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Elevated blood Lead impairs the Hematological and Heme Biosynthesis related parameters of Silver Jewellery Workers

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ABSTRACT

To see the present scenario of blood lead (BPb) levels of Silver Jewellery Workers (SJW) and its effects on haematological and heme biosynthesis parameters. Forty-two SJW of having an age range of 20-45 years were included for this study and compared with 50 healthy male subjects of the same age. Blood lead, RBC δ- aminolevulinic acid dehydratase (δ-ALDH), urinary δ- aminolevulinic acid (U- δ-ALA), urinary porphobilinogen (U-PBG) and haematological parameters were measured. Blood lead level of silver jewellery workers was significantly elevated ($p<0.001$, 325%) and non-activated δ-ALAD ($p<0.01$), activated δ-ALAD ($p<0.05$) were notably reduced and the ratio of A/NA δ-ALAD ($p<0.01$) was considerably enlarged in SJW as compared to control group subjects. Urinary excretion of δ-ALA ($p<0.001$) and PBG ($p<0.001$) levels were extensively increased in SJW as compared to subjects from the control group. Hb ($p<0.001$), PCV ($p<0.001$), MCV ($p<0.001$), MCH ($p<0.05$) and RBC ($p<0.001$) were notably reduced in SJW, while total WBC count ($p<0.001$, 29.8%) was considerably elevated as compared to subjects from the control group. Conclusions: Increased BPb level in SJW indicates the more absorption of lead from the GIT, which inhibits heme biosynthesis, and alters haematological parameters.

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INTRODUCTION

Lead is a multipurpose metal present everywhere and occurring naturally in the earth's crust and has been used by humans for over 9000 years. It is an inexpensive, soft, silvery grey metal with a low melting point of 327.5°C. Its properties include high resistance to corrosion, pliability, high density and thermal expansion, low elasticity, easy workability, and recyclability, with excellent antifriction capabilities. Due to these properties, lead finds usage in a variety of manufacturing processes like acid batteries, cable sheathing, ceramic glazing, soldering, and ship construction. Lead, and its alloys find usage in pipes, cable covers, shot and ammunition, and radiation shields. Lead compounds are used as pigments in paints and dyes, due to its anti-corrosive properties, Tetra-methyl and tetra-ethyl lead was used in gasoline additives as anti-knock agents and to enhance octane rating. Lead, along with other metals like mercury and arsenic, also finds its usage in cosmetics and traditional complementary and alternative medicine in India (Ayurveda) and China (ATSDR, 2017).

Lead after absorption through the respiratory and digestive systems, gets deposited in all tissues with maximal (>90%) accumulation found in bones. Half-

life of lead in blood and soft tissue is 30-45 days and in bones 27-30 years. It is primarily excreted through urine (90%) with small amounts found in faeces, sweat, hair, and nails. Depending on the blood lead level and duration of exposure, lead can cause widespread adverse health effects on numerous organ systems including hematopoietic, nervous, renal, reproductive, cardiovascular and immune systems. Three enzymes known as δ-aminolevulinic acid dehydratase, coproporphyrin oxidase, and ferrochelatase from heme biosynthesis, are inhibited by increased blood lead and also impairs the globulin synthesis, increased blood lead decreases serum levels of erythropoietin hormone and impairs the erythrocyte formation. Lead decreases erythrocyte survival rate by inhibiting the membrane-bound Na+/K+-ATPase, which results in impaired haemoglobin formation. Inhibition of heme biosynthesis by lead results in a reduction of heme pool and affect the nervous, renal and hepatic systems (ATSDR, 1998).

The occupational exposure to lead in developing countries like India is entirely unregulated. Workers employed in silver jewelry industries are required to perform activities like silver distillation from waste through smelting and alloying; manufacturing the silver wires, cutting, assembling, designing, polishing, and plating of silver rings and jewellery (ATSDR, 2007). These activities are usually performed at unregulated shops and homes without adequate training and protective gear, thereby potentially exposing them to toxic levels of lead fumes and dust. These also pose a health hazard for people, especially children, living near these shops.

