workers	All workers
ZPP (μmol/mol Hb)	(n = 555)	(n = 246)	(n = 801)
250	1.48-4.05	1.44-4.09	1.52-4.02
500	2.00-4.64	1.88-4.63	2.03-4.58
750	2.31-4.99	2.13-4.95	2.33-4.92
1000	2.52-5.23	2.31-5.17	2.54-5.16

Individual ZPP values may be used only as a very rough indicator of individual PbB, and the average ZPP of a group of workers only approximates the average PbB. This conclusion is supported by the work of Grandjean and Lintrup (1978) and Herber (1980). Accepting PbB as the best marker to estimate health risk implies that every worker who is possibly exposed to lead should be monitored for PbB at regular intervals. For this reason, in the U.S.A. as well as in the European Community assessment of PbB is required in workplace monitoring. However, detection of individuals with high PbB concentrations by screening for ZPP would be desirable because the latter is considerably less costly. Therefore, we further analyzed the relationship between PbB and ZPP.

Using the PbB and ZPP data of the 801 lead workers, we calculated the sensitivity and specificity of different ZPP cutoff points at the five standard values of PbB mentioned in the Directive of the European Community. (Within this context, sensitivity and specificity refer to the epidemiological characteristics of the relationship between PbB and ZPP. That is, the sensitivity is defined as the percentage of the cases with ZPP values above a chosen cutoff point, given a PbB value above a chosen standard value. The specificity is defined as the percentage of the cases with ZPP values below a chosen cutoff point, given a PbB value below a chosen standard value. These percentages depend on the characteristics of the population under study).

Appropriate cutoff points for ZPP at the five PbB standard values can be derived from plots of sensitivity against 1-specificity, the so-called receiver-operating characteristic (ROC) curves *(Figure 7.2)* [Metz (1978)]. The optimum cutoff point is determined as the point the farthest from the diagonal; this cutoff point maximizes the discriminative power of the test by minimizing the fractions of false-positive and false-negative results. *Table 7.2* gives the optimum ZPP cutoff points at the five PbB standard values as derived from *Figure 7.2.*

Screening with the aim of meeting the above Directive demands a sensitivity of 100% because every person with a PbB concentration exceeding an action level or the limit value should be identified. To have a sensitivity of 100%, one must use a lower cutoff point of ZPP and thus a lower specificity, leading to suboptimal discriminative power *(Table 7.2). Table 7.2* shows also that ZPP cannot be used as a discriminative indicator for the lowest PbB standard value (specificity = 0%).

TABLE 7.2 ZPP CUTOFF POINTS FOR OPTIMUM AND REQUIRED SENSITIVITY, AND RELATED SPECIFICITY, AT FIVE VALUES FOR PbB

PbB[b] (μmol/L)	ZPP[a] (μmol/mol Hb)	Optimum %		ZPP[a] (μmol/mol/Hb)	Required %	
		Sens.	Spec.		Sens.	Spec.
1.45	55	80	87	0	100	0
2.4	90	95	84	40	100	46
2.9	170	90	90	45	100	47
3.4	250	94	88	120	100	77
3.9	250	100	86	250	100	86

a. ZPP cutoff points as derived from Figure 7.2.
b. Action and limit values specified in European Community Directive (see text).

In that case all samples would have to be analyzed for PbB. This parallels the report of Meredith et al. (1979).

The positive predictive value of a screening result (i.e. the fraction of the samples for which PbB exceeds the standard PbB value of all samples for which ZPP exceeds the chosen cutoff point) depends not only on sensitivity and specificity, but also on the prevalence in the study population of values of PbB that exceed the standard value under consideration: the lower the prevalence, the greater the number of false-positive tests results. *Table 7.3* presents positive predictive values of ZPP cutoff points that yield a sensitivity of 100% at different prevalences.

TABLE 7.3 POSITIVE PREDICTIVE VALUE (PPV) OF ZPP CUTOFF POINTS AT DIFFERENT PREVALENCES OF FOUR VALUES FOR PbB

	PbB (μmol/L)							
	2.4		2.9		3.4		3.9	
ZPP (μmol/mol Hb)	40		45		120		250	
Sens. %[a]	100		100		100		100	
Spec. %[b]	46		47		77		86	
	Prev. %	PPV	Prev. %	PPV	Prev. %	PPV	Prev. %	PPV
	5	9	2	5	2	7	1	7
	10	17	4	9	5	19	2	13
	15	24	8	17	10	33	5	27
	20	31	10	21	15	43	10	44
	25	38	15	30	20	52	15	56
	30	44	20	37	25	59	20	64
	35	50	25	44	30	65	25	70
	40	55	30	51	40	74	30	75
	60	73	40	61	50	81	40	83
			50	70				

a. Sensitivity as required by the European Community Directive.
b. Specificity as derived from Figure 7.2.

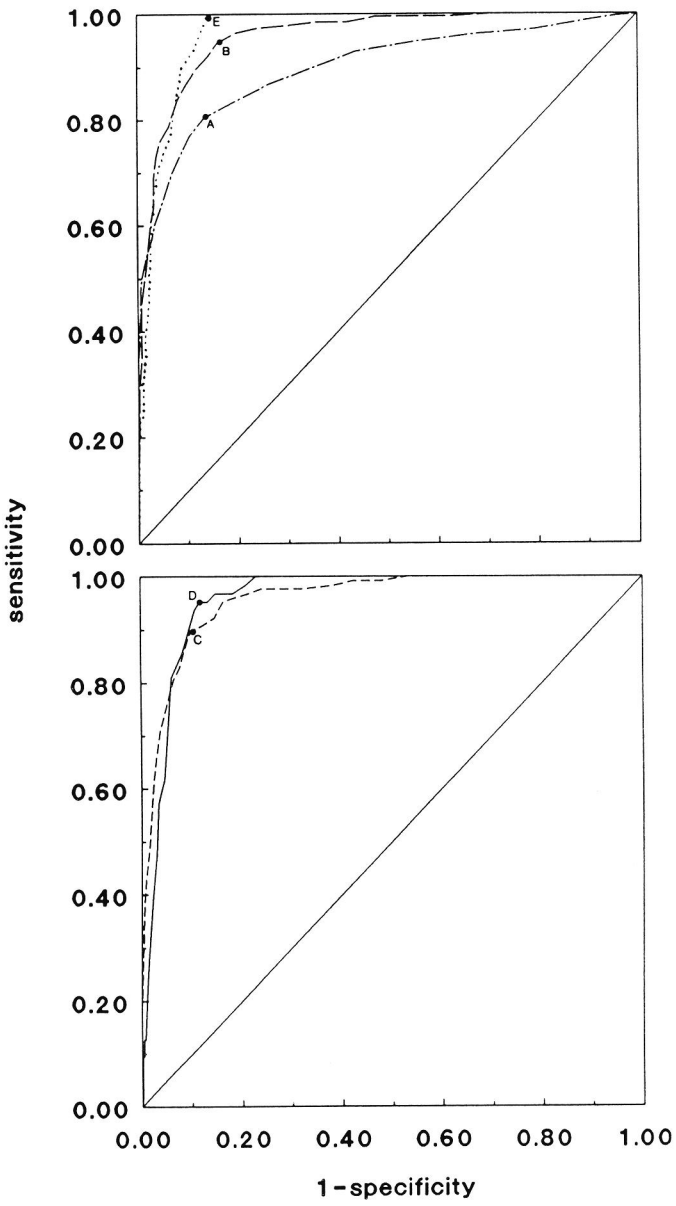

Figure 7.2 Receiver-operating characteristic curves for ZPP at the five standard PbB values of the European Community Directive: 1.45 µmol/L (—·—·—); 2.4 µmol/L (— — —); 2.9 µmol/L (– – – – –); 3.4 µmol/L (———); 3.9 µmol/L (·····).
The points in the Figures indicate the optimum cutoff points for ZPP and denote the values at maximum sensitivity and specificity: A = 55; B = 90; C = 170; D = 250; and E = 250 µmol/mol Hb.

ZPP can be used satisfactorily for pre-screening only if one can estimate reasonably accurately the prevalence of the standard PbB values in the population under study. Recently, health hazard surveys carried out in several lead-processing industries in the Netherlands [Zwennis (1985)] have reported prevalences of 60%, 33%, 19%, and 10% for PbB >2.4, >2.9, >3.4, and >3.9 μmol/L, respectively, in battery works (n = 200); 25%, 14%, 7%, and 3%, respectively, in pigment production (n = 556); and 100%, 87%, 87%, and 87%, respectively, in flame cutting of lead-painted constructions (n = 9) [*this thesis, Chapter 8*]. Rifle instructors showed no values for PbB >2.4 μmol/L (n = 125). Hernberg and Tola (1979) reported decile distributions and ranges of PbB values found in different types of work in Finland. These results allow an estimate of the prevalences in groups of lead workers.

The ultimate goal of the use of ZPP as a screening method is an economical one. Does the method save labor and time over screening via PbB?

Potential savings can be computed with the formula $S = nP - (nZ + fP)$, where S = savings, n = the number of samples, P = the cost of a PbB analysis, Z = the cost of a ZPP analysis, and f = the number of samples with true- and false-positive ZPP values that require additional PbB analysis.

Figure 7.3 gives the relation between savings and percentage of positive ZPP values, assuming that the cost of a ZPP analysis is about 10% of the cost of a PbB analysis, which is the case with our laboratory. Savings vary between 10% 'negative savings' (when all samples have positive ZPP values) and 90% of the costs of PbB analysis (when no samples have positive ZPP values).

In summary, measuring ZPP to screen lead workers for a PbB of 1.45 μmol/L has no economical value. All such samples must also be analyzed for PbB. However, screening for ZPP to detect the higher action levels and the limit values for PbB may save considerable costs.

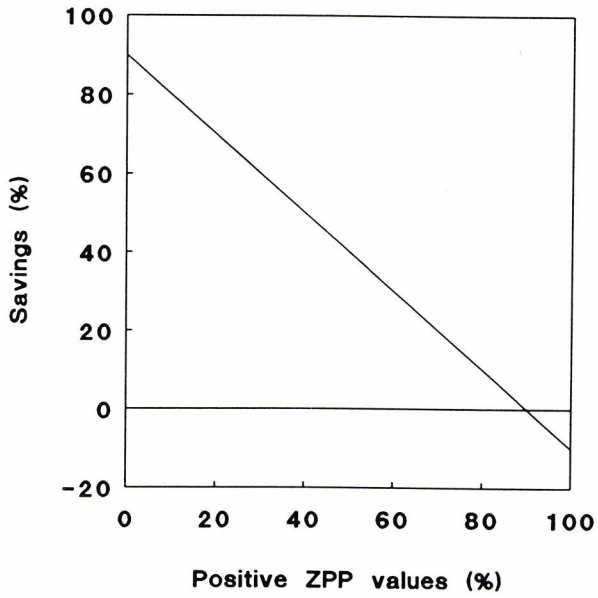

Figure 7.3 Savings in screening with ZPP instead of PbB for exposure to lead, assuming that costs of analysis are 10% of that for PbB.

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ACKNOWLEDGEMENT

The results in this study were obtained from a health hazard survey in lead-processing industries, financed by the Directorate General of Labour of the Ministry of Social Affairs and Employment in the Netherlands. We thank Mr. F.J.A. Kouwenberg and Mrs. M.E. van Pijkeren for their skillful assistance in carrying out the analyses of lead and zinc protoporphyrin, and Mr. J.F. Bleichrodt, Dr. J.J. van Hemmen, and Prof. Dr. R.L. Zielhuis for advice in preparing this manuscript.

CHAPTER 8

LEAD EXPOSURE DURING DEMOLITION OF A STEEL STRUCTURE COATED WITH LEAD-BASED PAINTS

I. ENVIRONMENTAL AND BIOLOGICAL MONITORING *

T. Spee** and W.C.M. Zwennis

Demolition of a steel railway bridge was carried out by nine workers using flame-torch cutting. The air in the breathing zone of the workers contained from 2 to 38 mg of lead/m^3, which is a very high level in comparison with the Dutch exposure limit of 0.15 mg/m^3 (MAC, 8 h time-weighted average). Without very effective respiratory protection these concentrations may result in acute lead poisoning. Upwind of the flame-torch the exposure level was below the exposure limit, whereas downwind lead concentrations of up to a hundred times the exposure limit were observed. Although filtering facepieces were used by the workers, average blood lead concentrations of about 4.5 µmol/L were rapidly attained. Possibly under these work conditions this value represents a maximum concentration attainable in blood. After termination of the exposure, there was a fast decrease of lead in the blood. This finding indicates that lead was mainly present in rapidly exchangeable compartments like blood. No stable correlation between the concentration of lead in blood and the concentration of zinc protoporphyrin in blood was found.

Key terms: industrial hygiene, occupational hygiene, zinc protoporphyrin

8.1 INTRODUCTION

Although it is known that the flame cutting of lead-painted steel constructions may cause severe lead poisoning [Feldman et al. (1977); NIOSH (1980) Fischbein et al. (1984)], exposure to lead fumes during this type of work still occurs. Such exposure may be due to the fact that the workers are insufficiently aware of the risk involved or the fact that the respiratory protection of the workers may be insufficient or may turn out to be difficult in practice.

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* Published in Scandinavian Journal Work & Environmental Health 13:52-55 (1987).

** Present adress: Arbouw Foundation, Amsterdam, the Netherlands.

The Dutch Labour Inspectorate has requested the TNO Medical Biological Laboratory to monitor lead exposure of workers involved in the demolition of a railway bridge. At the same time the health effects due to exposure to lead were studied through measurements of the delay and repair of nerve conduction velocity in the workers [Muijser et al. (1987)].

The arched steel railway bridge, built in 1902, consisted of two fixed bridges and a swivel bridge. Its total length was 265 m and the maximum height of the arches amounted to about 11.5 m. The bridge had been painted seven times with a 0.1 mm coating of a primer containing 60% lead and with a 0.02-mm top coat containing green chromium oxide. The bridge was taken down during the period October 1983 through February 1984 by flame-torch cutting (oxypropane flame). Seven workers cut on the bridge, and two cut parts to smaller pieces. One of the latter worked mainly on a deckship, the other mainly on the bank of the canal.

Several authors have reported on the relationship between the concentration of lead in the breathing zone (PbA) and that in the blood (PbB) of occupationally exposed workers. According to Coenen and Drasche (1978) and Alessio and Foà (1980) there is a linear relationship in groups of workers for PbA up to 0.2 mg/m^3. However, on an individual basis the PbB concentration cannot be derived from the PbA concentration [Gartside et al. (1982)]. Unpublished investigations by us in three lead-battery factories support the latter observation.

8.2 SUBJECTS AND METHODS

Air sampling

Air was sampled from the breathing zone by pumping air through a stainless steel filter holder fixed to the lapel of the worker (personal air sampling). The holder, with an opening 7 mm in diameter, contained a cellulose nitrate membrane and was connected to an air sampling pump (Du Pont P 2500) by Tygon tubing. The flow rate was 2 L/min. With this equipment particles with an aerodynamic diameter up to 30 μm are sampled. This method meets the criteria specified in the directive on inorganic lead issued by the Council of the European Community [EC (1982)].

Large air samples were taken by means of portable high-volume samplers (Portikon, Sartorius) at a flow rate of 200 L/min.

The lead concentrations were determined by atomic absorption spectrophotometric analysis (Varian Techtron AA 9751) after the filters were dissolved in *aqua regia*. The measurements were performed during dry weather and wind velocities of 1-2 m/s.

Analysis of lead and zinc protoporphyrin in blood

Venous blood was collected in 5-ml lead-free tubes containing 7.5 mg of ethylenediaminetetraacetate-disodium salt (EDTA-2Na) (Venoject, Terumo). The PbB concentration was determined after addition of 100 μl of blood to 900 μl of

1 Mol/L nitric acid and swirling [Stoeppler et al. (1978)]. The precipitate was centrifuged, and in the clear supernatant the lead was determined by atomic absorption spectrophotometric analysis (Perkin Elmer Model 4000, HGA Model 500). The day-to-day coefficient of variation of the method was 7% at 2.07 µmol/L and 6% at 3.75 µmol/L (N = 17).

The concentration of zinc protoporphyrin (ZPP) was determined with a direct-reading haematofluorometer (Environmental Sciences Association, model 4000) after oxygenation of a 100 µl blood sample by swirling on a shaker.

8.3 RESULTS

Environmental monitoring

Table 8.1 shows the concentration of lead in the breathing zone of the workers for three periods on the same day, 13 weeks after the start of the demolition. Workers 1 to 4 were engaged in flame cutting on the bridge. Worker 5 was cutting demolished parts of the bridge into smaller pieces on a deckship.

Air samples were also taken upwind and downwind of the torch simultaneously. The results are shown in *Table 8.2.*

Biological monitoring

The PbB and ZPP concentrations were determined in blood collected before the work started (between weeks 0 and 2), four times during the demolition (weeks

TABLE 8.1 LEAD CONCENTRATIONS IN THE BREATHING ZONE OF FIVE WORKERS[a] DURING THE DEMOLITION OF A LEAD-PAINTED STEEL STRUCTURE

Worker	Lead concentration (mg/m^3)		
	Period 1 (08.00-09.45)	Period 2 (10.15-12.30)	Period 3 (13.15-14.45)
1	10.8	..[b]	..[b]
2	32.3	17.3	5.7
3	20.5	2.3	3.2
4	38.1	13.4	6.0
5	3.1	3.3	3.7

a. Sampling on January 20 1984.
b. Measurement not successful.

TABLE 8.2 RESULTS OF SIMULTANEOUS SAMPLING UPWIND AND DOWNWIND AT A DISTANCE OF 50 CM FROM THE FLAME TORCH. THE SAMPLING WAS PERFORMED DURING PERIOD 2 (TABLE 8.1) BY MEANS OF PORTABLE HIGH-VOLUME SAMPLERS[a]

Worker	Sampling time (min)	Lead concentration (mg/m^3)	
		Upwind	Downwind
1	7	0.08	1.7
2	14	0.06	14.0

a. In order to avoid disturbing effects, only one worker was at work during the sampling.

7-20) and two times after the job was finished (weeks 39 or 40 and week 89). Due to absence some workers did not participate in the sampling on every occasion. The results are presented in *Table 8.3* (PbB) and *Figure 8.1* (ZPP).

TABLE 8.3 BLOOD LEAD CONCENTRATIONS (μMOL/L) OF THE WORKERS DURING DEMOLITION OF A RAILWAY BRIDGE

	Week						
Worker	0 or 2	7 or 8	11[a]	14 or 15	20[b]	39 or 40	89
1	1.7	4.5	5.1	4.2	5.0	2.6	1.3
2	2.3	...	...	2.7	4.5	3.0	1.5
3	1.9	4.9	5.5	4.2	4.3	2.4	1.4
4	1.3	3.8	4.5	3.8	4.2	2.4	1.1
5	2.5	5.3	3.4	4.7	5.0	3.0	2.7
6	1.9	4.5	3.8	3.3	...	2.1	1.2
7	1.6	3.3	4.4	3.4	3.2	2.3	1.3
8	1.2	4.8	4.3	3.8	...	1.8	...
9	...	3.7	3.1	2.4	2.5	1.6	0.7
Mean	1.80	4.35	4.51	3.61	4.08	2.36	1.40
SD	0.45	0.68	0.82	0.74	0.94	0.49	0.57

a. Season holidays during week 12.
b. The work was completed shortly after week 20.