Keeping these points in perspective, we conducted this study to understand the level of lead exposure among silver jewellery manufacturing workers from the unorganised sector of Maharashtra, India by ascertaining their blood levels of lead and its adverse effects on heme biosynthetic pathway and haematological parameters.

MATERIALS AND METHODS

Forty-two male silver jewellery manufacturing workers from village Hupari (Kolhapur) situated in Western Maharashtra, India were enrolled in this study as a study group. And for comparison, 50 non-lead exposed healthy male subjects from the same area were included as a control group. The subjects of study and control groups were recruited after satisfying the inclusion criteria. The age range of both group subjects was 20-45 years. Those who are on medication for significant diseases were excluded from this study. Silver jewellery manufacturing workers with a history of lead exposure more than five years were enrolled in this study. The study and control group subjects were on the same dietary intake, food habits, and socioeconomic status. From both group subjects, the occupational, clinical and demographic data were collected by taking their interviews during blood collection time and by using pre-structured questionnaires. Before enrolling study and control groups subject, the informed consent was collected, and all the study participants were informed about the health hazards of lead exposure. Ethical study approval [2016-2017/075] was taken, and all the study was carried out as per guidelines of the 1964 Helsinki Declaration (World Health Organization, 1995).

From study and control group subjects, the 10 ml blood was collected in EDTA bulb and a tube containing heparin for quantification of blood lead levels, haematological and heme biosynthesis-related investigations. Blood lead analyser special instrument, manufactured by Magellan Diagnostics, USA was mainly used for accurate quantification of blood lead levels. The principle of this instrument is based on Anodic Stripping Voltammetry. Lead care treatment reagent was used to lyse the red blood cells and releases the lead particles. These particles then accumulate on the test electrode. Then a negative potential was applied to the sensor. The potential was quickly reversed, which releases the lead particles. The current formed was directly proportional to the number of lead particles in the blood (Patil *et al.*, 2006a). The δ -aminolevulinic acid dehydratase (δ -ALAD) from red blood cells was determined by using the Julian Chisolan et al. method (Patil *et al.*, 2006b). On δ-aminolevulinic acid (δ-ALA) substrate, the enzyme δ - aminolevulinic acid dehydratase (δ -ALAD) acts and form porphobilinogen (PBG), which further reacted with Ehrlich's reagent to give pink coloured complex. The intensity of the colour was measured at 555 nm on a spectrophotometer.

Urinary δ-aminolevulinic acid (U δ-ALA) was determined by using the Osamu et al. method (Declaration of Helsinki, 1964). U δ-ALA reacts with acetylacetone to forms pyrrole, which then reacts with Ehrlich reagent to give red colour. This compound was extracted with chloroform, and the colour intensity was measured spectrophotometrically at 555 nm. Urinary porphobilinogen (U- PBG) was determined by using Mauzerall and Granick method (Magellan Diagnostics, 2015). U- PBG reacts with P-dimethyl amino benzaldehyde in acidic condition to form a red complex, the colour intensity was measured at 555 nm precisely after five min-

utes and PBG were calculated by using Rimington formula (1958) (Chisolm *et al.*, 1985).

Haematological parameters were estimated by using fully automated Hematology analyser Sysmex (Wada *et al.*, 1969). Statistical comparison between heme biosynthesis and haematological parameters of controls and silver jewellery workers was made by Student's t-test using Instat GraphPad software.

RESULTS

Blood lead levels of silver jewellery manufacturing workers were significantly elevated, and red blood cells non-activated δ-ALAD, activated δ-ALAD were notably reduced. The ratio was considerably enlarged in SJW as compared to control group subjects. Urinary excretion of PBG and δ-ALA were extensively elevated in SJW when compared with controls.

Significant negative correlation between blood lead with activated δ-ALAD, non-activated δ-ALAD and positive correlation with Act / N-Act δ-ALAD ratio, U–δ-ALA and U- PBG were observed in the study group.

Hematological parameters such as Hb, PCV, MCV and RBC count were notably reduced in silver jewelry workers, while total WBC Count was considerably elevated as compared to control.

A significant negative correlation between blood lead level with Hb, HCT, MCV, RBCs and a positive correlation with WBCs were observed in the study group.

Discussion

Blood levels of lead among subjects belonging to the study group were notably elevated (325%, p<0.001) when compared with the controls shows that the absorption of lead from the gastrointestinal tract was more in silver jewelry manufacturing workers. Such increased levels have also been described in another study (ATSDR, 2007). In the silver jewellery industry, lead is mainly used for making silver rings, an ornament, usually worn around ankles/toes. Lead melts at high temperatures which are then poured in 3 feet mold thereby making lead wires. These wires are then stretched to 15 feet by machine and coated with silver plating. When reheated, lead melts leaving behind silver wires which are made into rings. The molten lead is then reused. The lead smelting process releases fumes/vapors which are inhaled by workers and their family members, considering most of these work are done in their household without adequate protective gear.

Absorbed lead is mainly excreting in feces and urine and very less quantity of lead is excreting through, saliva, sweat, breast milk, nails and hair (Mauzerall and Granick, 1956). It gets accumulated in soft tissues and bones. The ninety-eight percent of lead in erythrocytes is mainly bound to proteins within the cell and roughly 40–75% of lead is bound with albumin (Rimington, 1971). The clinical symptoms including fatigue, peripheral neuropathy, encephalopathy, abdominal colic, dyspepsia, anemia, and have been reported in the literature due to increased blood lead levels (ATSDR, 2017).

Red blood cells non-activated δ-ALAD (-21.28%, p<0.01) and activated δ-ALAD (-18.47%, p<0.05) were considerably reduced and A /NA ratio of δ-ALAD (9.6 %, p<0.01) were notably elevated in the study group when compared with controls (Table 1 and Figure 1). The decreased activity of red blood cell δ-ALAD might be due to lead. Lead inhibits this enzyme by binding to sulfhydryl group of δ-ALAD. Earlier studies reported that lead inhibits 3 enzymes of heme biosynthesis pathway i.e. δ-aminolevulinic acid dehydratase, coproporphyrin oxidase, and ferrochelatase (Trester, 1999). Our results are similar to the earlier study, reported in the literature (Stauber *et al.*, 1994).

The urinary δ-ALA (124%, p<0.001) and urinary-PBG (53%, p<0.001) excretion were notably elevated in the silver jewellery manufacturing workers when compared with the controls, which might be because of inhibition of enzymes of heme biosynthesis pathway. These findings also consistent with an earlier study (Al-Modhefer *et al.*, 1979).

Hematological Investigations such as HB (-13.93%, p<0.001), PCV(-12.04%, p<0.001), MCV (-7.19%, p<0.001),MCH (-5.9%, p<0.05), and red blood cell (-10.07 %, p<0.001) were notably reduced and total WBC (29.8%, p<0.001) were considerably elevated in silver jewellery manufacturing workers as compared to the controls (Table 3 and Figure 2), which is because of inhibition of enzymes of heme biosynthesis pathway.