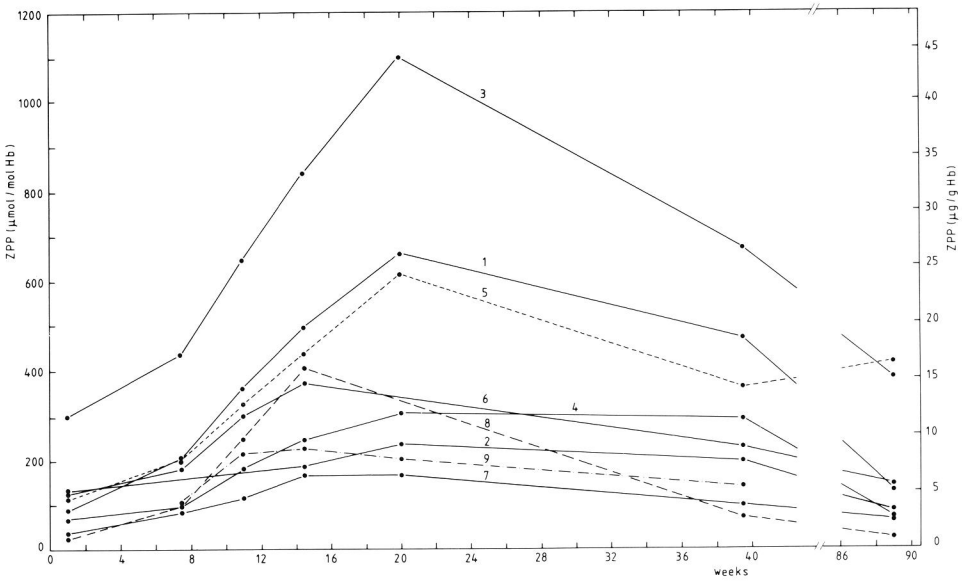

Figure 8.1 Zinc protoporphyrin concentration in the blood of the workers (marked by the number in the figure) during demolition of the railway bridge. (The worker numbers correspond with those in the tables).

8.4 DISCUSSION

During the first three months of the job the respiratory tract of the workers was protected by filtering facepieces. Thereafter half-mask respirators in combination with dust filters, class P2, were available. The results of the environmental monitoring show that concentrations of 100 times the Dutch exposure limit for lead (0.15 mg/m^3, TWA-8 h) were not exceptional. Other authors have also observed high exposure during flame-torch cutting in open air [Feldman et al. (1984); Shackleton et al. (1985)]. Even with protection by a half-mask respirator the situation was unacceptable, since for this type of mask a protection factor of only 10 to 12 is specified [NIOSH (1976); Labour (1983)]. The high exposure of workers 2 and 4 compared to that of workers 1 and 3 may not only have been due to exposure to lead fumes produced by themselves, but also to those produced by their colleagues. Workers 2 and 4 worked downwind of workers 1 and 3 at a distance of about 10 m.

It is often stated that, if a worker stays upwind of the torch, the exposure to lead fumes should remain at an acceptable level. To determine the validity of this statement, we took simultaneous air samples at a distance of 50 cm upwind and downwind of the torch. The results in *Table 8.2* indicate that indeed upwind the exposure is below the exposure limit, the more so because usually the distance between the worker and the torch is about 1 m. However, when working on a 30-cm broad bar, 10 m above ground level, a worker is not always able to take the right position with respect to the wind.

All workers had raised PbB levels (normal value <0.75 μmol/L) and most of them also had raised ZPP levels (normal value <38 μmol/mol Hb) before the work started, and therefore they had evidently been exposed to lead previously. However, during the first weeks of the demolition of the bridge the PbB concentrations increased sharply to 3.5-5.5 μmol/L.

Shortly after the collection of the blood samples in week 11, the work was interrupted for a week of season holidays. After resumption of the work half-mask respirators in combination with a dust filter, class P2, were available to the workers instead of the filtering facepieces used during the preceding period. The average PbB decreased significantly. (Compare weeks 14/15 to week 11; $\alpha = 0.05$, multiple comparison analysis according to Friedman). This finding indicates that the use of the masks resulted in a diminished uptake of lead. Nevertheless the average PbB was still above the value of 2.9 μmol/L and therefore suggested that the protection provided by the half-masks was also insufficient [Shackleton et al. (1985]. However, notwithstanding an exposure that notably exceeded the exposure limit, the average PbB did not exceed 4.5 μmol/L. Apparently, at this level a plateau is reached where a dynamic equilibrium exists between uptake, storage in deposits, and excretion. Such a plateau could explain why worker 5, whose exposure was much lower than that of workers 1-4, showed a similar PbB during weeks 7/8 through 20. No linear relation was found between PbA and PbB.

After the job was finished (week 20) the PbB level decreased, the average concentration in week 89 differing significantly from that in weeks 0 or 2 (t-test for pairs, $P_2 = 0.022$). Apparently, exposure between weeks 20 and 89 was less than during the period preceding the demolition. Usually the workers followed by us are involved in other types of demolition work, demolition of steel constructions being only a minor fraction.

The PbB values in weeks 0 or 2 show that, during the preceding period, exposure to lead was relatively low compared to the exposure during demolition. Other compartments, therefore, played a minor part in the decrement of PbB.

While in week 11 the average PbB reached its maximum, the ZPP concentration was still increasing. Since ZPP is formed in the bone marrow and the life-span of erythrocytes is about 120 days, the exchange of the entire population of erythrocytes requires that length of time. Therefore, at the end of the work in week 20, the concentration of ZPP was still in an increasing phase. The EC-Directive on inorganic lead states a limit value of 3.4 µmol/L for PbB, but a concentration between 3.4 and 3.9 µmol/L is acceptable if the ZPP concentration is lower than 500 µmol/mol Hb [EC (1982)]. With the exception of the value of worker 9, the PbB level of all workers exceeded the first criterion at one or more times.

8.5 CONCLUDING REMARKS

The demolition, by flame-torch cutting, of a steel structure coated with lead-based paints resulted in very high concentrations of lead in the breathing zone of the workers. Without the use of effective respiratory protection such concentrations may result in acute lead poisoning.

Exposure to these high concentrations of lead resulted in a fast increase of the concentration of PbB. After the exposure was terminated, there was a fast decrease in the PbB values indicating that the body burden of lead was mainly present in rapidly exchangeable compartments.

The use of half-mask respirators during the work resulted in significantly lower PbB concentrations. In addition working upwind of the torch also provided a substantially lower exposure to lead.

Exposure to PbA concentrations ranging from 3 to 38 mg/m^3 resulted in average PbB concentrations of about 4.5 µmol/L. This finding might indicate that this value represents an equilibrium concentration attainable in blood under the conditions given.

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ACKNOWLEDGEMENT

This study has been performed on behalf of and financed by the Directorate General of Labour (Ministry of Social Affairs and Employment) in the Netherlands. The dedicated technical assistance of Ms. A.Ch. Franssen, Ms. M.E. van Pijkeren and Mr. J. vd Tuin is gratefully acknowledged. Thanks are also due to Ms. M.J. Wijnans for the statistical analysis of the results.

CHAPTER 9

EFFECT OF TECHNICAL AND HYGIENIC MEASURES ON EXPOSURE TO LEAD IN A LEAD-RECYCLING PLANT: A CASE STUDY *

W.C.M. Zwennis, A.Ch. Franssen, T. Spee ** and A.W. Hol ***

9.1 INTRODUCTION

Occupational exposure to lead (Pb) has decreased considerably during recent decades. This has been effected by adaptations of technical processes as well as by hygienic measures initiated by the increased awareness of the health risks of exposure to lead. However, there is still reason for concern, because in several branches of industry workers are even yet often exposed to levels of Pb which may result in adverse health effects. It has become clear that such effects already may occur at levels which were considered without harm a few years ago [Landrigan (1989); Davis et al. (1993)].

In this study results are presented of technical measures and hygienic provisions on the levels of lead in blood (PbB) of the workers at a plant where scrap-lead is smelted, refined and made into lead sheet.

9.2 SUBJECTS AND METHODS

Housing and production process

The plant was housed in two adjacent halls. In one (100x40 m) the foundry and the rolling departments were housed in two separate sections and in the other (100x20 m) the storehouse and, separated by a wall, the sawing department. The canteen, washing facilities and changing-room were situated next to the rolling department *(Figure 9.1)*.

The stock of scrap-lead (tubings, roof lead, cable lead etc.) was kept in the open air until 1985, after which it was stored in a depot open at one side. The scrap was conveyed with a shovel to a shredder which was situated in the open air. With a conveyor-belt batches of about 100 tons of shredded lead were transported to an oven and smelted at 450°C. Dross like sand, copper etc. was allowed to float to the

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*** Bedrijfsgezondheidsdienst Den Haag e/o, Rijswijk, the Netherlands.

surface. The combustible part was burned by adding diesel oil to the smelt. The remaining was skimmed off; larger objects like wire were removed manually. In 1988 shredding was ended and the scrap was conveyed straight into the smelting oven.

Sixty tons of the smelt was drained along closed gutters into forms where it solidified to lead slabs of circa 6 ton. The smelting oven was filled up and the process started again. The slabs were cold-rolled to lead sheet. This was subsequently rolled up and sawed into the desired lengths. Finally, the rolls were weighed, packed in polyethylene foil and transported to the storehouse.

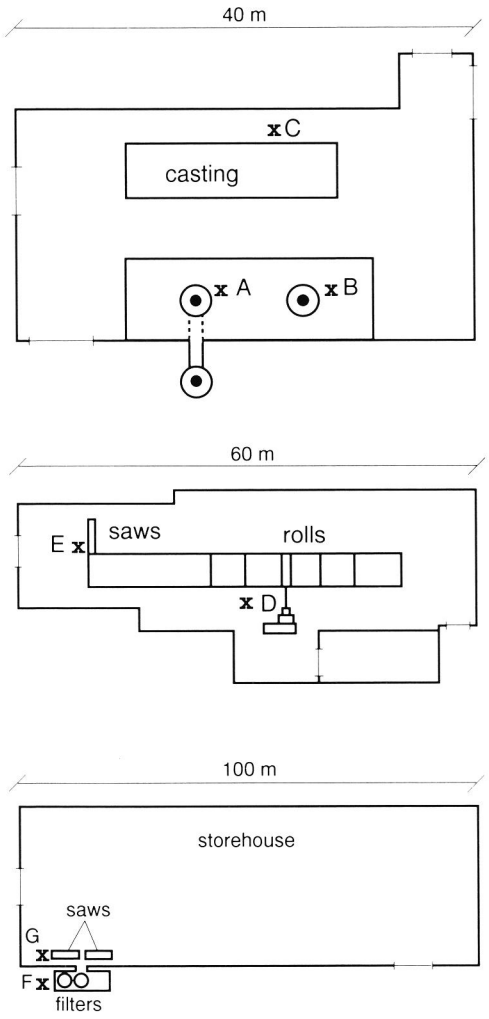

Figure 9.1 Ground-plan of the plant. Measures are not to scale.

In 1982 a refining oven with a capacity of 100 tons of Pb was installed. About 60% of the so-called pre-smelt was pumped into this oven and was refined by the 'Harris procedure'. According to this process sodium nitrate and sodium hydroxide are added at circa 500°C. In this way other metals are converted into their oxides, forming the 'chemical dross'. This was removed from the surface with an enclosed screw conveyor, stored in drums and handled further outside the plant. About 60% of the refined lead, which had a purity of >99.9%, was drained to cast slabs. The refining oven was filled up from the smelting oven and the cycle restarted.

The smelting and refining steps were each carried out two times per 24 hours. An outline of the production process is given in *Figure 9.2*.

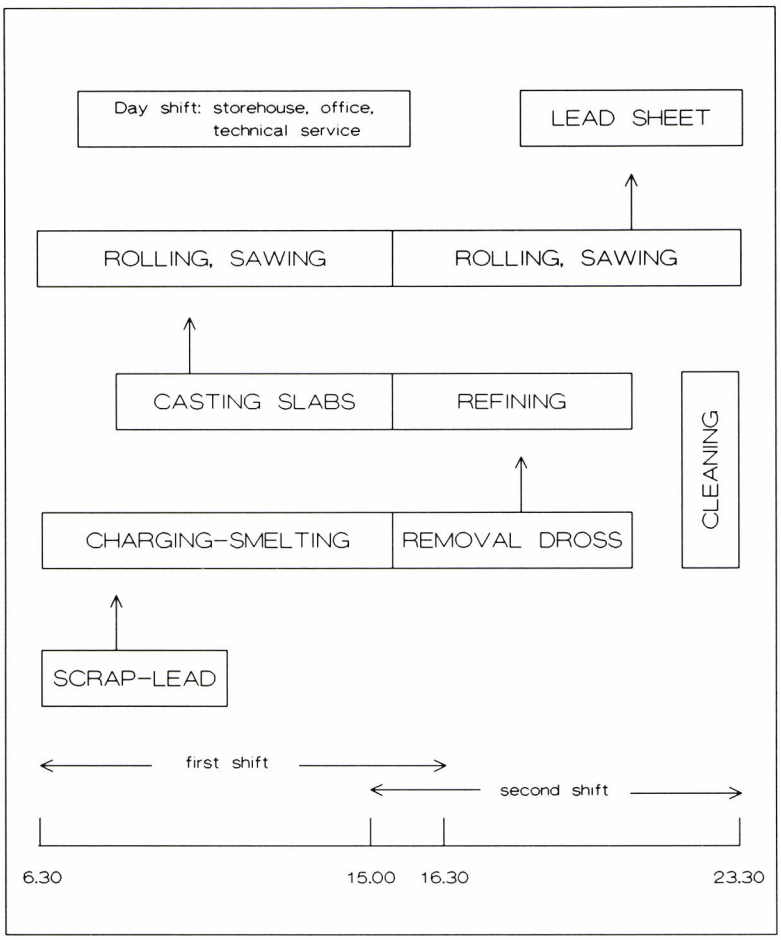

Figure 9.2 Outline of the production process.

Occupational hygiene survey

In the survey, exposure of the workers to Pb was estimated by regularly measuring the concentration of PbB and of the effect parameter zinc protoporphyrin (ZPP). An Occupational Health Service was responsible for these measurements.

Levels of Pb in the breathing zone were measured by personal air sampling (PAS), monitoring some parts of the work with supposedly high exposure (removing dross, cleaning) separately. Assumed sources of lead dust were monitored by stationary air sampling (SAS).

Analysis of PbB and ZPP in blood

Venous blood was collected in 5-ml lead-free tubes containing 7.5 mg of EDTA (Venoject, Terumo). The concentration of Pb was determined after mixing 100 µl of blood and 900 µl of 1 Mol/L nitric acid solution by swirling on a shaker. The precipitate was centrifuged and in the clear supernatant Pb was determined by atomic absorption spectrophotometry (Perkin Elmer Model 4000, equipped with a Model 500 graphite atomizer) [Stoeppler et al. (1978)]. The quality of the analysis was checked by participation in the U.K. External Quality Assessment Scheme of the Queen Elizabeth Hospital, Birmingham, U.K. At three-weekly intervals lead-supplemented human blood samples were analysed. On the average, the results differed by 7.7% (range 0-22.6%) from the average results of about 80 participants, but there was no systematic deviation.

The concentration of ZPP was determined with a direct-reading haemato-fluorometer (Model 4000; Environmental Sciences Ass., Bedford, MA, U.S.A) after oxygenation of a 100 µl blood sample by swirling on a shaker. The coefficient of variation of 15 analyses performed in the same samples on five successive days was 2.6% at a ZPP content of 95 µmol/mol Hb and about 1.5% at concentrations of ZPP between 150 and 625 µmol/mol Hb.

Personal air sampling

Air in the breathing zone was sampled by pumping air through a filter in a holder fixed to the lapel of the worker. The holder, with an opening of 6 mm diameter, contained a glass-fibre filter and was connected to an air sampling pump (Du Pont P 2500) by Tygon tubing. The flow rate was 2 L/min and the sampling time 3-4 h. This method meets the criteria specified in a directive on inorganic lead issued by the European Community [EC (1982)].

Stationary air sampling

SAS was carried out at fixed spots by pumping air through a holder containing a glass-fibre filter with a sampling pump (Du Pont P 4000). After dissolving in *aqua regia,* the Pb on the filters was determined by atomic absorption spectrophotometry (Varian Techtron AA 9751). Concentrations are reported as $\mu g/m^3$. The possible error in air sampling and the analysis amounted to about 10%.

Hand-washing
Workers were invited to wash their hands with distilled water and lead-free soap just before lunch-time. The collected washing-water was filtered and Pb was determined in both the filtrate and insoluble fraction.

Statistical analysis
The decrease of the PbB level of each worker in time was tested with the rank correlation test of Spearman with $p = 0.05$ as level for statistical significance. The relation between PbB and ZPP in individuals was tested with linear regression analysis ($p = 0.05$).

9.3 RESULTS

During the survey (1982-1990), Pb and ZPP were determined in 563 blood samples collected from 99 workers. Because of the time of their employment or the low level of PbB, 22 workers were screened only once, whereas the others were screened more frequently (one worker was screened as many as 33 times).

The ranges of $R_{Spearman}$ of the PbB levels of 28 workers, screened seven times or more, are given in *Table 9.1*. Also presented are the numbers of these workers showing a statistically significant decrease of PbB during the time of the survey.

Thirteen other workers have been screened three to six times. These numbers are too low for a Spearman rank correlation analysis. However, PbB levels of five workers showed a decreasing trend. Conditions were: at least five decreasing levels of PbB, successively in a period of more than a year, or all measurements decreasing when only three or four screenings were available.

PbB data of office workers are not shown. The values ($n = 37$) were lower than 0.9 µmol/L except two of 1.27 and 1.81 µmol/L, respectively.

The concentration of lead in the breathing zone of 20 workers was measured by PAS during four periods on two successive days (*Table 9.2*). Ten of the workers were employed in two shifts of about equal size whereas the others were working during day-time. Also the data of PAS measurements during removal of dross and during cleaning of the smelting department are shown. The concentrations of Pb during SAS measurements presented in *Table 9.2* refer to the places marked with a letter in *Figure 9.1*.

The concentrations of lead in water collected after hand-washing are also given in *Table 9.2*.

In the same week that PAS measurements were carried out, blood samples were collected. *Table 9.3* shows the mean and range of the PbA and PbB levels that were measured.

Linear regression of ZPP on PbB for 19 workers screened ten times or more, revealed a statistically significant relation for 17 of them ($p = 0.05$). In *Figure 9.3* four representative examples are plotted.

TABLE 9.1 DEVELOPMENT OF PbB LEVELS OF 28 WORKERS BETWEEN 1982-1990

Department	Number of workers	$R_{Spearman}$ (range)	Number of workers with significant decrease[a]	Remarks
Smelting	6	.24/.72	3	b, c
Rolling	8	.18/.86	3	
Sawing	3	.32/.71	0	
Storehouse/ forwarding	5	.07/.61	1	d
Miscellaneous lead products	2	.02/.60	0	
Technical service	1	.79	1	
Management support office	3	.28/.70	1	

a. $P_2 \leq 0.05$.
b. One person showed a significant increase of PbB.
c. One person showed an increase of PbB until 1983, and from then a signficant decrease.
d. One person showed an increase of PbB until 1987, and from then a significant decrease.

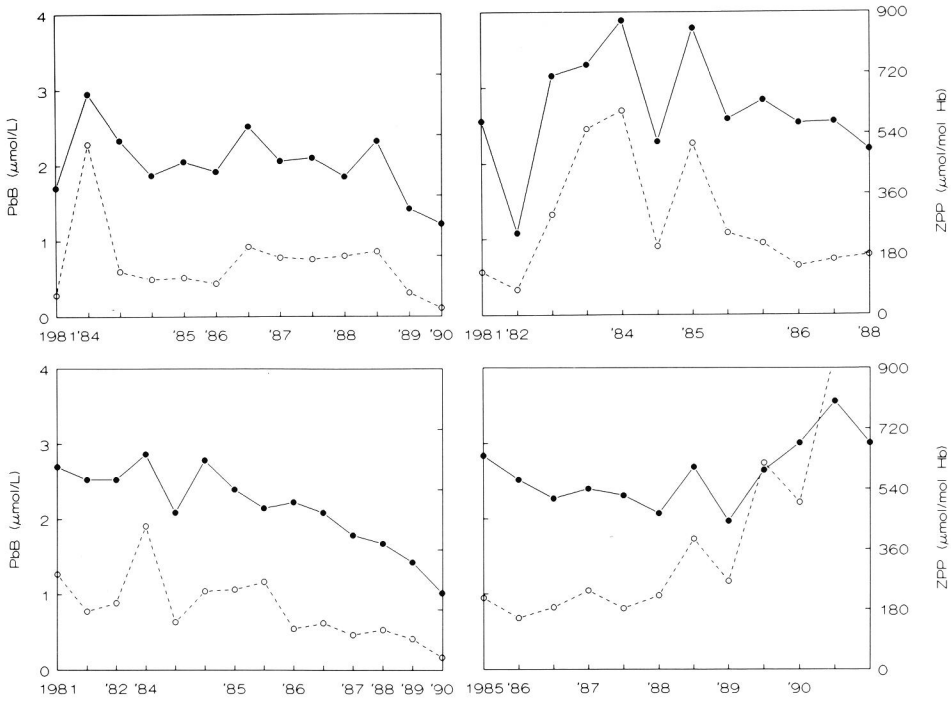

Figure 9.3 PbB levels, µmol/L (drawn line) and ZPP levels, µmol/mol Hb (dotted line) of four workers.

TABLE 9.2 RESULTS OF PERSONAL AND STATIONARY AIR SAMPLING OF LEAD AND OF LEAD IN WASHING-WATER

Job function/ Department	Number of operator/ Place of sampling	Type of sampling	Number of samples	PbA (µg/m³)		Percentage of values >MAC[a]	Pb in washing-water (mg)	
				median	range		median	range
Smelter	1,2,3,4	PAS	16	620	170-1970	100	15.2	4.1-102
Shovel driver	5	PAS	4	680	570-750	100	11.1	
Removal dross[b]	3	PAS	1		1800	100		
Other work[b]	3	PAS	1		360	100		
Cleaning[b]	4	PAS	1		610	100		
Other work[b]	4	PAS	1		950	100		
Rolling master	6,9	PAS	8	55	40-60	0		7.8-8.6
Rolling/sawing	7,10,11	PAS	12	95	60-110	0	18.1	9.7-26.8
Sawing	12,13,14	PAS	12	300	200-380	100	3.3	3.1-11.8
Storehouse	16,17,18,19	PAS	15	400	260-680	100	2.2	1.5-10.6
Technical service	20,21,22	PAS	11	320	210-920	100	30.6	9.5-59.0
Smelting oven	A[c]	SAS	8	410	90-1620	88		
Refining oven	B	SAS	8	305	120-620	75		
Casting lead	C	SAS	7	160	80-240	57		
Rolling department	D	SAS	8	20	10-60	0		
Rolling department	E	SAS	8	60	20-80	0		
Filters	F	SAS	3	10	10-20	0		
Sawing department	G	SAS	4	200	180-2940[d]	100		
Charging shovel		SAS	2		40-130	0		
Canteen		SAS	4	35	20-50	0		

a. MAC: 150 µg/m³ (TWA-8 h).
b. Sampling during the job indicated.
c. Letters refer to Figure 9.1.
d. Value of 2940 µg/m³ excluded from calculation of median.

TABLE 9.3 MEAN AND RANGE OF LEVELS OF LEAD IN AIR AND IN BLOOD

Department	PbA ($\mu g/m^3$)[a]			PbB ($\mu mol/L$)		
	Number of workers	Mean	Range	Number of workers	Mean	Range
Smelting	4	780	330-1350	3	2.76	2.44-3.30
Rolling	5	70	40-100	5	1.90	1.09-2.79
Sawing	3	280	220-330	1	3.10	
Storehouse	3	390	220-495	2	2.20	1.87-2.53

a. TWA-8 h.

9.4 DISCUSSION

In 1982 the European Community issued a directive on the protection of workers from health risks of exposure to lead. It was implemented in the legislation of the Netherlands in 1988 [EC (1982); Labour (1988)]. In *Table 9.4* the measures described in the Directive are summarized. In this context environmental air monitoring and biological monitoring are the tools for health surveillance of workers occupationally exposed to lead.

TABLE 9.4 ACTION LEVELS AND THRESHOLD LEVELS OF PbA AND PbB FOR OCCUPATIONALLY EXPOSED PERSONS

Lead in air ($\mu g/m^3$)[a]		Lead in blood ($\mu mol/L$)	Requirements
>40	and/or	>1.4	Information to all workers Opportunity to undergo medical examination. Hygienic measures
>75	and/or	>2.4	Information to all workers Opportunity to undergo medical examination. Additional hygienic measures
		>2.9	Intensive medical examination[b]
150[c]		3.4[c] 3.4-3.9[d]	If occurring twice within three months, withdrawal from exposure

a. TWA-40 h.
b. Applies only to the worker concerned.
c. Threshold level.
d. Acceptable, provided the level of δ-aminolaevulinic acid in urine <20 mg/g creatinine, of δ-aminolaevulinic acid dehydratase in blood >6 European units or of zinc protoporphyrin in blood <500 μmol/mol Hb.

The knowledge of adverse health effects of Pb has increased considerably during the last decades. Effects on spermatogenesis [Koëter et al. (1989)], on kidney function [Verschoor et al. (1987)] and on the central and peripheral nervous system [Seppäläinen (1988)] may occur at lower PbB levels than was assumed a few years ago. So the permissible levels of PbB (3.4 μmol/L) and of lead in

environmental air (150 μg/m^3, TWA-40 h) mentioned in the EC-Directive may not prevent the occurrence of health impairment. (By order of the European Community, Apostoli (1991) updated the data of the Directive. He proposed a reduction of the present permissible levels of PbA to 70 μg/m^3 for male lead workers and to 35 μg/m^3 for female workers. The proposed biological limit values for PbB were 1.9 and 1.4 μmol/L, respectively).

The general policy of the management of the present plant is that measures to improve work conditions will also have a positive effect on the volume and quality of the production. Therefore, such measures are continuously carried out aiming to reduce the intake of lead by the workers. Between 1982 and 1990 the most important measures were the following. The year in brackets is the one in which the provision was introduced.

- The ovens are exhaust ventilated at a rate of 20.000 m^3/h (1988).
- The smelting and refining ovens are equipped with covers and central exhaust ventilation. Ventilation at the rims was introduced in 1989.
- Instead of being sawn, rolls of lead are cut into parts (1990).
- During the working-day the stock of scrap-lead is sprinkled automatically every 15 minutes (1988).
- The changing room was rebuilt in 1985 and now has separated sections for personal and protective clothing.
- No protective clothing is allowed in the canteen.
- Smoking is prohibited, except in the canteen (1988).
- Coffee and tea breaks are only allowed in the canteen, instead of distribution of drinks at the workplace (1988).
- Eating and drinking during work is prohibited (1988). In the smelting department this was already prohibited in 1984.
- Since 1982, workers of the smelting department are permitted to shower during working-time. Other workers are also allowed to shower at the plant but not during working-time.
- Since 1984 workers engaged in removing dross use an air-stream helmet.
- Workers on the platform near the smelting and refining ovens use a P2 filter-mask.
- In the smelting department protective clothing is supplied three times a week, in the other departments once a week (1983).

Whether measures relating to personal hygiene are observed, depends on the worker himself. He is responsible for his own personal hygiene. In general such measures are reasonably well observed. If PbB of a new-comer becomes higher than 2.4 μmol/L, the consequences of the non-hygienic behaviour are pointed out to him. Newly arrived workers generally show an increase of PbB at first, which often stabilizes at a lower level after they get used to good hygienic working practice.

Statistical analysis of the results of PbB assays shows that between 1982 and 1990 nine workers of a group of 28 showed a significant decrease of PbB *(Table 9.1)*, whereas five of a group of 13 other workers showed a decreasing trend. So, altogether the PbB level of 14 workers of a group of 41 declined. These were active in several departments. Therefore, it is not possible to point to the particular measures which contributed most to their decreased PbB. However, personal hygiene especially is considered an important factor with regard to the intake of Pb [Chavalitnitikul (1981); Ulenbelt (1990)]. Also, between 1982 and 1984, 48 of the PbB levels measured were >2.9 μmol/L, whereas between 1988 and 1990 this number decreased to 12. Overall, the PbB levels measured are comparable to those measured by Ulenbelt et al. (1991) in 24 smelters and 13 refining workers in a similar type of plant.

PAS and SAS measurements were carried out on two days with an average production of lead sheet in December 1984. PAS in the smelting department frequently showed extremely high Pb levels; all levels were higher than the limit value *(Table 9.2)*. Stationary sampling indicated as the main sources the ovens and, in particular the removal of dross. As the latter is carried out manually, the worker is very close to the smelting oven. In the rolling department all levels of Pb were lower than the permissible limit value. In the sawing department, where all Pb levels were higher than the permissible limit, the circular saw was provided with ventilation equipment. However, without casing, ventilation is only effective when the capacity is very large, since the particles emitted have a high velocity. In the storehouse Pb levels were 2-3 times the permissible limit. This is unusual for places where finished lead products are stored. The Pb was supposed to originate from the sawing which was carried out in the same hall or from lead whirled up into the air by the fork lift trucks used in this room. Mechanics were exposed to PbA levels of 2-6 times the permissible limit. However, the ambulatory nature of the job made it difficult to establish a representative picture of their exposure.

Whereas the PbA levels exceeded the limit value by 100-400% (with the exception of the rolling department), only one of the PbB levels was higher than the action value of 2.9 μmol/L, and none exceeded the permissible limit *(Table 9.3)*. A clear relation between PbA and PbB is seldom observed [Alessio et al. (1983); Booher (1988)]. PbB reflects mainly the deposits in two main tissue categories: one in blood and soft tissues (half-time 20-40 days), and another in bone (half-time about 10 years). The impact of the latter on the PbB level is relatively large after prolonged exposure, but small after a short exposure. Thus PbB reflects current exposure better in the initial stages of exposure than later on [Hernberg (1980)]. Furthermore, it has been recognized that in the lead industry personal protection devices will help to achieve lower PbB levels [Ulenbelt et al. (1991)]. A negative trend between PbA and PbB was shown for people wearing an air-stream helmet and for those spitting during work, and a positive trend for smokers. These and other factors may affect the ratio between the actual

concentration of lead in environmental air and the ultimate intake. It is concluded that establishing PbA in relation to health effects has a limited purpose.

The concentration of lead in water used for hand-washing furnished data on the possibility of oral uptake of Pb by hand-to-mouth contact ('finger-shunt'), also out of working time. Sampling was carried out on the same days as the PAS and SAS measurements. The results indicate that uptake of lead by contaminated food and smoking of cigarettes may occur. In this respect, however, it should also be realized that in a plant like the present one, where thousands of tons of lead are processed each month, intake of amounts in the mg range or less may be inevitable.

The assay of ZPP in blood has been used as a fast and inexpensive method to screen occupationally exposed workers with regard to their PbB level [Herber (1980); Wildt et al. (1987); Zwennis et al. (1990)]. However, to estimate PbB in an individual on the basis of his ZPP level, the relation between PbB and ZPP of a large cohort of lead workers is required.

The PbB and ZPP levels of 19 workers who were screened at least 10 times over a period of several years, thus with relatively large intervals, showed a statistically significant relation for 17 of them (*Figure 9.3*). Using these data, the sensitivity and specificity of the ZPP analysis to establish a change in the PbB level of an individual was calculated. In this context, sensitivity and specificity are defined as the ability (in per cents) of the ZPP test to identify correctly an increase, respectively decrease of the PbB level in a sample. Altogether in 226 PbB and ZPP pairs sensitivity was 78.0% and specificity 68.0%. These results suggest that in the health surveillance of a single lead worker a change in the level of ZPP may possibly be used as an indicator for a change of PbB. An increment of ZPP, which will indicate an increment of PbB, will then require additional assay of the concentration of PbB. This approach can only be applied when the subject involved has been exposed to lead for a time sufficiently long to reach an equilibrium between the deposits. The procedure has to be worked out further but may facilitate and promote the frequent screening of lead workers.

From this study it is concluded that the comprehensive measures carried out to reduce exposure to lead have effected a general decline of the PbB levels. No particular measures can be indicated which led to this result. No relation between Pb levels in environmental air at the workplace and in blood has been established. Therefore, in this study the use of PbA in relation to health effects has only a limited meaning.

Under conditions of prolonged exposure, a change in the level of ZPP of an individual worker may possibly be applied as an indicator of a change in the level of PbB.

9.5 SUMMARY

Results are presented of a survey in a lead-recycling plant that produces mainly lead sheet. The survey covers the determination of lead and zinc protoporphyrin in blood of the workers over a period of ten years. At one occasion stationary air sampling and personal air sampling were carried out. The possibility of intake of Pb by 'finger shunt' was also considered. During the survey a programme of technical changes and hygienic measures was carried out with the goal of reducing the uptake of Pb by the workers. The effect of these measures, as reflected in PbB levels, indicated a general decline in the uptake. It is suggested that a change in the ZPP level of an individual worker exposed for prolonged periods may be applied as an indication of a change in PbB.

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ACKNOWLEDGEMENT

The survey was financed in part by the Ministry of Social Affairs and Employment, The Hague, the Netherlands. The authors thank the management and the workers of the plant for their co-operation. We are indebted to Mr J.F. Bleichrodt and Dr. J.J. van Hemmen for advice in preparing this manuscript. The dedicated technical assistance of Ms. U. Soekhoe is gratefully acknowledged.

CHAPTER 10

TOXICITY OF LEAD ACETATE TO FEMALE RABBITS AFTER CHRONIC SUBCUTANEOUS ADMINISTRATION

I. BIOCHEMICAL AND CLINICAL EFFECTS*

H. Falke** and W.C.M. Zwennis

Abstract. The effect of chronic subcutaneous administration of lead acetate was studied in female rabbits. The low-dose group (15 animals) received three times a week 0.10-0.20 mg/kg body weight and the high-dose group (15 animals) 0.80-1.20 mg/kg. The control group received the vehicle only. Concentrations of lead in blood in the low-dose group increased to ca. 400 µg/L*** after 70 days and in the high-dose group to ca. 900 µg/L after 110 days. After 7.5 months 8 animals of each group were sacrificed. The remaining rabbits were kept for an additional 4 months without treatment. Blood lead concentrations decreased with a half-time of 60-70 days. During exposure the gain in body weight was lower in the high-dose group than in the control group and the low-dose group. The high-dose group developed slight anaemia and low MCV, MCH and MCHC, and basophilic stippling of erythrocytes. These effects disappeared during recovery. ALAD activity in erythrocytes was very low during exposure in both exposed groups and did not reach control values during recovery. During exposure the concentrations of ZPP and ALAU increased, but only ALAU returned to normal during recovery. No other effects of lead on the composition of the urine were observed. No effects were observed on plasma urea and creatinine concentrations. In the high-dose group the concentration of ALAD in the liver decreased by 30%. During recovery this effect was no longer present. No effects were seen in cytochrome P-450 content or cytochrome P-450-dependent enzyme activities. Lead was mainly stored in bones, but some also in several soft tissues. After recovery the concentrations in soft tissues decreased to a variable degree. In the high-dose group the relative weights of heart and liver increased. These effects disappeared during recovery. At 400 µg lead/L blood no adverse effects were observed that did occur at the high dose level.

Key words: Lead - Rabbit - Chronic exposure - Biochemical effects - Clinical effects

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* Published in Archives of Toxicology 64:522-529 (1990).

** Present adress: Board for the Authorization of Pesticides, Wageningen, the Netherlands.

*** Note added: 100 µg lead corresponds to 0.48 µmol.

10.1 INTRODUCTION

The rabbit is considered a very suitable animal model for lead intoxication with regard to haem synthesis and nephrotoxicity [Hass et al. (1964); Scharding and Oehme (1973); Chiba and Kikuchi (1974)]. In 1981, Spit et al. described changes in the proximal tubules of kidneys of rabbits that had been treated subcutaneously with lead acetate for 3 months, resulting in concentrations of lead in blood (PbB) of 500-600 µg/L during the last month. At this level no adverse effect of lead on the kidneys had been detected before. Therefore, it was decided to perform a second experiment in a somewhat different way, using additional parameters for kidney function that could possibly be implemented in a protocol for biological monitoring of lead workers.

This is a report of an experiment of 11.5 months, including a recovery period of 4 months, in which female rabbits were treated subcutaneously with lead acetate in doses resulting in a PbB of 400 (low-dose) or >800 µg/L (high-dose). The PbB level of 400 µg/L was chosen because it is accepted by the WHO as a no-adverse-effect level for lead workers, whereas a level of >800 µg/L was chosen to yield clear effects. The study included kidney function tests, haematological and biochemical analyses and determination of lead in several organs at the end of exposure and after recovery.

10.2 MATERIALS AND METHODS

Animals

Fifty-five young adult female rabbits (New Zealand White), weighing 2.5-2.8 kg, were obtained from ENKI-Konijnenfarm, Someren, Netherlands. The rabbits were caged individually in a room with constant temperature (18 ± 2°C) and relative humidity (50-70%). A 12-hour light-dark cycle was maintained. At the start of the study 45 rabbits were selected and divided into three groups of 15 animals each: a control group, a low-dose group (intended PbB 400 µg/L) and a high-dose group (intended PbB >800 µg/L). The rabbits were allocated to the various groups by computer randomization. A standard rabbit diet was provided ad libitum. Tap water was supplied ad libitum in macrolon bottles which were filled daily with fresh water and cleaned weekly.

Test material

Lead acetate (PbAc) was purchased from BDH Chemicals Ltd., Poole, England (N° 10142). The material was stated to contain at least 99.5% lead acetate.

Administration of test material

Lead (Pb) was administered as a lead acetate solution in sterile 5% glucose solutions by subcutaneous injection in the neck of the animals. Fresh solutions were prepared every Monday. Control animals received the vehicle only. Solutions

prepared contained 0.20 mg lead acetate per ml for the low-dose group and 0.80 mg/ml (initially), 1.0 mg/ml (after 7 weeks) and 1.20 mg/ml (after 11 weeks) for the high-dose group.

The animals were injected three times a week, on Monday, Wednesday and Friday. The low-dose group received per injection 0.20 mg/kg body weight, the high-dose group 0.80-1.20 mg/kg. In order to keep PbB of the low-dose group at the intended 400 µg/L, dosing was occasionally decreased to 0.15 mg/kg or 0.10 mg/kg dependent on PbB measured in the previous week. During the recovery period no injections were given.

Experimental design

The exposure to Pb for the main group lasted 7.5 months. The recovery period lasted four months. Each animal was identified by a six-digit computer reference number and a number written in its left ear. The animals were distributed randomly in a cage rack.

Body weight

Individual body weights were recorded at the start of the study, then once a week and at termination of exposure and after the recovery period.

Blood sampling

Blood samples were collected from the central ear artery after rubbing the ear with xylene to stimulate the blood flow. Blood was collected in heparinized vacutainer tubes at day 0 and thereafter once a week for determination of the lead level. At days 0, 48, 97 and thereafter at approximately monthly intervals a second sample was taken for haematological and biochemical analyses.

Analysis of lead in blood

Analysis of lead in blood was done by atomic absorption spectrometry (AAS) according to Schaller (1983).

Analysis of lead in tissues

At day 223 eight rabbits per group and at day 336 the animals that were allowed to recover were anaesthetized by intravenous injection of Nembutal followed by exsanguination from the abdominal aorta. Samples were taken from a number of tissues (see Results) for determination of lead. Lead was also determined in the remainders of each animal after homogenization in a Stephan Cutter. The concentration was determined by AAS after destruction with nitric acid and H_2O_2.

Haematology

Haematological parameters (see Results) were determined by conventional methods.

Clinical chemistry

Blood samples for clinical chemistry were centrifuged for 15 minutes at 1250 g. In the plasma the concentrations of creatinine and urea were determined by conventional methods. The concentration of zinc protoporphyrin (ZPP) in blood was determined according to Blumberg et al. (1977), the activity of δ-amino-laevulinic acid dehydratase (ALAD) in erythrocytes according to Burch and Siegel (1971).