The adverse effects of lead on the hematopoietic system in both humans and animals, resulting in increased urinary excretions of porphyrins, coproporphyrins, δ-aminolevulinic acid, erythropoietic protoporphyria, zinc protoporphyrin, and anemia has been well documented in the literature (Trester, 1999). A noticeable interference with heme biosynthesis pathway enzymes leads to reduction in the Hb concentration in blood. Our results are also consisting with another study (EPA, 1986). Impaired erythropoietin hormone production due to renal damage, resulting in insufficient maturation of erythroid

Table 1: Blood Lead and Heme Biosynthesis-Related Investigations in Study and Control Groups

Sr. No.	Investigations	Control Group (N= 50)	Silver Jewelry Workers (N= 42)
1.	Blood Lead (ug/dl)	5.46 ± 2.58 (3.3 – 11.9)	23.23 ± 5.91*** (11.9 - 37)
2. Erythrocyte δ-ALAD (mmol δ-ALA utilised)/ (min/L of erythrocytes)			
A.	Activated δ-ALAD	35.08 ± 11.25 (16.7 – 62.83)	28.6 ±10.18* (10.92 - 42.6)
B.	Non-activated δ-ALAD	27.29 ± 8.7 (13.73 – 45.11)	21.48 ± 8.15** (7.12 - 34.3)
C.	Act / N-Act δ-ALAD Ratio	1.25 ± 0.081 (0.9 - 1.57)	1.37 ± 0.25** (1.1 -2.5)
3.	U-δ-ALA (mg/L)	5.00 ± 0.79 (3.99 – 6.90)	11.2 ± 5.59*** (3.8 – 24.3)
4.	U- PBG (mg/l)	12.7 ± 2.89 (6.38 - 18.27)	19.52 ± 3.67*** (14.4 - 29.6)

Figures mention Mean ± SD values and range of values given in parenthesis, *** P< 0.001, ** P< 0.01, * P< 0.0

Table 2: Correlation Coefficient (r) between PbB and Heme Biosynthesis related Parameters of Silver Jewelry Workers

Sr.	Biochemical Investigation	Correlation Coefficient (r)	P-Value
1. Erythrocyte δ-ALAD (mmol δ-ALA utilised) / (Min/L of erythrocytes)			
A	Activated δ-ALAD	-0.45	P<0.01
	Non-activated δ-ALAD	-0.49	P<0.001
C	A / NA δ-ALAD Ratio	0.37	P<0.05
2.	U-δ-ALA (mg/L)	0.38	P<0.01
3.	U- PBG (mg/l)	0.45	P<0.01

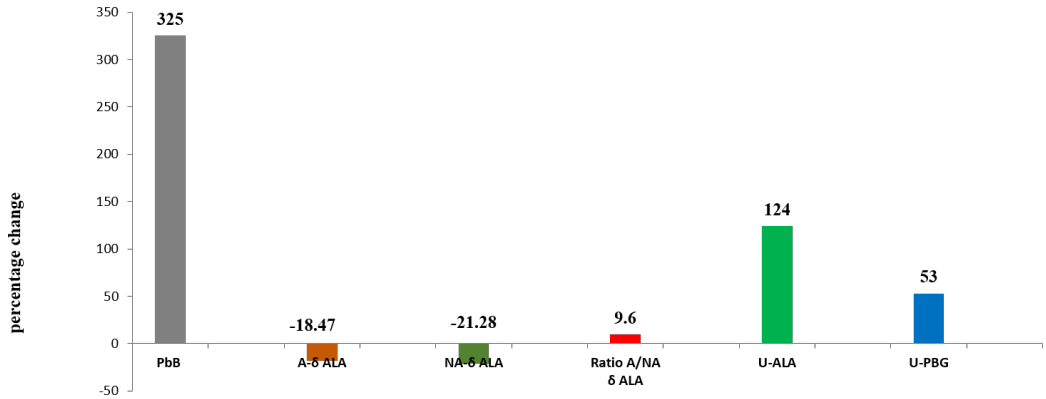
Table 3: Hematological Parameters in the Study and Control groups