Urinalysis

Urine was collected from individual animals at approximately monthly intervals. Until day 175 urine was collected by catheterization of the bladder. The bladder was emptied between 08.00 and 09.00 and the urine discarded. Further collections were made between 12.00 and 13.00 and between 16.00 and 17.00. These two portions of urine were pooled for analysis. From day 175 on, the rabbits were placed in metabolic cages for urine collection. After 1-3 days of acclimatization 24-h urine samples were collected. Sample volume and pH were measured and after adjustment to pH 5 with nitric acid, the concentration of lead was determined according to the method used for the blood samples. Additionally, the following parameters were determined: creatinine, urea, 5-aminolaevulinic acid (ALAU) according to Davis and Andelman (1967). The kallikrein activity was measured with the slightly modified method for human urine using the chromogenic substrate S-2266 from KabiVitrum. N-acetylaminoglucosaminidase activity was determined according to Tucker et al. (1975) after desalting the urine samples over a Sephadex G-50 column. Protein concentrations were determined with the Bio-Rad protein kit using bovine serum albumin as a standard. The protein spectrum was estimated by SDS-PAGE. At the end of exposure and at the end of the recovery amino acid concentrations were measured by ion exchange chromotography, based on the method of Tutschek et al. (1977).

Biochemistry of the liver

Part of the liver of each animal was used for biochemical analysis. The tissue was weighed, collected in ice-cold isotonic (1.15%) KCl solution and cut into small pieces. The latter were washed once with cold isotonic KCl and subsequently homogenized in three volumes of cold KCl solution with a glass-teflon Potter type homogenizer. The homogenates were centrifuged for 15 minutes at 10,000 g and 4°C to obtain the post-mitochondrial fraction. In this fraction the following measurements were made: total protein with the Bio-Rad protein kit using a Kjeldahl-calibrated post-mitochondrial rat liver fraction as a standard, cytochrome P-450 according to Schoene et al. (1972), aniline-4-hydroxylase (AH) activity according to Imai et al. (1966), aminopyrine-N-demethylase (APDM) activity according to Gram et al. (1968), ALAD activity (ALAD-liver) by a modified method of Burch and Siegel (1971) for erythrocytes.

Statistical analysis
Data on body weights, organ weights, haematology, clinical chemistry and biochemistry were evaluated by one-way analysis of (co-)variance, followed by Dunnett's multiple comparison test. Data on reticulocytes and basophilic stippling were evaluated with the Mann-Whitney U test and the Fisher exact test, respectively.

10.3 RESULTS

Clinical signs
During the study some animals, apparently infected with Pasteurella spp., died or were killed. Animals of the exposed groups developed subcutaneous nodules at the injection sites. Hardly any reactions of this kind were observed in the control group. The size of the nodules was dose-dependent. The nodules disappeared during recovery in about three months and were not observed at autopsy of the recovered animals.

Blood lead concentrations (Figure 10.1)
On average, the low-dose group reached the intended PbB at about day 70 and was kept at this concentration for each animal through weekly adjustment of the amount of PbAc injected. The animals showed large variation in their response. Some animals never reached 400 µg lead/L blood while in others the amount of injected PbAc had to be decreased by 25% or sometimes even 50% to keep it on the intended concentration. The high-dose group passed 800 µg lead/L blood at about day 110 and stabilized at approximately 900 µg/L. During recovery PbB gradually decreased until day 300. Thereafter PbB seemed to stabilize at approximately 150 µg/L in the low-dose group and 440 µg/L in the high-dose group. Mean PbB in the control group was almost always lower than 40 µg/L.

Body weights (Figure 10.2)
During the main study the high-dose group gained less weight than the other groups. This delay of growth became apparent after about day 80 when PbB was approximately 750 µg/L. Due to intercurrent deaths the number of animals in the recovery phase was small. The body weights of the animals selected for recovery were not representative of the mean body weights of the total group. Therefore, the apparent increase in body weights in the high-dose group after recovery cannot be attributed to the termination of exposure. The apparent decline of the body weights in the control group after termination of the exposure period was caused by the computer programme, which, accidentally, selected the heaviest animals in this group for killing.

Haematology (Table 10.1)
Haemoglobin concentrations in the high-dose group were significantly lower than

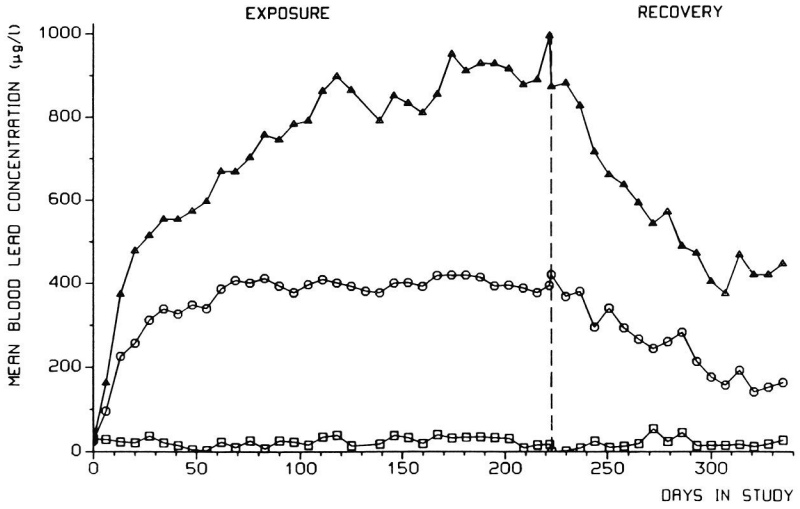

Figure 10.1 Mean concentrations of lead in blood of female rabbits during exposure and recovery. □——□ control, ○——○ low dose, ▲——▲ high dose. Statistics: ANOVA + Dunnett tests. The data of the low-dose and the high-dose differ statistically from the control ($p < 0.01$).

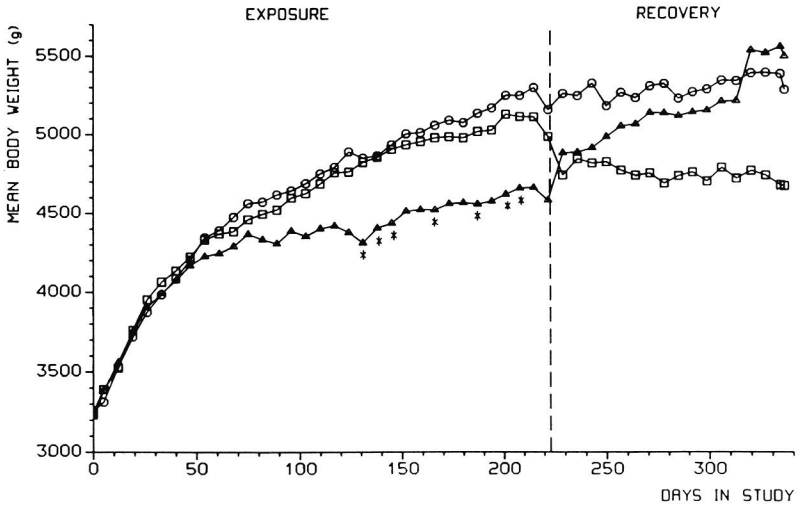

Figure 10.2 Mean body weights during exposure and recovery. Symbols and statistics as in Fig. 10.1. The data marked with * differ statistically from the control (* $p < 0.05$, ** $p < 0.01$).

in controls from day 125 onwards. At that day the mean PbB was 865 µg/L. During the recovery period the haemoglobin concentrations rapidly increased to control levels. Packed cell volumes in the high-dose group were somewhat lower than in the controls from day 125 onwards. The differences disappeared in the recovery period. Red blood cell counts were not influenced by lead. In the high-dose group, red blood cell indices (MCV, MCH, MCHC) were clearly affected by

lead. MCV and MCH were depressed from day 97 (PbB 784 µg/L) and MCHC from day 125 (PbB 865 µg/L). In the recovery period the effects on the red blood cell indices rapidly disappeared. Reticulocyte counts showed a tendency towards higher values in the high-dose group during the second half of the main study, but the difference was statistically significant only on day 216. Basophilic stippling of the erythrocytes was observed in most animals of the high-dose group from day 125 onwards. This phenomenon gradually disappeared during the recovery period. No consistent differences in these haematological variables were observed between the control- and low-dose groups.

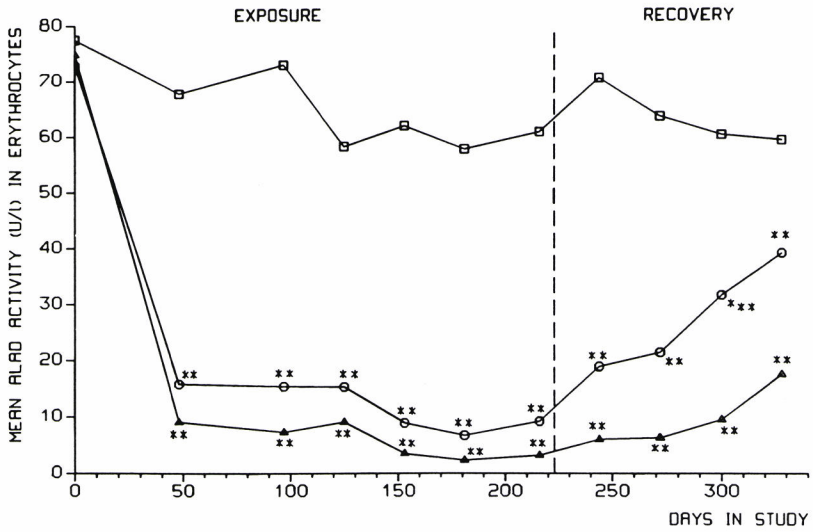

Figure 10.3 Mean aminolaevulinic acid dehydratase activity in erythrocytes during exposure and recovery. Symbols and statistics as in Figures 10.1 and 10.2.

Clinical chemistry (Figures 10.3 and 10.4)

The mean activity of ALAD in the erythrocytes on day 48 was in the low-dose group 23.3% and in the high-dose group 13.4% of that in the controls. At that time mean PbB in the low-dose group was 349 µg/L and in the high-dose group 574 µg/L. During the whole main study ALAD activity remained very low. In the recovery period the activities in both groups gradually increased (somewhat faster in the low-dose group than in the high-dose group) but never reached control values. At the end of the study, when PbB was 163 µg/L, ALAD activity in the low-dose group was only 66% of that in the controls. ZPP in the high-dose group gradually increased after the start of the study. Mean ZPP in the low-dose group was consistently higher than in the controls but the difference was statistically significant only at day 48. During recovery ZPP in the high-dose group decreased

TABLE 10.1 HAEMATOLOGICAL EFFECTS OF LEAD ACETATE IN FEMALE RABBITS [MEAN AND (SEM)]

		Exposure (days in study)							Recovery (days in study)			
Variable		0	48	97	125	153	181	216	244	272	300	328
Haemoglobin (mmol/L)	control	7.7 (0.1)	7.8 (0.1)	8.1 (0.1)	8.1 (0.1)	8.2 (0.1)	8.1 (0.1)	7.8 (0.1)	7.5 (0.2)	7.8 (0.5)	7.8 (0.3)	7.7 (0.3)
	high dose	7.8 (0.2)	7.6 (0.1)	8.0 (0.1)	7.4** (0.1)	7.5** (0.1)	7.4** (0.1)	7.2* (0.1)	6.9* (0.1)	7.6 (0.1)	7.7 (0.2)	7.9 (0.2)
Haematocrit (L/L)	control	0.388 (0.005)	0.386 (0.005)	0.384 (0.004)	0.391 (0.005)	0.391 (0.005)	0.387 (0.004)	0.371 (0.007)	0.349 (0.012)	0.365 (0.022)	0.377 (0.015)	0.381 (0.014)
	high dose	0.394 (0.007)	0.375 (0.005)	0.383 (0.007)	0.371 (0.007)	0.373 (0.007)	0.369* (0.004)	0.358 (0.007)	0.342 (0.003)	0.359 (0.003)	0.375 (0.005)	0.383 (0.006)
Red blood cell count (10^{12}/L)	control	5.1 (0.1)	5.1 (0.1)	5.3 (0.1)	5.4 (0.1)	5.2 (0.1)	5.2 (0.1)	5.3 (0.1)	4.9 (0.2)	5.4 (0.4)	5.5 (0.3)	5.3 (0.3)
	high dose	5.2 (0.1)	5.2 (0.1)	5.7 (0.1)	5.4 (0.1)	5.3 (0.1)	5.3 (0.1)	5.3 (0.2)	4.8 (0.1)	5.2 (0.1)	5.4 (0.1)	5.4 (0.2)
MCV (fL)	control	75.6 (0.8)	75.4 (1.4)	72.0 (0.6)	73.2 (0.7)	75.2 (0.9)	74.2 (0.8)	70.8 (0.7)	70.9 (1.2)	68.4 (0.8)	69.1 (1.6)	71.6 (2.5)
	high dose	75.8 (1.0)	72.5 (1.1)	67.6** (1.0)	68.7** (1.1)	71.2** (1.2)	69.7** (1.0)	67.4** (0.8)	71.0 (1.2)	68.9 (1.2)	69.7 (0.9)	70.8 (1.1)
MCH (fmol)	control	1.50 (0.02)	1.53 (0.02)	1.53 (0.01)	1.51 (0.01)	1.58 (0.02)	1.56 (0.02)	1.49 (0.01)	1.52 (0.03)	1.47 (0.02)	1.43 (0.03)	1.44 (0.03)
	high dose	1.50 (0.02)	1.46 (0.02)	1.42** (0.01)	1.38** (0.02)	1.43** (0.02)	1.39** (0.02)	1.35** (0.02)	1.43 (0.03)	1.46 (0.02)	1.43 (0.02)	1.45 (0.01)
MCHC (mmol/L)	control	19.9 (0.1)	20.3 (0.3)	21.2 (0.2)	20.7 (0.1)	21.1 (0.1)	21.0 (0.1)	21.1 (0.1)	21.5 (0.3)	21.5 (0.0)	20.7 (0.1)	20.1 (0.3)
	high dose	19.9 (0.1)	20.2 (0.2)	21.1 (0.2)	20.1** (0.1)	20.1** (0.1)	19.9** (0.1)	20.1** (0.1)	20.1** (0.2)	21.1 (0.2)	20.4 (0.2)	20.6 (0.3)
Reticulocytes (1/1000)	control	15.3 (2.3)	20.4 (2.6)	30.0 (4.9)	28.2 (2.9)	21.9 (3.3)	19.6 (2.7)	29.2 (3.2)	28.8 (10.9)	28.8 (10.3)	26.3 (3.8)	13.8 (4.3)
	high dose	16.7 (2.6)	20.3 (2.2)	27.1 (3.5)	33.2 (2.9)	27.5 (3.7)	27.1 (3.1)	44.3** (3.8)	26.7 (6.0)	31.7 (6.1)	34.2 (1.5)	32.0* (3.7)
Basophilic stippling (fraction of animals)	control	nm	nm	1/14	2/14	1/14	2/14	1/13	0/4	0/4	0/4	nm
	high dose	nm	nm	6/14	11/14**	10/14**	11/14**	13/14**	5/6*	3/6	2/6	nm
Number of animals	control	15	14	14	14	14	14	13	4	4	4	4
	high dose	15	15	14	14	14	14	14	6	6	6	5

Statistics: ANOVA + Dunnett tests * p <0.05 ** p <0.01 two sided; for reticulocytes: Mann/Whitney *U* test * p <0.05 ** P <0.02 two sided; for basophilic stippling: Fisher exact test * p <0.05 ** p <0.01; nm = not measured

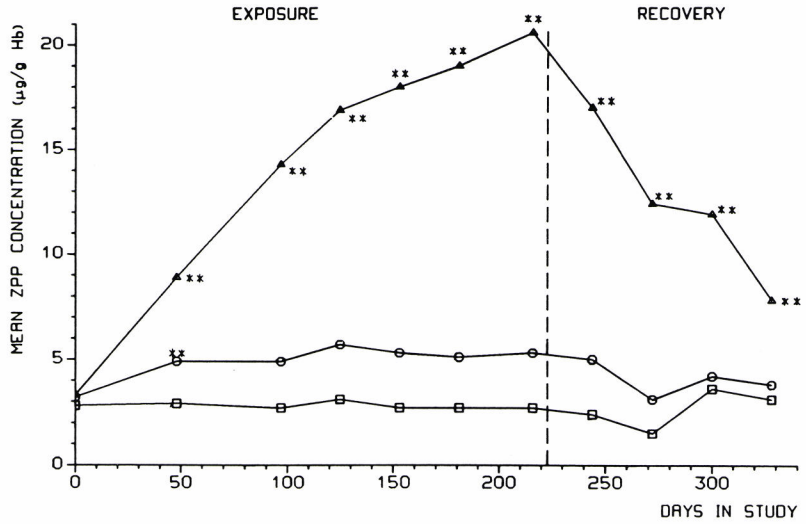

Figure 10.4 Mean zinc protoporphyrin concentrations during exposure and recovery. Symbols and statistics as in Figures 10.1 and 10.2.

but did not reach control values at the end of the study. In the low-dose group ZPP completely returned to control values. Plasma urea and creatinine concentrations showed no differences with control values (data not shown).

Urinalysis

There were no significant differences in mean urinary volumes between the three groups. ALAU of the high-dose group increased during the first part of the study and stabilized somewhat during the second half *(Fig. 10.5)*. During the recovery period ALAU rapidly returned to control values. No significant increase was observed in the low-dose group. No differences were found between the groups in pH, urinary creatinine concentrations, kallikrein activity, protein concentrations, N-acetylglucosaminidase activity, protein spectrum or amino acid concentrations (data not shown). Mean concentrations of lead in urine increased with increasing amounts of lead administered. The range in concentrations was very wide. Therefore, no statistical evaluation was performed.

Biochemistry of the liver (Table 10.2)

ALAD activity was decreased in the liver of the high-dose group at the end of the exposure phase, but was not affected in the low-dose group. The difference was no longer observed after the recovery phase. No differences were seen in concentrations of cytochrome P-450, AH, APDM or post-mitochondrial proteins.

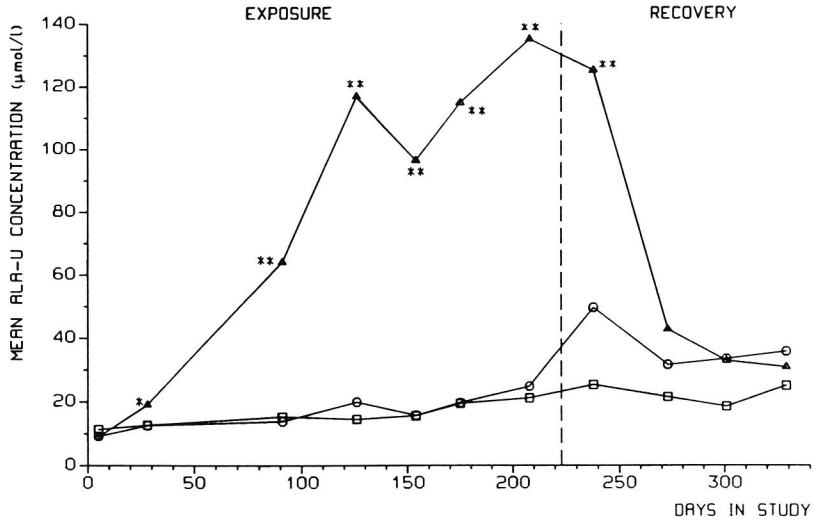

Figure 10.5 Mean urinary aminolaevulinic acid concentrations during exposure and recovery. Symbols and statistics as in Figures 10.1 and 10.2.

Lead distribution (Tables 10.3 and 10.4)

The concentrations of lead measured in several tissues at the end of the exposure phase increased with increasing amounts of lead administered, with the exception of skeletal muscle. In the control group relatively high levels were observed in lungs, kidneys and femur. The highest concentrations of lead in the low- and high-dose groups were found in the femur, liver, kidneys and lungs. Relatively low concentrations were found in the other tissues but the increase as compared to the controls was usually ten times or more in the high-dose group. Very high concentrations were found in the subcutaneous nodules. After the recovery phase, the lead concentrations were generally lower than after the exposure phase, with the exception of the femur. The decrease in the other tissues was 30-50%, with the exception of the lungs, which showed very low concentrations of lead after the recovery phase.

The total amount of lead in the body increased with dose. After the recovery phase a decrease of 20-30% was seen. Most of this lead seemed to reside in the skeleton. In this study the weight of the skeleton was estimated to be 5-10% of total body weight. Therefore, the lead content of the whole body and of several tissues are given for relative carcass weights of 5 and 10%. The other organs accounted for 1% or less of total body burden. After recovery almost all lead in the rabbits seemed to be located in the skeleton. The total amount in the skeleton was even higher than at the end of the exposure phase. However, this might be an artefact due to the large scatter of the data and the small number of animals in the recovery group.

TABLE 10.2 EFFECTS OF LEAD ACETATE ON BIOCHEMICAL PARAMETERS IN THE LIVER OF FEMALE RABBITS [MEAN AND (SEM)]

		Day 222 end of exposure	Day 336 end of recovery
Cytochrome P-450 (μmol/g protein)	control	0.53 (0.05)	0.33 (0.04)
	high dose	0.63 (0.05)	0.35 (0.03)
Aniline hydroxylase (U/g protein)	control	0.223 (0.024)	0.224 (0.022)
	high dose	0.213 (0.011)	0.194 (0.021)
Aminopyrine N-demethylase (U/g protein)	control	1.08 (0.11)	1.22 (0.15)
	high dose	1.08 (0.05)	1.06 (0.10)
Aminolaevulinic acid dehydratase U/g protein	control	0.37 (0.01)	0.29 (0.03)
	high dose	0.26* (0.01)	0.27 (0.01)
Number of animals	control	8	4
	high dose	8	5

Statistics: ANOVA + Dunnett tests.
* $p < 0.01$ two-sided.

Organ weights

Relative weights of heart and liver in the high-dose group were higher than in the control group. At the end of the recovery phase this difference was no longer observed (data not shown). No other differences in organ weights were observed.

TABLE 10.3 MEAN LEAD CONCENTRATION (MG/KG) IN THE CARCASS AND SEVERAL TISSUES OF RABBITS AFTER EXPOSURE TO LEAD ACETATE. IN BRACKETS THE RANGE OF VALUES OBSERVED

	Day 222 (end of exposure)			Day 336 (end of recovery)		
Variable	Control (n = 8)	Low dose (n = 8)	High dose (n = 8)	Control (n = 4)	Low dose (n = 5)	High dose (n = 5)
Carcass	0.21 (0.14-0.43)	3.37 (1.12-6.14)	18.40 (8.74-35.15)	0.65 (0.18-1.77)	2.39 (1.49-3.49)	9.09 (4.52-22.48)
Femur	1.64 (0.80-2.90)	16.74 (13.00-22.40)	113.91 (78.80-148.50)	1.71 (1.08-2.52)	15.36 (11.80-18.91)	140.73 (117.69-158.41)
Liver	0.15 (0.00-0.32)	3.48 (2.24-4.78)	8.74 (4.47-12.56)	1.13 (0.50-2.50)	1.62 (0.80-2.70)	5.76 (4.20-7.80)
Kidneys	1.22 (0.24-2.17)	2.43 (1.81-3.11)	18.35 (12.74-22.94)	0.37 (0.02-1.28)	1.15 (0.85-1.75)	6.89 (4.48-13.70)
Lungs	2.93 (1.50-6.40)	2.94 (2.00-5.20)	6.11 (3.50-7.00)	0.56 (0.36-0.91)	0.30 (0.04-0.69)	0.78 (0.56-1.12)
Brains	0.18 (0.02-0.90)	0.23 (0.11-0.64)	2.80 (0.64-15.61)	0.45 (0.29-0.75)	0.56 (0.34-0.81)	1.49 (0.88-2.84)
Spleen	0.19 (0.06-0.34)	0.78 (0.30-1.38)	2.44 (1.46-4.14)	0.33 (0.23-0.43)	0.44 (0.28-0.66)	1.27 (0.50-2.46)
Aorta	0.12 (0.05-0.24)	0.31 (0.14-0.72)	0.83 (0.60-1.65)			
Pancreas	0.18 (0.06-0.27)	0.38 (0.17-0.61)	1.86 (0.73-3.95)			
Thymus	0.09 (0.05-0.12)	0.11 (0.06-0.14)	0.22 (0.16-0.36)			
Bladder	0.01 (0.01-0.02)	0.03 (0.02-0.30)	0.08 (0.06-0.12)			
Muscle	0.06 (0.03-0.12)	0.12 (0.02-0.30)	0.07 (0.05-0.11)			
Ovaries	0.06 (0.00-0.13)	0.23 (0.11-0.60)	1.70 (0.82-4.35)			
Uterus	0.02 (0.01-0.05)	0.04 (0.00-0.15)	0.17 (0.12-0.23)			
Subcutaneous nodulus	0.70 (0.30-1.10)	99.60 (0.60-240.70)	509.20 (392.0-648.2)			

No statistics done because of very large scatter of data.

TABLE 10.4 MEAN LEAD CONTENT (MG) OF THE CARCASS AND SEVERAL TISSUES OF FEMALE RABBITS AFTER EXPOSURE TO LEAD ACETATE

	Day 222 (end of exposure)			Day 336 (end of recovery)		
Variable	Control (n = 8)	Low dose (n = 8)	High dose (n = 8)	Control (n = 4)	Low dose (n = 5)	High dose (n = 5)
Whole body (calculated)	1.11	16.97	78.55	1.29	13.14	52.22
Bone (est. 10%)	0.83	8.54	49.66	0.80	8.11	77.26
(est. 5%)	0.42	4.27	24.83	0.40	4.06	38.63
Subcutaneous nodulus	0.07	0.35	4.13			
Liver	0.01	0.34	0.96	0.11	0.15	0.77
Kidney	0.02	0.05	0.34	0.00	0.02	0.18
Lungs	0.04	0.04	0.10	0.01	0.01	0.02
Blood (est. 7%)	0.01	0.14	0.31	0.01	0.06	0.17
Muscle (est. 50%)	0.19	0.38	0.20			

Values calculated from the means in Table 10.3.
Percentages given for some tissues are estimated relative weights used for calculation.

10.4 DISCUSSION

The effects of exposure of female rabbits to lead for 7.5 months, resulting in concentrations of lead in blood equal to the WHO limit for lead workers (400 µg/L) and higher were investigated. The rabbits received lead by subcutaneous injections of lead acetate. This route circumvents the dietary effects on gastro-intestinal lead absorption [Mahaffey (1983); Tsuchiya (1986)] and allows better control of concentrations of lead in blood. These advantages may be offset to some extent by the appearance at the injection sites of subcutaneous nodules accumulating significant amounts of lead. However, it appeared possible to keep the PbB level of the low-dose animals very close to the intended concentration of 400 µg/L when the dose of lead acetate was adjusted weekly, based on the PbB measured in the previous week. Animals receiving the same amount of lead showed very different responses with respect to their PbB values. Differences in release of lead from the nodules and in number of nodules might be among the causes of the different responses; differences between animals with respect to lead metabolism may also contribute to this phenomenon.

Injection of 0.10-0.20 mg lead acetate/kg body weight three times a week was required for blood lead concentrations of 400 µg/L (low-dose group) whereas 1.20 mg lead acetate/kg resulted in mean lead levels of 900 µg/L (high-dose group). A similar result was described by Wibowo and Zielhuis (1980). In their experiment

doubling of the amount of lead given to rabbits (0.50 vs. 0.25 mg lead acetate, three times a week) resulted in a 20% rise of PbB. These rabbits also developed subcutaneous nodules at the injection sites [Zielhuis (1988)]. Rabbits of the high-dose group stored approximately six times as much lead in their bones as did the animals of the low-dose group, and ten times as much in the subcutaneous nodules.

In the recovery phase PbB decreased with a half-time of 60-70 days. This is much longer than the reported half-time for lead in human blood, which is approximately 20 days [Task Group (1976)]. Most probably this difference is due to slow leakage of lead from the nodules. After about 12 weeks of recovery PbB seemed to stabilize at approximately 40-50% of the concentration maintained in the exposure phase. Since this apparent stabilization was measured during an additional period of only four weeks no conclusions can be drawn as to the further course of PbB.

At the end of the exposure phase lead concentrations were highest in the subcutaneous nodules and in bone. Other organs with relatively high lead concentrations were liver, kidneys, lungs, brain and spleen. With the exception of the lungs, this is in accordance with published results on lead accumulation in man and rat [Goyer (1981); Hejtmancik et al. (1982); Mahaffey (1983)]. No values for the concentration of lead in lungs were found in the literature. The organs were not perfused prior to collection, so part of the lead measured in the organs will have been erythrocyte-bound. After the recovery phase the lead content decreased in all tissues analysed except bone. In bone there even seemed to occur some further accumulation of lead in the high-dose group. Although an artefact cannot be excluded, reallocation of lead may have occurred during the recovery phase.

The inhibitory effects of lead on haem synthesis are well known. Our results are in accordance with current knowledge. Effects were almost entirely limited to the high-dose group. From day 125 on the haemoglobin concentration decreased and the erythrocytes showed increased frequency of basophilic stippling. The effects on red blood cell indices were present already on day 97 (lowered MCV and MCH). These phenomena are considered to be the earliest signs of anaemia.

No haematological effects were seen in the low-dose group. Wibowo and Zielhuis (1980) found no effect on haemoglobin concentration and haematocrit with a PbB of 500 and 600 μg/L. In adult people, effects on haemoglobin concentration are usually found at a PbB higher than 800 μg/L [Zielhuis (1977)]. During recovery the haematological values rapidly returned to control levels.

Some biochemical parameters of haem synthesis showed larger effects of lead. ALAD was severely inhibited already after about 50 days in both the low- and high-dose group. However, indications of decreased haem synthesis (increased ZPP and ALAU and lowered haemoglobin concentration) were seen only in the high-dose group. ZPP in the low-dose group was higher than in the controls during the whole main study, but the difference was statistically significant only at day 49. Therefore, the increase of ZPP in the low-dose group, if real, is much less

pronounced than the effect on ALAD. In female rabbits the effects seem to occur at higher levels of PbB than in women.

Inhibition of ALAD in liver required a much higher PbB than inhibition of this enzyme in erythrocytes. This is inversely related to the lead content of both 'tissues'. In the low-dose group the lead content of the erythrocytes was approximately 0.8 mg/kg whereas the livers of the same animals contained 3.5 mg/kg. Nevertheless, erythrocyte ALAD activity was only 23% of control values while no inhibition was found in the liver. The difference may be caused by a different distribution of lead in the cell. In nucleated cells, including the liver parenchymal cell, lead is fixed in the nuclei by acidic non-histon proteins [Cherian and Nordberg (1981)]. Apparently, this fixation mechanism is able to keep cytoplasmatic lead concentrations low enough to prevent inhibition of liver ALAD, in spite of considerable concentrations of lead in tissues. Mature erythrocytes have lost their nuclei and thus a lead-inactivating mechanism, resulting in inhibition of erythrocyte ALAD at low PbB. The relatively small effect of lead on haemoglobin concentrations may be explained by the presence of a nucleus in the erythrocyte precursor (normoblasts) were most of the haemoglobin is synthesized.

In summary, subcutaneous injection of lead acetate in female rabbits during a period of 7.5 months resulted in a number of effects, mainly in the high-dose animals. The only effects observed in the low-dose animals were a clear inhibition of ALAD and a marginal increase of ZPP in erythrocytes. Since in these animals the haemoglobin concentration was not affected, the effects apparently had no physiological consequences. Therefore, using various parameters at a level of 400 µg lead/L blood no adverse effects were observed. In particular, the kidney function was not affected.

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ACKNOWLEDGEMENT

The authors thank Prof. Dr. V.J. Feron and Prof. Dr. R.L. Zielhuis for their help in preparing this manuscript and Mrs. A.Ch. Franssen, Mr. F.J.A. Kouwenberg and Mr. H. van Vulpen for their skilled assistance. This study was supported financially by the Dutch Directorate General of the Ministry of Social Affairs and Employment.

CHAPTER 11

GENERAL DISCUSSION AND CONCLUDING REMARKS

In lead-processing industries in the Netherlands, an overall decline in the levels of exposure of the workers has been effected, among others, by improving working conditions. The increased attention of occupational physicians and hygienists, the more adequate legislation and the measures taken by the Directorate General of Labour contributed also to reduce the problem of disruption to health by occupational exposure to lead. So, nowadays, in industry intoxications by lead with pronounced clinical symptoms are seldomly encountered any more (personal communication, W.L.A.M. de Kort). However, in many lead workers levels of lead in blood (PbB) that may cause adverse effects still occur frequently *(Chapter 4, Table 4.9),* e.g. effects on the male reproduction system and on the central and peripheral nervous system. It implies that in many industries a 'lead problem', be it reduced, still exists, continuously requiring the vigilance of the authorities responsible, as well as of the employers, employees and trade unions.

Vital information on the extent of lead exposure can be obtained from the lead content of the blood. Understandably, when biological monitoring (BM) of lead in blood is performed, the analytical quality of the assay, i.e. its accuracy and reproducibility, is of paramount importance. As is pointed out in the Lead Decree, issued by the Ministry of Social Affairs and Employment, the results of the analysis of PbB of an individual worker should be interpreted with respect to the so-called action levels and the limit values of PbB [Labour (1988)]. When these levels are exceeded, measures are required to reduce or to prevent further uptake of lead. This implies that results of the analysis of PbB may have great economical and social consequences for employees as well as for employers. When incorrectly high PbB values are reported, suggesting unacceptable levels of exposure, this may lead to unjustifiably drastic measures, such as costly technical changes or adaptations of processes. Ultimately the worker concerned may be unnecessarily removed from his job. On the other hand, if values are reported which are too low, necessary measures to decrease exposure will probably be omitted, possibly leading to undesirable and hazardous situations.

The round robin exercises described in *Chapter 3* indicate that in view of these considerations the performance of the analysis of PbB by the participating laboratories was, in general, insufficient with regard to precision (reproducibility) and accuracy (free from bias). In order to be assured of valid data, it is suggested that the assay of lead in biological samples for health surveillance purposes should be restricted to laboratories that have demonstrated proficiency in measurements

of low-level trace elements in this type of sample, and that carry out the analyses regularly. Versieck and Cornelis (1980) and Versieck (1985) have shown that improved control of contamination and other factors resulted in considerably lower and more consistent reference values of metals in biological samples than were reported earlier. It is reasonable to assume that similar effects, resulting in generally more reliable results, will be achieved when such measures are applied on samples with increased levels of the metals, including lead.

Of great importance in obtaining demonstrably more reliable results is also the continuous participation in external quality control programmes. This allows comparison of the skill of a laboratory with that of other participants and assessment of the reliability of the analytical method used. Satisfactory performance in such a programme will inspire confidence that the analysis of other samples also is carried out with comparable quality awareness.

In daily routine, so-called internal quality control samples, should be analysed in the same series as the actual samples. The former are 'spiked' samples containing a known quantity of added lead. The results help in identifying any trends in the performance which may occur in course of time and to check intralaboratory precision and accuracy.

Following the above recommendations may result in a coefficient of variation of the PbB analysis of 5-10% [Bullock et al. (1986)], instead of the values described in *Chapter 3*, which ranged from 10 to 36%.

In many studies it has been established that lead is nephrotoxic and that it influences blood pressure. Effects on blood pressure have been established in lead workers at levels of PbB of about 2 µmol/L *(Chapter 2)*. This is only marginally above the limit value for males of 1.9 µmol/L proposed by the Dutch Expert Committee on Occupational Standards [DECOS (1980)]. The aim of the study presented in *Chapter 4* was to establish whether the levels of PbB that occur in lead workers in the Netherlands, make it desirable to include the routine measurement of blood pressure in health surveillance programmes. As is shown in the overview given in *Chapter 4*, in practice these levels of PbB were sometimes considerably higher than the proposed limit value.

De Kort et al. (1987) observed an increase of systolic blood pressure of about 1.8 mm Hg when PbB increases by 0.5 µmol Pb/L. In view of this small effect, it is concluded that the present levels of PbB in the Netherlands, which probably will decline further in the future, would make it very difficult or even impossible to establish epidemiologically effects of lead on blood pressure in occupationally exposed workers. Only longitudinal prospective studies in large cohorts might possibly reveal such effects.

Therefore, incidentally measuring blood pressure in lead workers with the aim of detecting an effect of lead exposure, serves no practical purpose and is not recommended.

Anaemia caused by exposure to lead only occurs as a result of high exposure levels. In the study presented in *Chapter 5* a statistically significant linear relation between PbB and haemoglobin concentration in Dutch lead workers was observed. Over the range of PbB of 0-5 µmol/L the average decrease in the level of Hb amounted to 0.28 mmol/L. The upper limit of the 95% confidence interval amounted to a decrease of 0.59 mmol/L. Although there is no clear consensus as to the exact dose-response relationship between PbB and the decrease of Hb [Moore and Goldberg (1985)], these results may have implications for people having a low-to-normal Hb level (low-reference value is 8.6 mmol/L) at the start of their work and who are highly exposed.

There still is a number of occupational activities which may lead to levels of exposure of about 3 µmol/L and higher. The demolition work described in *Chapter 8* is such an example. Also specific activities in the production of electric batteries and in the process of re-smelting scrap-lead presented in *Chapter 9* appeared to result frequently in very high levels of exposure. These situations occur in practice notwithstanding interventions taken to decrease exposure and regular inspections by the occupational physicians responsible. In this respect it may be mentioned that Ulenbelt et al. (1990) have shown that the hygienic behaviour of workers in an electric battery factory was an important factor in the intake of lead. Hand-washing before visiting the toilet, spitting and nose blowing without using a handkerchief were considered examples of good hygienic behaviour. So, despite moderate levels of environmental concentrations of lead dust, due to carelessness avoidable additional uptake may occur, resulting ultimately in health impairment like anaemia.

It is concluded that measurement of Hb is desirable in future lead workers to detect those at extra risk of developing anaemia.

Exposure to lead may occur in various ways, with different consequences. A particular situation arises when the exposition involves an insoluble lead compound, such as lead chromate. The study presented in *Chapter 6* shows that after inhalation of lead chromate dust by rats, the greater part forms a deposit in the lungs. In such a case PbB levels will reflect only that -small- part of the inhaled quantity of the compound that is absorbed into the blood. Because the deposit in the lungs is not accounted for, the PbB data give an underestimation of the actual intake and uptake in the body.

These results suggest that inhalatory exposure of man to lead chromate may eventually result in little adverse systemic effects, in conformity with the low PbB level. However, there is uncertainty as to the health effect of the deposit in the lung tissues, among others in view of the suspected carcinogenicity of lead chromate.

Health surveillance of workers exposed to lead chromate, but probably also of those exposed to other insoluble lead compounds, requires an approach different from that of workers exposed to metallic lead or soluble lead compounds. It is

desirable to estimate the total quantity of the compound taken up by the body and not to rely solely on PbB as the index of internal dose. Therefore, it is recommended that personal air sampling (PAS) is also carried out, systematically and frequently, to allow an estimate of the intake and thus of the deposit. The data obtained should be related to the MAC of lead chromate [MAC (1994)].

Exposure to lead will affect the synthesis of haemoglobin, and will result in the formation of another metalloporphyrin compound, viz., zinc protoporphyrin (ZPP), which then occurs at an elevated level in the blood. This effect shows great interindividual variation as has been demonstrated in *Chapter 7*.

The determination of ZPP in relation to lead exposure has found two applications. Firstly, ZPP is applied frequently in biological-effect-monitoring (BEM). An increased concentration of ZPP in occupationally exposed people indicates an effect of lead on the haematopoietic system and is considered to reflect an 'active lead deposit' in the body. This has been discussed in *Chapters 2 and 7*.

The other application of the assay of ZPP is as a simple and cheap pre-screening method. *Chapter 7* presents an investigation into the possibility of using ZPP to estimate whether PbB in a sample exceeds one of the action levels or the limit values of the EC-Directive. In that case additional determination of PbB would be required. However, the low specificity and sensitivity of ZPP at the first action level mentioned in the EC-Directive (PbB = 1.45 μmol/L) makes it an inadequate substitute for the PbB measurement around this concentration. Because of the high proportion of workers with low to moderate exposure to lead, nowadays the applicability of ZPP for pre-screening individual workers has decreased.

However, this does not rule out the use of ZPP in detecting an increased concentration of PbB in lead workers. An average increased concentration of ZPP in a group of people indicates almost with certainty an average increased level of PbB. Since anaemia induced by iron shortage also results in higher levels of ZPP, however, it is recommended in screening an individual for ZPP to concomitantly determine Hb. But neither in a group, nor on individual basis is it possible to estimate the actual level of PbB merely on the basis of ZPP measurement.

The effects of occupational exposure to lead on PbB and ZPP in practical situations, is described in *Chapters 8 and 9*. There, the results are presented of BM of PbB and of BEM of ZPP in lead workers in two different working conditions. The data in *Chapter 8* show that, despite the extremely heavy exposure (the time-weighted average concentration in the breathing zone amounted to more than 100 times the present MAC of 0.15 mg/m^3), the PbB level of the workers did not exceed 5.5 μmol/L. Apparently, after a more or less continuous exposure at this level, a plateau is reached with an equilibrium between uptake, deposition and excretion. The data suggest that the very high intake is reflected only partly in the level of PbB.

Essentially this result is in agreement with those of Skerfving (1993) who used available data. He established a curvilinear relationship between uptake of Pb (maximum 2.9 µmol/day) and PbB, which levelled off asymptotically at about 2.5-4 µmol/L. Thus, the increase of PbB per unit increase of the concentration of Pb in air (PbA) gradually becomes smaller.

This may have implications for establishing the 'true' intake of lead during heavy exposure. Under working conditions in which high levels of exposure are likely to occur, it is unwise to estimate exposure only by measuring PbB. Therefore, it is suggested that personal air sampling and determination of Pb in urine (PbU) are also included. The latter has been recommended in particular to establish a severe exposure [Roots (1979)]. PAS and PbU data complement the results of the PbB analyses in decisions on managing the exposure.

A low excretion of lead in urine in a situation of high intake will be a signal of the formation of a deposit in the body. This may require examination of the kidney function and further measures to control intake of lead.

It appears from the results in *Chapter 8* that induction of ZPP by lead exposure shows great differences between individuals when related to the PbB values reached. Similar results have been described in *Chapter 7.* The PbB levels of the workers increased to levels between 3.5 and 5.5 µmol/L within a few weeks while the concentrations of ZPP reached values between 175 and 1100 µmol/mol Hb. The ratio of ZPP and PbB at the highest level of PbB of the workers varied between 36 and 207. Because there is a time-lag in the formation of ZPP after exposure to lead, the levels of ZPP were probably still increasing during the following weeks. This indicates that not only the maximum allowable level of PbB, but also that of ZPP stated in the Lead Decree (500 µmol/mol Hb) in most cases has been exceeded grossly.

The second study in a practical situation *(Chapter 9)* concerned a lead-recycling plant, where an extensive programme was initiated of hygienic and technical measures and of elaborate precautions to reduce the levels of PbB. Although this resulted in a general decline of exposure, the study made clear the difficulty of reaching a level of exposure that complies with the permissible level of PbB.

More important from the health surveillance point of view was the observation made in this survey that it is difficult to demonstrate a decrease in intake of lead in chronically exposed subjects solely on basis of PbB data. Under conditions of chronic exposure, the level of PbB is determined by two sources: the absorbed lead originating from recently experienced exposure and, secondly, a continuously present 'background' built up over time, which is determined by the equilibrium of lead in the different compartments in the body (in addition to PbB, the deposits in soft tissues, trabeculae and, to a lesser extent, in compacta). It has been discussed in *Chapter 2* that this background decreases only slowly when exposure is reduced and that it may contribute considerably to the PbB level. This implies that, although technical measures as well as improved personal and work hygiene

may have resulted in a substantially decreased intake of lead, their actual effect on the PbB level of workers with a record of prolonged exposure is only reflected in the long term. Therefore, in addition to the assay of PbB, PAS will be required to monitor the short-term effect of these measures and the expected decline in intake of lead.

In a group of 19 workers who were screened more than ten times for PbB and ZPP, 17 showed a statistically significant relation between these parameters. The workers had been chronically exposed to reasonably constant levels of Pb for several years and the PbB and ZPP screening had been performed at relatively long intervals. In 72% of the measurements, an increase or decrease of PbB resulted in a corresponding change of ZPP. In 18% of the cases an increase of ZPP went together with a decrease of PbB, thus yielding a false positive indication. Only 10% of the measurements showed a false negative result (decreasing ZPP versus increasing PbB). This suggests that in workers exposed for a prolonged period, variations in the ZPP level of an individual may be used to establish changes in his PbB level. Such an approach might considerably simplify health surveillance of lead workers.

An animal study was undertaken to see whether under conditions of long term exposure to lead, any physiological parameter would be affected in such a way that it might be considered indicative of the extent of exposure or of the total lead burden *(Chapter 10)*. In this study two groups of female rabbits were exposed to lead acetate causing levels of PbB of about 1.9 and 4.3 μmol/L, respectively, for 7.5 months. Thereafter, a part of the animals was kept for 4 months without treatment.

During exposure, gain in body weight was lower in the 'high-dose' group than in the 'low-dose' group, whereas the relative weights of heart and liver in the 'high-dose' group increased. The first group also developed anaemia and effects on several other haematological parameters (inhibition of ALAD, increased levels of ZPP and ALAU). Also basophilic stippling and lowered MCV and MCH in red blood cells were observed. No haematological effects occurred in the 'low-dose' group, with exception of ALAD inhibition. In neither of the groups effects on kidney or liver function tests or on clinical parameters were observed. The experiment did not indicate any parameters that could possibly be applied in the health surveillance of lead workers.

The objective of the present study on the labour conditions of workers in the lead-processing industry was to investigate experimentally specific topics that could already be applied in health surveillance programmes, but which demanded a renewed evaluation. Further, it was considered desirable to pay attention to a number of topics that are recommended to become integrated parts of these programmes. In this context the aspects described in *Chapters 3-10* have been investigated. The subjects vary greatly in character, but each in itself is considered

an essential element in health surveillance. The results of the studies, when applied in practical situations, will contribute to a more reliable health surveillance of people occupationally exposed to lead, and may help to prevent avoidable health risks. The following conclusions are drawn.

- The results of the analysis of PbB may have radical consequences for people involved. In this respect it is essential that quality control of the assay of lead in blood constitutes an integrated part of health surveillance programmes.
- Because high exposure to lead may induce anaemia, it is strongly advised that the potential occurrence of this effect in (future) lead workers is established. Therefore, measurement of haemoglobin should be a part of the pre-placement surveillance of these people.
- In case of exposure to lead chromate or other insoluble lead compounds, one cannot rely on PbB measurements. Additional indicators of uptake are required to prevent the occurrence of health impairment. Therefore, establishment of the exposure by PAS should be included in the surveillance programme.
- Due to its simplicity, the measurement of ZPP as (rough) indicator for PbB allows a frequent estimation of exposure. It is recommended that this screening should be incorporated into health surveillance.
- During heavy exposure to lead the level of PbB does not increase correspondingly. In these cases one should not rely solely on PbB as an indicator of body burden. In addition, the intake should be estimated by PAS and the uptake also by PbU.

A protocol for health surveillance of occupationallly exposed lead workers in which these recommendations have been incorporated is suggested in the *Annex*.

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ANNEX

PROTOCOL FOR HEALTH SURVEILLANCE OF WORKERS EXPOSED TO LEAD

INTRODUCTION

In many countries the great number of people occupationally exposed to lead and the knowledge of the related risks to health, have led to the issuing of maximum permissible levels of PbB and PbA to ensure protection of workers (*Table A1*).

In the Netherlands such regulations, just as other occupational health-based standards, are developed in the following three-step procedure.

1. The Dutch Expert Committee on Occupational Standards (DECOS) advises in terms of exposure levels with respect to *health effects*.
2. The Subcommittee on Occupational Exposure Limits of the Social and Economic Council (SOEL, the former National MAC-Commission) judges the advice of DECOS with regard to its social and economical implications.
3. Finally, the Government lays down by rule, or decides, on the acceptable levels of exposure.

In 1980, DECOS proposed permissible biological limit values of PbB for male and female lead workers. The Committee concluded that levels of PbB higher than 1.9 μmol/L may lead to disturbed nerve conduction velocity (NCV) and/or a clearly increased level of zinc protoporphyrin (ZPP) in blood, the latter indicating the presence of an 'active' deposit of lead in the body. In view of the adverse effects of lead on the central nervous system of the foetus, for fertile women a permissible level of PbB of 1.4 μmol/L was proposed. In the proposal also the potential induction of chromosomal aberrations in lead workers was considered.

In particular the effect on NCV was considered injurious to health. Health consequences of the increased level of ZPP were not clear, while the literature on the occurrence of chromosomal aberrations was conflicting.

The Committee also proposed permissible levels of lead in environmental air at the workplace: for men of 60 $\mu g/m^3$ and for women of 40 $\mu g/m^3$ (TWA-8 h, particle size <5 μm). It was emphasized, however, that the main criterion in health surveillance should be the level of PbB.

Since the proposal of DECOS in 1980, no other health-based permissible levels of PbB and PbA have been issued by official bodies in the Netherlands. However, several recent studies, which are discussed in *Chapter 2*, have shown that adverse

TABLE A1 STANDARDS AND GUIDELINES FOR LEAD IN BLOOD AND IN ENVIRONMENTAL AIR, AND FOR EFFECT PARAMETERS

PbB	PbA	Status				
(µmol/L)	(µg/m^3)	legally binding	guide line	Country	Effective since*	Remarks
1.9	60		+	Netherl.	1980	Men PbA:TWA-8h
1.4	40		+			Women PbA:TWA-8h
	150	+		Canada	1980	
	0	+		Germany	1980	Fertile women may not be exposed to material containing >2% Pb
1.9			+		1982*	Men, and women above reproductive age
1.4			+			Women in reproductive age ALAU <6 mg/g creatinine ZPP ≤50% raised (Recommended by WHO)
	50	+		U.S.A.	1983	
3.4	150	+		E.C.	1988	
3.9						Prov. ALAU <20 mg/g creat. or ZPP <500 µmol/mol Hb or ALAD <6 European units
	150		+	U.S.A.	1987*	Inorganic lead dust
2.4			+	U.S.A.	1987*	PbU: 150 µg/g creatinine ZPP: 1 µg/ml blood
	150		+	Belgium	1987*	
	50	+		Sweden	1989	Respirable dust of Pb and inorganic lead compounds
	100	+		Finland	1989	
3.4			+	Germany	1992*	ALAU <15 mg/g creatinine (men)
1.4						Women <45 years: ALAU <6 mg/g creatinine
	100					TWA-8 h
	1000					STEL-30 min
						Exposure to Pb < MAC and < permissible level of PbB cannot exclude adverse effects to offspring
	150		+	U.K.	1993*	TWA-8 h

For abbreviations see list on page 141.

* The year indicated with * is the year that the guideline was issued.

health effects may occur at levels of exposure that in the recent past were considered without risks. In the light of these newly acquired insights, a re-evaluation of the proposed levels by an expert group is recommended*.

In 1982, the SOEL advised permissible biological levels of PbB for men and women of 2.9 and 2.4 μmol/L, respectively, and a permissible level of PbA of 100 $\mu g/m^3$. These proposals should have taken effect in 1985 but were superseded by a Directive of the European Community (see below). Although the SOEL agreed with the opinions of the DECOS, the levels of PbB proposed by the latter were at that time considered not to be attainable in practice [SOEL (1982)]. However, they advised a further reduction of the permissible levels in the future.

At present, health surveillance of lead workers in the countries of the European Community is based on the requirements of an EC-Directive [EC (1982)]. This directive was implemented in the Dutch legislation as the 'Lead Decree' ('Het Loodbesluit') which took effect in 1988 [Labour (1988)]. It applies to work in which persons may be exposed to lead and aims at assessment and control of exposure and prevention of health impairment. However, the permissible limit values and the so-called 'action' levels for PbB and PbA defined in the decree *(Table 9.3 in Chapter 9)* were not based on published arguments. In view of current knowledge on the relation between exposure and occurrence of adverse health effects, as discussed in *Chapter 2*, these values and levels are considered too high.

By order of the European Community the data of the EC-Directive were updated by Apostoli (1991). On the basis of recent studies the adverse effects of lead in adults were reconsidered. It was proposed to reduce the biological limit value of PbB for men to 2.4 μmol/L and for women to 1.4 μmol/L. Standards of PbA at the workplace were reduced to 70 and 35 $\mu g/m^3$ for men and woman, respectively. However, in the report no argumentation is given for these environmental air levels. It is emphasized further that the proposals are under discussion, now, and not accepted yet.

Programmes for health surveillance of occupationally exposed workers are often based on the standards presented in *Table A1*. Generally, PbB and/or PbA are determined in these programmes and sometimes one or more effect parameters. Also more comprehensive programmes are available which cover additional aspects related to the health risks of occupational exposure. In the Lead Decree a protocol is described which is based on both the action and permissible levels of the EC-Directive.

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* Recently, DECOS started the procedure for updating the recommendations issued in 1980.

PROPOSAL OF A PROTOCOL

Introductory remarks

Health surveillance of lead workers is usually not performed on a regular basis. However, systematic collection of data in a longitudinal programme allows a more reliable assessment of exposure and earlier recognition of health impairment than incidental measurements [Zielhuis and Henderson (1986)].

The following protocol aims at obtaining a continuous and reliable insight into the exposure of workers in order to prevent the occurrence of early health impairment. It is based on that of Lerner (no year). In addition, the relevant results of the studies presented in *Chapters 3-9* have been incorporated. Also several recommendations made in Publication P 170-1 [Labour (1988)] and in the Handbook of Occupational Hygiene [Harvey (1993)] have been taken into account.

Alessio and Foà (1980) recommended that in health surveillance of lead workers two biological tests are used simultaneously. The first should indicate the internal dose or exposure as reflected in the concentration of PbB, the second should indicate an effect.

Since it was argued before that the action values and permissible limit levels of PbB and PbA mentioned in the Lead Decree [Labour (1988)] are considered too high, the limit values proposed by DECOS (1980), i.e. PbB: 1.9 and 1.4 μmol/L and PbA: 60 and 40 $\mu g/m^3$ for men and women, respectively, are adopted in the following protocol. A consequence of this is that the traditionally used biological effect parameters, like ALAU, CPU and to a smaller extent also ZPP, have become less important since they only respond at higher levels of PbB. They may have to be replaced by more sensitive parameters that have greater significance at lower levels of exposure, when available.

In *Chapter 8* it has been argued that on an individual basis the concentration of PbB cannot be derived from PbA. WHO (1980) also concluded that it is not possible to indicate a level of PbA which guarantees that the permissible level of PbB is not exceeded. Therefore, following DECOS (1980), in the following protocol PbB is the primary criterion for assessing exposure and preventing toxic effects, whereas PbA is used as a means to ascertain adherence to limit values.

THE PROTOCOL (see also Table A2)

A. PRE-PLACEMENT ASSESSMENT

The following biological tests should be performed preferably prior to the commencement, but in any event within the first two weeks of the job.

TABLE A2 SCHEME OF HEALTH SURVEILLANCE

	New employees		All employees				
	Pre-placement	Monthly for 4 months	PbA < limit value A	PbA > limit value B	PbB > limit value C	Exposure to lead chromate D	High exposure D
PbB	x	x	x	x	x		
ZPP[d]	x	x	x	x	x		
ALAD[d]	x	x	x	x	x		
P_5N[d]	x	x	x	x	x		
Hb	x		x	x	x		
Questionnaire	x						
Medical examination	x						
PAS		x	x	x	x	x	
PbU							x
EM[e]				x	x	x	

For abbreviations see list on page 141.

A. Annually, on condition that PbB's of all workers are <1.9 µmol/L.
B. At the decision of the occupational physician.
C. Repetition of PbB, offered to the worker concerned within three months.
D. Additional measurements
d. One of these three BEM parameters.
e. To identify sources of emission.

- Assay of lead in blood
  This analysis will reveal previous exposure in work or leisure time. On the condition that the analysis is performed before the first day of employment, it may also be used as a baseline value for further prospective studies.

  In the author's experience the assay of PbB by the method of Stoeppler et al. (1978) has proven to be reliable and applicable for the measurement of large series of samples. The precision of the method, expressed as the relative standard deviation in parallel determinations at different times, is about 5% and the detection limit about 0.05 µmol/L blood. Also, the procedure of Schaller (1983) which uses extraction of the sample and flame atomic absorption spectrometry may be recommended (precision, is about 5%; detection limit 0.07-0.25 µmol/L blood.

The necessity of participation in an external quality control programme has been argued in *Chapter 3.*

- Determination of haemoglobin in blood *(Chapter 5)*
  Persons with a low-to-normal or a Hb-level below the reference value, who are potentially at risk of (further) developing anaemia, can thus be identified. This

applies in particular to workers who are likely to be significantly exposed to lead. Knowledge of the level of Hb also avoids a possibly wrong conclusion that an increased level of ZPP in blood actually resulting from anaemia is due to lead exposure *(see Chapter 7)*.

- Determination of at least one of the following biological effect parameters
- Zinc protoporphyrin in blood *(Chapter 7)*
  It is recommended that PbB and ZPP are always determined jointly in a single blood sample. Recommended method of analysis: Blumberg et al. (1977).

- δ-Aminolaevulinic acid dehydratase in blood
  References for the analysis of ALAD by the 'European Standardized Method' of Berlin et al. (1977) are given in *Chapter 2*. The precision of the method is about 3%.

- Pyrimidine 5'-nucleotidase in blood
  Details of the method of analysis and its application are given in *Chapter 2*.

Of these three methods the assay of ZPP has found the most general application in health surveillance programmes. The analysis is cheap and easy to perform and the relation between PbB and ZPP has been studied extensively. However, as has been discussed above and in *Chapter 7*, at lower levels of exposure its meaning is limited. In those cases determination of ALAD or of P_5N in blood is preferable.

In *Table A3* a summary is presented of reference values and available limit values of the parameters discussed in the proposed protocol. The reference values may give an indication of the levels that may be expected in non-exposed groups. However, it is strongly recommended that together with the screening of exposed workers, the levels in matched local control groups are established.

PRE-PLACEMENT MEDICAL SURVEILLANCE

Lerner (no year) and Labour (1988) recommend that in the medical surveillance of each worker to be employed attention should be given to the following issues.

- Determination of any past occupational exposure to lead. This may be achieved by analysis of PbB and/or by a questionnaire on occupational history.
- The physical status of the worker, to identify possible conditions that may be aggravated by exposure to lead. In this connection it is recommended that attention is given to

  - the blood and the haematopoietic system;
  - the nervous system;

- the kidneys;
- the heart and the vascular system.

The Directorate General of Labour has published the protocol 'Lead and its ion compounds' (Protocol S 30-8) giving particulars on the medical examination of workers exposed to lead and its ion compounds.

TABLE A3 REFERENCE VALUES AND LIMIT VALUES OF PARAMETERS APPLIED IN THE PROPOSED PROTOCOL

	Reference value	Limit value
PbB (μmol/L)	<0.75[a]	1.9[b] (men) 1.4[b] (women in reproductive age)
PbU (μmol/mol creatinine) (μmol/L)	1.9[c] <0.61[e]	82[d] N.A.
ZPP (μmol/mol Hb)	38 ± 13 (men)[f] 48 ± 16 (women)[f]	N.A. N.A.
ALAD (European units)	38.3[g]	N.A.
P_5N (International units)	12 ± 0.7[h]	N.A.
Hb (mmol/L)	8.4-10.9[i] (men) 7.3-9.8[i] (women)	
PbA (μg/m³, <5 μm, TWA-8h)[j]		60[b] (men) 40[b] (women in reproductive age)
Lead chromate		25[k]

For abbreviations see list on page 141.

a. Based on a great number of PbB levels of non-occupationally exposed Dutch males established by MBL.
b. DECOS (1980).
c. Gerhardsson et al. (1992).
d. ACGIH (1986).
e. Zhang (1993).
f. Wildt et al. (1987).
g. Berlin et al. (1977).
h. Cook et al. (1986).
i. Local reference values.
j. Particles <5 μm are considered respirable.
k. TWA-15 min (MAC 1994).

N.A. Not available.

- Identification of factors which may affect or contribute to exposure. As has been discussed before, poor personal or work hygiene like untidyness, smoking, eating and drinking during work, are often the most important factors determining intake of lead.

- The ability of a subject to use a respiratory protective device when required. The physical status of a worker may restrict the use of a respirator mask. Facial hair e.g. may interfere with its proper fitting.
- Special attention should be given to the working conditions of pregnant and nursing women. Removal of women from work associated with exposure to lead in the early phase of pregnancy may be too late to prevent adverse effects to the central nervous system of the foetus.

B. PERIODIC ASSESSMENT

Periodic assessment aims at preventing the development of acute or chronic effects of lead. As has been discussed in *Chapter 2*, individuals may show different responses or symptoms and biological effects at similar levels of exposure. This implies that there is no level of PbB synonymous with lead poisoning. The diagnosis requires identification of early impairment, such as changes of nerve conduction velocity or kidney function.

New employees

New employees are subjected monthly to a programme of biological tests similar to that applied before employment. Along with this screening, levels of Pb in the breathing zone are also measured with PAS*. When after four months the average of the PbB (and PbA measurements) are below the permissible limit values, the frequency of screening is reduced to once a year. It is assumed that exposure will not change significantly if the workplace of a subject and his work conditions remain the same. However, employment of a worker at another place or in another job requires that the monthly test programme is restarted [Labour (1988)].

Already employed workers

When data of four PbA measurements, performed earlier in four successive quarters, are below the limit value (in this protocol 60 or 40 $\mu g/m^3$ for male and female workers, respectively), and PbB of all workers is below the limit value (1.9 and 1.4 µmol/L, for men and women, respectively), the worker concerned is subjected annually to the programme A shown in *Table A2*.

When no measurements are available, PbA should be determined four times, each with an interval of three months. When the results of the three last results are below the limit value, a regimen similar as described above takes effect.

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* Monitoring of lead in the breathing zone should be carried out according to the Dutch National Tentative Norm NVN 2938.

The Lead Decree describes that when a PbA level exceeds the limit value the occupational physician decides on the necessity and time of repeating the assessment of the biological tests.

Also, when a PbB level exceeds the limit value the employee is offered the opportunity to have the analysis of PbB repeated in newly obtained blood samples within three months. When the first result is confirmed, suspension of further exposure or transfer to a workplace with less exposure are indicated, together with repeated measurements of PbB until the latter has fallen below the permissible level. It is of essential importance that this step is followed by an investigation into the causes of the high exposure and that appropriate measures are taken to reduce further uptake. Counselling of the workers concerned may be required to obtain more insight into the causes of the inadmissible extent of exposure. Suspension of further exposure serves only a limited purpose if the causes of the high exposure are not eliminated. In *Chapter 9* and *below* several work practices and procedures are described to avoid contamination of the workplace and the subsequent intake of lead.

C. GENERAL CONSIDERATIONS

The purpose of the following considerations is to improve further the quality and reliability of the proposed health surveillance programme.

- The protocol is applicable under conditions of more or less regular levels of exposure. However, in practice the conditions of exposure may vary in such a way that the application of the time-schedule suggested in the protocol will yield unsufficient insight into the actual intake of Pb. Under these circumstances it may be necessary to increase the frequency of the recommended measurements. The proper execution of the protocol depends on the actual circumstances and possibilities and rests with the occupational physician responsible and the management concerned.

- The annual screening discussed above, which is based on the directions of the Lead Decree, may imply a considerable risk that workers are exposed to unnoticed too high levels of Pb during the interval between two screenings. Therefore, contrary to these directions, it is suggested that the frequency of the screening is increased to several times a year.

- The Lead Decree requires that biological tests and medical surveillance are performed by or under the responsibility of an Occupational Hygiene Service. If work conditions do not change during subsequent employment, it is recommended that the medical examination is carried out once a year.

- The biological tests should be carried out on persons who work in normal situations and who work during the usual time in the workplace [Harvey (1993)]. The situation at a plant in respect to exposure should not be based on results of BM measurements that are performed on workers with deviating work-time or work conditions.

- For analysis of PbB the time of sampling during the day is not critical. In order to correct for diurese and circadian rhythm, however, collection of urine and sampling of blood for BEM parameters should each time be performed at about the same time of the day.

- Although BM of lead provides an accessible measure for estimating exposure, interpretation of measured PbB levels should take into account the fact that they reflect only one point in time. Thus, if external exposure is highly variable in time and level, a single BM value may easily be misinterpreted. This should be taken into account when incidentally a large deviation from the usual exposure levels occurs. Under these conditions more frequent applications of the biological tests may be necessary. Also, when BM is carried out regularly, deviating higher results may indicate peaks in exposure which otherwise would not have been noticed. In *Chapters 2 and 9* it is discussed that a decrease in exposure is not necessarily reflected, in the short term, in a lower level of PbB.

- When in a group of workers with similar tasks, PbB of one subject is permanently significantly higher than that of the others, this may indicate poor personal or work-hygienic behaviour of the worker concerned. The single high PbB level does not necessarily reflect a general high concentration of lead in environmental air. Results of BM should also be considered from this angle.

- In handling solid lead, the usual precautions with regard to personal hygiene, protective measures and cleanliness of the workplace usually prevent significant intake [Harvey (1993)]. In general, when personal hygiene is maintained, intake of lead by primary ingestion is considered negligible compared to intake by inhalation.

- In connection with the adverse effects of lead on a foetus, particularly during the first weeks of pregnancy *(Chapter 2)*, it is recommended that fertile women do not experience exposure resulting in PbB levels exceeding the proposed limit value of 1.4 µmol/L. The Dutch Government intends to insert a bill into the Lead Decree stating that pregnant and nursing women may not be obliged to work with lead and lead compounds when the possibility exists of uptake of lead in the body [Bill 14[a], Staatscourant 33, 1993].

- Because of the suspected carcinogenicity of lead chromate, it is proposed that health surveillance of lead workers who are exposed to this insoluble compound includes personal air sampling *(Chapter 6)*. Very likely this applies also to exposure to other insoluble lead salts. In contrast with PbB, PAS will give information as to the actual intake of the compound and the presence of a deposit. The PAS-data may be interpreted on basis of the MAC-values stated in the MAC-list (1994).

- In particular types of work, heavy exposure to lead is almost inevitable. The results of the study presented in *Chapter 8* suggest that the PbB levels of the workers concerned do not reflect this very high intake. Therefore, it is absolutely required that under such circumstances workers are also monitored regularly by PAS.

- When working conditions may lead to heavy exposure, as described in *Chapter 8,* it is proposed to determine also PbU [Cf. Roots (1979)]. The PbU and PAS data will give additional information as to the intake and excretion of lead which cannot be derived from the PbB measurements only (see above). The results may also support decisions in managing the exposure.

- PbU should preferably be determined in 24-hour urine samples. However, collecting urine over a 24-hour period is difficult to realize in practice. When spot samples are used, interpretation of PbU data may require correction for the fluctuations of the Pb concentration caused by differences in the concentration of the urine. To this end PbU levels are often expressed per mol of creatinine* or at a certain urinary density, usually 1.020 [Kowal and Zirkes (1983)]. In principle, urine samples with levels of creatinine <4.4 and >26.5 mmol/L, or with a density <1.015 and >1.030, are considered inappropriate for establishing the correct excretion of Pb [Dell Orto et al. (1987)].
  Persistently low PbU levels in combination with high levels of PbB may indicate a decreased capacity of the kidneys to excrete lead. Workers concerned should be medically examined [Lerner (no year)].

- Data of BM and of BEM should be recorded numerically as well as in diagrams. The occupational physician should consider individual data of biological assessments as confidential.

- The Directorate General of Labour in the Netherlands generally follows the principle that exposure of workers should be prevented by intervention in the

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* The quantity of creatinine excreted in urine per unit of time is grosso modo constant and largely independent of the volume of the urine.

transfer of dust from processes and sources to the worker. This goal may be reached by appropriate technical measures or by adaptation of processes, as well as by sufficient ventilation near the sources or the use of closed processes. Only when these measures do not result in acceptably low levels of exposure, should personal respiratory protective equipment be applied, but only temporarily. An exception is made for those situations in which exposure can only be prevented by personal protection [Labour (1988)].
In this strategy, personal protection is the last resort on the route from emission to intake of lead by workers. However, in view of the proposed large reduction of the permissible levels of PbB and PbA in this protocol, the use of these personal protection devices should temporarily be given a higher priority.

Compared to the present situation, in many lead-processing industries a considerable further decline in the level of exposure is required to conform to the proposed biological limit values of PbB and the limit values of PbA. To pursue this goal in addition to addressing exposure by an adequate surveillance programme, the work procedures (and their inadequacy!) require the attention of the management and the occupational physician or hygienist responsible.
In this respect, when appropriate the following aspects discussed by Harvey (1993) and those in *Chapter 9* should be considered to control and reduce exposure.

- Reduction of emission and transmission of lead-containing dust and aerosols in processes by improved exhaust ventilation as close to the sources as possible. Total enclosure of processes is to be preferred to exhaust ventilation.
- Failure of temperature control, presence of dust on the floors and drying up of battery grids are examples of sources in battery plants resulting in avoidable uptake of lead.
- Above 500 °C, lead vapour may be given off.
- Use of granulated instead of powdery compounds.
- Substitution of lead-containing materials by lead-free material.
- Application of sprinkler systems to wet particular stocks, floors, etc.
- Avoidance of dry sweeping.
- Availability of respirator protection devices.
- Washing and changing facilities divided into a clean section without risk of exposure and a 'dirty' section where exposure may occur. It is important that the washing facilities are used by all workers before leaving the premises.
- Canteen facilities should be situated in the clean section. Workers should change clothing before entering the canteen.
- Information, instruction and training of the workers to effectively manage exposure and to impress on them the health risks of ignoring precautions.

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ABBREVIATIONS

AH	=	aniline-4-hydroxylase
ALA	=	δ-aminolaevulinic acid
ALAD	=	δ-aminolaevulinic acid dehydratase
ALAU	=	δ-aminolaevulinic acid in urine
APDM	=	aminopyrine-N-demethylase
BEM	=	biological-effect monitoring
BM	=	biological monitoring
CP	=	coproporphyrin
CPU	=	coproporphyrin in urine
CV	=	coefficient of variation
EC	=	European Community
EM	=	environmental monitoring
EDTA	=	ęthylenediaminetetraacetate-disodium salt
Hb	=	haemoglobin
Hg	=	mercury
H_2O_2	=	hydrogen peroxide
KCl	=	potassium chloride
MAC (TWA-8h)	=	maximum accepted average concentration during 8 hours/day, and not more than 40 hours/week
MBL	=	Medical Biological Laboratory
MCH	=	mean corpuscular haemoglobin
MCHC	=	mean corpuscular haemoglobin concentration
MCV	=	mean corpuscular volume
PAS	=	personal air sampling
Pb	=	lead
PbA	=	lead in air
PbB	=	lead in blood
PbU	=	lead in urine
P_5N	=	pyrimidine 5'-nucleotidase
SDS-PAGE	=	sodium dodecylsulphate-polyacrylamide gel-electrophoresis
STEL	=	short-term exposure limit
TNO	=	Nederlandse organisatie voor toegepast natuur-wetenschappelijk onderzoek (Netherlands organization for applied scientific research)
TWA	=	time-weighted average
WHO	=	World Health Organization
ZPP	=	zinc protoporphyrin

UNITS AND CONVERSION FACTORS

Molecular weight ZPP	=	626
Hb	=	16,125 (Hb/4)
creatinine	=	113

1 µmol Pb	=	207 µg
100 µg Pb	=	0.48 µmol
1 µmol ZPP/mol Hb	=	0.04 µg/g Hb
1 µmol Pb/mol creatinine	=	1.83 µg/g creatinine

SUMMARY

During the last decades the industrial application of metallic lead and lead compounds has decreased considerably. In addition, present legislation and the measures taken by employers and trade unions to improve occupational hygiene have contributed to the disappearance of cases of overt intoxication of lead workers. On the other hand, the application of new and more sensitive (biochemical) tests, i.e. on neurological responsiveness and on renal metabolism, has made clear that health impairment by lead may already occur at levels of exposure which recently were considered harmless.

The object of the studies presented in this thesis is to find conditions that can be applied to improve health surveillance of workers occupationally exposed to lead, in order to prevent the occurrence of adverse health effects, also in the long term. To this end, the topics indicated in the following paragraphs have been studied.

In *Chapter 2* a review is presented of the literature on health impairment induced by lead in man and on the methods which may be applied to estimate exposure. These are monitoring of lead in environmental air at the workplace (EM of PbA) and in the breathing zone of workers (personal air sampling, PAS) or biological monitoring (BM) of lead in blood (PbB) and of lead in urine (PbU). Also methods for routine assessment of effects of lead on the haematopoietic system (biological-effect monitoring, BEM) are discussed.

There is general consensus that the level of PbB is a measure of exposure to lead, and that it is also reflecting the body burden. To prevent adverse health effects in lead workers, in many countries maximum allowable levels of PbA and/or PbB have been issued. In the European Community so-called 'action' levels and limit values of PbB and PbA are legally binding. When these levels or values are exceeded, particular measures must be carried out to prevent risks of (further) health impairment. Furthermore, the effect of technical and hygienic measures to reduce exposure of workers in lead-processing industries is often judged on the basis of a change of the PbB level.

For either application the quality of the assay of PbB is of paramount importance. In *Chapter 3* two interlaboratory surveys are described that gave insight into the quality of the analysis of PbB in the Netherlands. Almost all laboratories determining lead in blood more or less regularly at that point in time (1986/87), took part. The results of the assays performed by the greater part of the 12 and 14 participants in these surveys, respectively, were insufficient in terms of precision and accuracy. The coefficient of variation (CV) of the average results ranged from 10 to 36%.

A CV of about 6% is attainable as appears from the results of an interlaboratory quality control programme organized in Great Britain. Therefore, it is proposed that laboratories analysing PbB within the frame-work of health surveillance of lead workers participate in a continuously running external quality control programme. The CV of the results, obtained over a longer period, should be lower than 10%.

In several studies, a positive relation between PbB and blood pressure has been established. De Kort et al. (1987) estimated an increase of systolic blood pressure of about 1.8 mm mercury (Hg) and of diastolic blood pressure of 1 mm Hg per 0.5 µmol increase of lead/L of blood *(Chapter 4)*. In the general population the standard deviation of blood pressure varies between 15 and 25 mm Hg. To establish a statistically significant effect of the size of 1.8 mm Hg, blood pressure should be measured in a cohort of at least 3200 people. The present levels of exposure in the Netherlands (P_{50} of PbB of Dutch lead workers, according to age, lies between 1.4 and 1.6 µmol/L; P_{50} of PbB of mediterranean lead workers between 1.8 and 2.2 µmol/L) do not justify such an investigation. The results also imply that occasional measurement of blood pressure of lead workers, for the purpose of establishing the effect of lead, has no practical meaning.

In *Chapter 5* a study is presented on the incidence of anaemia in 494 Dutch lead workers. A statistically significant linear relation between PbB and haemoglobin (Hb) was established with a decrease of the Hb level by 0.28 mmol/L when PbB increased from 0 to 5 µmol/L. The upper limit of the 95% confidence interval amounted to a decrease of 0.59 mmol/L. These results suggest that occupational exposure to lead of people with a low-to-normal Hb value may lead to anaemia. Therefore, it is proposed that the assay of Hb is part of the pre-placement medical examination of new lead workers.

When rats are exposed to lead chromate by inhalation, only a small fraction of this insoluble compound is absorbed into the blood, the greater part forming a deposit in lung tissue *(Chapter 6)*. Extrapolation of these results to man implies that in case of exposure to lead chromate, the concentration of PbB represents an underestimation of the actual intake of the compound. Owing to the relatively low level of PbB, minor systemic effects will occur. However, it is desirable to obtain insight into the presence and magnitude of the deposit in the lungs, among others in view of the suspected carcinogenicity of lead chromate. Therefore, in addition to BM of PbB, also the intake, reflected in the concentration of PbA in the breathing zone of the workers, should be measured by PAS. This is apart from the recommendations to avoid exposure completely.

An increased level of zinc protoporphyrin (ZPP) in blood generally reflects an effect of lead on the haematopoietic system. In *Chapter 7* it is stated that the assay of ZPP in blood may be applied also to estimate whether the concentration of PbB

in that sample is higher or lower than one of the action levels or permissible values stated in the Lead Decree (Loodbesluit). The latter aims to protect workers to health risks of occupationally exposure to lead. Only when ZPP exceeds a predetermined level, does the concentration of PbB need to be determined to establish the correct value in the sample. The method, which may save cost and labour, is not applicable for the lowest action level of PbB (1.45 µmol/L) due to the low sensitivity and specificity of ZPP at low levels of exposures.

In *Chapter 8* a study is presented on the exposure to lead during demolition by flame-torch cutting of a steel structure coated with lead-based paint. The nine workers concerned showed levels of PbA in the breathing zone between 2 and 38 mg/m^3. These extremely high concentrations (the present MAC is 0.15 mg/m^3), resulted in levels of PbB of 3.5-5.5 µmol/L, which suggests that at these levels the PbB values show a saturation phenomenon and no longer fluctuate in accordance to exposure variations. Probably, at such very high intake the material is partly excreted in urine or deposited in the body. Therefore, it is proposed that under conditions of high exposure, the intake is not only estimated by determining PbB, but also by measuring PbU and by performing PAS measurements.
The use of filtering facepieces was unsuitable for preventing uptake of lead dust and lead aerosols. Also dust filters, with a protection factor of 10 to 12, only partly prevented the unwanted intake of lead.

To reduce exposure to lead and to maintain a low level of exposure in a secondary lead smeltery joined to a rolling mill, a programme of hygienic measures and technical adaptations of the production process was carried out over several years *(Chapter 9)*. As a result, PbB levels of thirteen employees out of a group of 28 showed a statistically significant decline between 1982 and 1990. PbB levels of five out of thirteen others showed a declining trend. Also, the number of PbB levels >2.9 µmol/L observed in workers decreased from 48 in the period 1982 - 1984 to 12 in 1988 - 1990. Specific technical or hygienic measures which resulted in this generally lower level of exposure could not be indicated.

Over a period of several years 19 workers of the plant were screened for PbB and ZPP more than 10 times. In 17 of them levels of PbB and ZPP showed a statistically significant relation. This suggests that after prolonged exposure, resulting in an equilibrium of lead in the deposits, the ZPP of an individual lead worker may be used as an indicator of a change in the level of PbB. This approach would simplify health surveillance of lead workers considerably.

In *Chapter 10* a study is presented on the effects of two concentrations of lead acetate injected subcutaneously in two groups of rabbits three times a week. The levels of PbB increased to about 1.9 and 4.3 µmol/L after 70 and 110 days, respectively, and were maintained at these levels during 7.5 months. Hereafter, a part of the animals was subjected to a recovery period of four months. This study

aimed at the identification of any physiological criterion that might be used to indicate early health effects of lead exposure.

The animals of both groups were subjected to a similar regimen. In the high-exposed animals effects on the haematological system and on red blood cells were observed (anaemia, increased levels of ZPP and ALAU, basophilic stippling and lowered MCV and MCH). Gain in body weight was lower and relative weights of heart and liver increased. In both groups ALAD activity was very low. In neither of the groups effects on kidney function and on the haematopoietic system were observed which might be applicable as biological effect parameters in a programme for health surveillance of lead workers.

The general discussion on the data from *Chapters 3-10* and the concluding remarks are presented in *Chapter 11*.

The results of the studies in *Chapters 3-9* have been incorporated into a protocol for health surveillance of lead workers which is suggested in the *Annex*. The accent lies on prevention of adverse health effects by periodically assessing the exposure. Dependent on the workers involved and the extent and kind of exposure, the programme should be carried out monthly to annually.

SAMENVATTING

De industriële toepassing van metallisch lood en loodverbindingen is in de loop van de laatste decennia aanzienlijk afgenomen. Daarnaast hebben wijzigingen van technische processen, alsmede wetgeving en maatregelen van werkgevers en vakbonden ertoe bijgedragen dat acute vergiftigingen van personen die in hun beroep worden blootgesteld aan lood, thans niet meer of nog slechts zelden voorkomen. Door de toepassing van nieuwe en gevoeliger (biochemische) bepalingsmethoden, zoals die welke het functioneren van het zenuwstelsel en van de nieren meten, is echter duidelijk geworden dat er bij blootstellingsniveaus die nog niet zo lang geleden als risicoloos werden beschouwd toch gezondheidsschade kan optreden.

Het doel van het onderzoek dat is beschreven in dit proefschrift was om informatie te verkrijgen die gebruikt zou kunnen worden om te komen tot een betere gezondheidsbewaking van personen die beroepsmatig aan lood worden blootgesteld. Hierdoor zou het optreden van nadelige gezondheidseffecten worden voorkomen, ook op de langere termijn. In dit kader zijn de onderwerpen die in het volgende zijn aangegeven nader onderzocht.

In *Hoofdstuk 2* wordt een kort overzicht gegeven van de literatuur over gezondheidseffecten van lood. Tevens worden methoden beschreven waarmee de blootstelling kan worden geschat. Dit zijn 'monitoring' van lood in de lucht (PbA) op de werkplek en in inademingslucht (personal air sampling, PAS), alsmede biologische monitoring (BM) van lood in bloed (PbB) en van lood in urine (PbU). Tevens worden methoden besproken die worden gebruikt om routinematig effecten van lood op het bloedvormend systeem te bepalen (biologisch-effect monitoring, BEM).

Het wordt algemeen aanvaard dat de concentratie van PbB een maat is voor de blootstelling en voor de opname van lood in het lichaam. Om nadelige gezondheidseffecten door beroepsmatige blootstelling te voorkomen, zijn in een groot aantal landen grenzen voor de maximaal toelaatbare concentraties van PbA en PbB vastgesteld. Ook in de Europese Gemeenschap zijn wettelijk aanvaarde z.g. 'actieniveaus' en grenswaarden voor PbA en PbB van kracht. In Nederland zijn deze opgenomen in het 'Loodbesluit'. Bij overschrijding dienen bepaalde wel omschreven maatregelen te worden genomen om de blootstelling te verminderen en daarmee het (verder) optreden van gezondheidsrisico's te voorkomen. Het effect van deze maatregelen wordt vooral afgeleid uit de mate waarin deze leiden tot een verandering van de PbB waarde. Eén en ander impliceert dat een juiste uitvoering van de PbB bepaling van groot belang is, omdat het resultaat belangrijke consequenties kan hebben voor de betrokken werknemers en werkgevers.

In *Hoofdstuk 3* worden twee vergelijkende onderzoeken beschreven naar de kwaliteit van de PbB bepaling zoals die in Nederland werd uitgevoerd. Aan deze onderzoeken werd deelgenomen door bijna alle laboratoria in Nederland (15-20) die de bepaling met een zekere regelmaat uitvoerden.

De variatiecoëfficiënten (CV) van de gemiddelde resultaten van de 12, respectievelijk 14 deelnemers aan de onderzoeken varieerden tussen 10 en 36%. Een CV van ca. 6% is echter haalbaar, zoals blijkt uit de resultaten van een doorlopend programma voor kwaliteitsbewaking dat in Engeland is georganiseerd. Voor de meerderheid van de deelnemers aan het Nederlandse onderzoek waren de resultaten van hun loodbepalingen dan ook onvoldoende in termen van precisie en juistheid.

Voorgesteld wordt dat laboratoria waar bepalingen van lood in bloed worden uitgevoerd in het kader van de gezondheidsbewaking van loodwerkers, deelnemen aan een z.g. extern kwaliteitscontroleprogramma. De CV van de resultaten die daarin over een langere periode worden behaald, dient lager te zijn dan 10%.

In de literatuur zijn verscheidene onderzoeken beschreven waarin een positieve relatie tussen PbB en de bloeddruk werd vastgesteld. De Kort e.a. (1987) schatten de stijging van de systolische bloeddruk op ongeveer 1,8 mm kwik (Hg) per 0,5 µmol toename van lood/L bloed, en van de diastolische bloeddruk op ongeveer 1 mm per 0,5 µmol/L *(Hoofdstuk 4)*. In de algemene bevolking varieert de standaarddeviatie van de bloeddruk tussen 15 en 25 mm Hg. Om een statistisch significant effect ter grootte van 1,8 mm Hg aan te tonen, zou derhalve de bloeddruk van een cohort van 3200 personen onderzocht moeten worden. Afgezien van de vraag of in Nederland een dergelijk aantal personen bereid is deel te nemen, wordt een zo omvangrijk onderzoek niet gerechtvaardigd door de blootstellingsniveaus die in Nederland momenteel voorkomen (P_{50} voor PbB van Nederlandse loodwerkers, afhankelijk van leeftijd, tussen 1,4 en 1,6 µmol/L; voor mediterrane loodwerkers tussen 1,8 en 2,2 µmol/L). Dit houdt tevens in dat incidentele meting van de bloeddruk bij loodwerkers met het doel een effect van lood op de bloeddruk te meten, geen practische betekenis heeft.

In *Hoofdstuk 5* wordt een onderzoek beschreven naar de hemoglobine concentratie (Hb) bij 494 Nederlandse loodwerkers. Hierbij werd een statistisch significant lineair verband gevonden tussen PbB en Hb, met een afname van de Hb-concentratie van 0,28 mmol/L bij een toename van de PbB concentratie van 0 tot 5 µmol/L. De bovengrens van het 95% betrouwbaarheidsinterval van deze afname bedroeg 0,59 mmol/L. In twee groepen van respectievelijk 59 in Nederland werkzame Marokkaanse en 18 Turkse loodwerkers werd een dergelijk verband tussen PbB en Hb niet gevonden.

Het resultaat voor de Nederlandse populatie duidt erop dat de beroepsmatige blootstelling aan lood van personen die reeds een laag-tot-normale Hb-waarde

hebben, kan leiden tot bloedarmoede. Daarom wordt voorgesteld de bepaling van Hb deel te laten uitmaken van het aanstellingsonderzoek van loodwerkers.

Bij inhalatoire blootstelling van ratten aan lood chromaat wordt slechts een klein deel van deze onoplosbare verbinding direct in het bloed opgenomen *(Hoofdstuk 6)*. Het grootste deel vormt een depot in het longweefsel. Extrapolatie van deze resultaten naar de mens betekent dat bij blootstelling aan lood chromaat met biologische monitoring van PbB een onderschatting van de blootstelling wordt verkregen. Als gevolg van de lage concentratie van lood in bloed zullen ook de systemische effecten klein zijn. Toch is het gewenst dat inzicht wordt verkregen in de aanwezigheid en grootte van het depot in de longen, vooral in verband met de mogelijke carcinogeniteit van lood chromaat. Bij blootstelling aan deze verbinding dient daarom, als aanvulling op BM van PbB, ook de concentratie in de inademingslucht (PAS) gemeten te worden. Dit staat los van de aanbeveling dat blootstelling geheel voorkomen dient te worden.

Bij blootstelling aan lood wijst een verhoogde concentratie van zink protoporfyrine in bloed (ZPP) bijna altijd op een effect op het bloedvormend systeem. In *Hoofdstuk 7* wordt beschreven dat met de eenvoudig uit te voeren meting van ZPP kan worden vastgesteld of PbB in een bloedmonster hoger of lager is dan één van de wettelijk vastgestelde actieniveaus of grenswaarden uit het Loodbesluit. Alleen als ZPP een vooraf gekozen, bepaalde waarde overschrijdt, dient alsnog de concentratie van lood in het monster bepaald te worden. Door de lage specificiteit en sensitiviteit van ZPP bij geringe blootstelling is de voorgestelde methode, die kosten en tijd bespaart, echter niet bruikbaar voor het eerste actieniveau (PbB = 1,45 µmol/L).

In *Hoofdstuk 8* wordt een onderzoek beschreven naar de blootstelling aan lood bij het met snijbranders slopen van een met loodhoudende verf behandelde stalen constructie. Bij de negen betrokken personen werden in de ademzone loodwaarden gemeten tussen 2 en 38 mg/m^3. Deze, in vergelijking met de huidige MAC (0,15 mg/m^3), zeer hoge concentraties leidden tot snel oplopende PbB waarden, die echter niet hoger stegen dan 3,5 - 5,5 µmol/L. Dit lijkt een aanwijzing dat deze concentraties een plateau weergeven, waarbij de extreem grote opname van lood slechts gedeeltelijk tot uiting komt in de PbB-waarde. De zeer hoge blootstelling leidt mogelijk tot een verhoogde uitscheiding in de urine of tot het optreden van depotvorming. Daarom wordt voorgesteld om in situaties met zeer ernstige blootstelling, bijv. zoals beschreven in *Hoofdstuk 8*, de opname van lood niet alleen te controleren door bepaling van PbB, maar ook door de concentratie van lood in de ademzone en in de urine te meten.
Het gebruik van gelaatsmaskers, z.g. 'snuitjes', was onvoldoende om de opname van lood te voorkomen. Ook stoffilters met een beschermingsfactor van 10-12, voorkwamen de opname van lood slechts gedeeltelijk.

Teneinde de blootstelling van de werknemers te verminderen en vervolgens op een lager niveau te handhaven, werd in een secundaire loodsmelterij en -pletterij tussen 1982 en 1990 een programma van wijzigingen in het productieproces en van arbeidshygiënische maatregelen uitgevoerd *(Hoofdstuk 9)*. In deze periode daalden de PbB waarden van 13 personen uit een groep van 28 werknemers statistisch significant. De PbB-waarden van vijf personen uit een groep van 13 anderen lieten een dalende trend zien. Terwijl tussen 1982 en 1984 nog 48 PbB waarden >2,9 µmol/L werden gemeten, waren dit er tussen 1988 en 1990 nog maar 12. Welke maatregelen in het bijzonder tot de algemene vermindering van de blootstelling hadden bijgedragen, kon echter niet worden vastgesteld.

In een periode van enkele jaren werd bij 19 personen meer dan tien keer PbB en ZPP gemeten. Bij 17 van hen was er een statistisch significant verband tussen beide parameters. Dit resultaat suggereert dat bij langdurig blootgestelde loodwerkers een stijging of daling van de individuele ZPP concentratie gebruikt kan worden als een indicatie voor een overeenkomstige verandering van de PbB waarde. Deze benadering, die nog verder uitgewerkt dient te worden, zou een aanzienlijke vereenvoudiging van het health surveillance programma betekenen.

In *Hoofdstuk 10* wordt een onderzoek beschreven waarin twee groepen konijnen gedurende 7,5 maanden door herhaalde subcutane injecties werden blootgesteld aan lood acetaat. PbB nam in de twee groepen respectievelijk toe tot circa 1,9 en 4,3 µmol/L; deze concentraties werden gehandhaafd door wekelijks lood acetaat toe te dienen. Na de blootstellingsperiode onderging een deel van de dieren een 'herstelperiode' van vier maanden waarin geen lood acetaat werd toegediend. In de hoogst blootgestelde groep dieren werden effecten waargenomen op de haematopoëse en op de rode bloedcellen (anemie, verhoogde concentratie van ZPP en ALAU, verlaagde MCV en MCH en basofiele stippeling). In beide groepen was de activiteit van ALAD sterk gedaald. In geen der groepen werden effecten op de nier- of leverfunctie of klinische afwijkingen gemeten. In de groep dieren met de laagste blootstelling werden geen effecten waargenomen die gebruikt zouden kunnen worden als effect-parameters bij de gezondheidsbewaking van loodwerkers.

De algemene discussie van de onderzoeken die zijn gepresenteerd in de *Hoofdstukken 3-10* en de conclusies zijn vermeld in *Hoofdstuk 11*.

De resultaten van de onderzoeken in de *Hoofdstukken 3-9* zijn verwerkt in een voorstel voor een protocol voor de gezondheidsbewaking van beroepsmatig aan lood blootgestelde personen dat wordt voorgesteld in de *Annex*. In het protocol ligt het accent op preventie van gezondheidsrisico's door, aan de hand van een tijdschema, regelmatig de blootstelling te volgen. Afhankelijk van de werkomstandigheden en de mate en aard van de blootstelling is het protocol maandelijks tot jaarlijks toe te passen.

NAWOORD

Aan de totstandkoming van dit proefschrift, als afsluiting van mijn loopbaan, is door velen een bijdrage geleverd. Mijn dank gaat in de eerste plaats uit naar prof. dr. W.R.F. Notten. Wilfried, ik heb het zeer op prijs gesteld dat je mijn promotor wilde zijn. Je adviezen hebben in belangrijke mate bijgedragen tot de uiteindelijke vorm van dit proefschrift.

Dr. J.J. van Hemmen, mijn co-promotor, ben ik zeer erkentelijk voor zijn waardevolle opmerkingen en suggesties. Joop, dat je altijd gelegenheid vond mijn teksten grondig en snel te beoordelen heb ik bijzonder gewaardeerd.

Dr. F. Berends en Ir. J.F. Bleichrodt dank ik voor hun bereidwilligheid het manuscript kritisch te lezen. Frits en Frits, jullie bijdragen waren belangrijk om de resultaten van de onderzoeken op de juiste wijze te verwoorden.

Ook wil ik graag dr. G. de Mik danken. Gerrit, je pragmatische opstelling bij de dagelijkse leiding van de afdeling Bedrijfstoxicologie van het MBL en het vertrouwen dat je je medewerkers gaf, heeft mede de basis gelegd voor dit proefschrift.

Nog vele anderen hebben mij op enigerlei wijze bij het onderzoek geholpen of advies gegeven. Zonder niet genoemden tekort te doen wil ik in het bijzonder bedanken Michel Boermans, Peter Bos, Ria van der Eik, prof. Henderson, Wim de Kort, Frans Kouwenberg, Frans van der Kreek, Marlies van Pijkeren, Usha Soekhoe, Hans Stouten, Anton Wibowo, Marja Wijnans en prof. Zielhuis. Een speciaal woord van dank voor Agnes Franssen. Onze samenwerking is begonnen op de afdeling biochemie van het MBL en heeft bijna 20 jaar geduurd. In die tijd was je voor het werk vaak mijn belangrijkste klankbord en gesprekspartner.

Terugkijkend op de meer dan 35 jaar die ik bij het MBL heb gewerkt, ben ik dankbaar voor de gelegenheid die ik had om in dit wetenschappelijk voor-aanstaande instituut een bijdrage aan het werkprogramma te leveren. De kritische leiding van dr. R.A. Oosterbaan en dr. F. Berends, heeft in de eerste jaren belangrijk bijgedragen aan mijn wetenschappelijke vorming.

CURRICULUM VITAE OF W.C.M. ZWENNIS

Willem Cornelis Marie Zwennis was born on July 29 1930 in The Hague. After graduating from secondary school he was educated as a scientific research technician in the fifties. Between 1950 and 1958 he was employed at the research laboratories of Gist-Brocades N.V., Delft (head Prof. W. Berends) and Unilever Research, Vlaardingen (head Prof. Dr. J. Boldingh).

In 1958 he became a research assistent at the department of biochemistry of the TNO Medical Biological Laboratory, Rijswijk. Under the direction of Dr. R.A. Oosterbaan and Dr. F. Berends he worked on the structure-analysis of hydrolytic enzymes. From 1970 he was employed at this institute as a research scientist. In 1978 he became co-worker of the newly established department of Industrial Toxicology (head Dr. G. de Mik) of the TNO-MBL until his retirement in 1993. Here he was head of a working group involved in biological monitoring of metals in workers occupationally exposed to metals.

In 1993 Zwennis graduated at the High School 'Rotterdam & Omstreken' (Polytechnical Faculty), Rotterdam.

STELLINGEN

1. De aan de Europese Gemeenschap voorgestelde verlaging van de biologische grenswaarde van lood-in-bloed en van lood in de omgevingslucht voor vrouwen is onvoldoende om de mogelijkheid van nadelige effecten van lood voor de foetus uit te sluiten.

   Council Directive 82/605/EEC. Proposal for updating, final report. P. Apostoli. Maart, 1991.

2. Onderzoek van het loodgehalte van faeces van loodwerkers geeft inzicht in de orale opname van lood en kan worden beschouwd als een parameter voor persoonlijke hygiëne en hygiënisch gedrag tijdens het werk. Daardoor kan het nemen van gerichte arbeidshygiënische maatregelen worden bevorderd.

3. Bij hun voorstel om voor de gezondheidsbewaking van loodwerkers de grenswaarden uit de 'Loodrichtlijn' van de EG te hanteren, houden Gennart e.a. onvoldoende rekening met de gezondheidsrisico's die al bij lagere blootstelling kunnen optreden.

   J-P. Gennart e.a.,
   Int. Arch. Environ. Health 64:49-57 (1992).

4. Door het ontbreken in het 'Loodbesluit' van argumenten waarop de actienivo's en grenswaarden voor de concentratie van lood in bloed en in lucht zijn gebaseerd, wordt een gezondheidskundig verantwoorde toepassing niet bevorderd.

   Het Loodbesluit, P 170-1 (1988).
   Directoraat-Generaal van de Arbeid, Ministerie van Sociale Zaken en Werkgelegenheid, Den Haag.

5. Wanneer in de Nationale MAC-lijst bij stoffen geen H-indicatie (voor resorptie door de huid) is vermeld, dient te worden aangegeven of dit is gebaseerd op wetenschappelijke gegevens of het ontbreken hiervan.

   Nationale MAC-lijst, P 145 (1994).

6. Bij de beoordeling van de toelaatbaarheid van feromonen en andere signaalstoffen als bestrijdingsmiddelen, dient rekening te worden gehouden met het feit dat het bestrijdingseffect van deze stoffen niet berust op toxiciteit.

7. Bij de bemonstering van deeltjes in de buitenlucht t.b.v. het mutageniteitsonderzoek d.m.v. filtratie, worden niet alleen mutagenen uit de deeltjes verzameld, maar kunnen ook mutagenen ontstaan door omzetting van geadsorbeerde gasvormige polycyclische aromatische koolwaterstoffen.

   W.K. de Raat e.a.,
   The Science of the Total Environment 63:176-189 (1987).
   W.K. de Raat e.a.,
   Atmospheric Environment 24A:2875-2887 (1990).

8. Behalve kameraden gaan ook voetbalhysterie en voetbalvandalisme hand in hand.

9. Het doorgaan van de voetbalwedstrijd Juventus-Liverpool te Brussel op 29 mei 1985 direct na een ongeluk waarbij in het stadion tientallen doden vielen, maar het afgelasten van de wedstrijd Feyenoord-PSV na een regenbui op 24 april 1994 in Rotterdam is een ergelijk voorbeeld van de huidige sportverdwazing.

10. Met volledige werkgelegenheid is de emancipatie van partners in een samenlevingsverband meer gediend dan met opgelegde maatregelen van de overheid.

11. Met name bij het schrijven van een proefschrift over lood wegen de laatste loodjes het zwaarst.

Stellingen behorende bij het proefschrift
'Health surveillance of workers exposed to lead'.
W.C.M. Zwennis