Sr. No.	Hematological Parameters	Controls (N=50)	Silver Jewellery Workers (N= 42)
1.	Hb (gm/dl)	15.57 ± 1.07 (12.7 - 17.6)	13.4 ± 1.93*** (8.8 - 16.1)
2.	HCT or PCV (%)	44.5± 3.42 (36.2.-51)	39.14 ± 3.87*** (28 - 45.3)
3.	MCV (fL)	85.42 ± 5.24 (76-98)	79.27 ±8.31*** (59.5 – 98.7)
4.	MCH (pg)	28.8 ± 2.47 (24- 34)	27.1 ± 4.41* (18.4 - 34.9)
5.	MCHC (gm/dl)	34.6± 1.16 (31.5 – 36.4)	34.2± 1.72 · (30.3 - 36.5)
6.	RBC count (million/ml)	5.46 ± 0.53 (4.3 - 6.5)	4.91 ± 0.49*** (3.95 - 6.43)
7.	WBC count (/cumm)	6.7± 1.44 (4.6 - 11.1)	8.7 ± 1.5*** (5.87 - 12)

Figures mention Mean ± SD values and the range of values givenin parenthesis. *** P< 0.001, * P< 0.05, · Non-significant as compared with controls

Table 4: Correlation Coefficient (r) between PbB and Hematological Parameters of Silver Jewelry Workers

Sr. No.	Haematological Investigations	Correlation Coefficient (r)	P-Value
1.	Hb (g/dl)	-0.37	P<0.01
2.	HCT or PCV (%)	-0.39	P<0.01
3.	MCV (fL)	-0.41	P<0.01
4.	MCH (pg)	-0.28	ns
5.	MCHC (g/dl)	-0.21	ns
6.	RBC count (million/ml)	-0.32	P<0.05
7.	WBC count (/cumm)	0.46	P<0.01



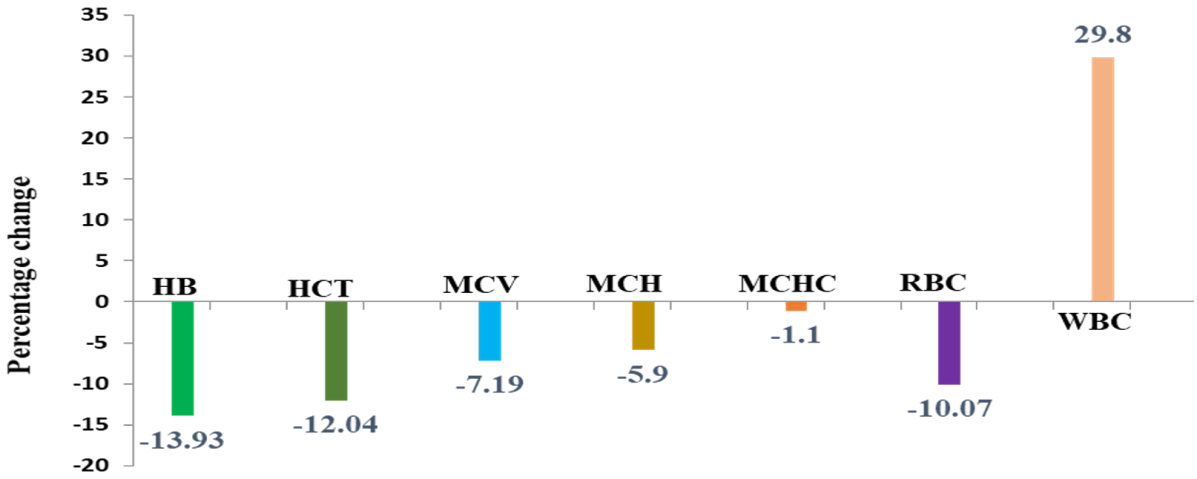
Blood lead (PbB), Activated δ- Aminolevulinic Acid Dehydratase (A- δ-ALAD), Non activated δ-Aminolevulinic Acid Dehydratase (NA- δ-ALAD), Ratio of Activated δ-Aminolevulinic Acid Dehydratase to Non activated - aminolevulinic Acid Dehydratase (Ratio A / NA- δ-ALAD), Urinary δ- aminolevulinic acid (U- δ-ALA), Urinary Porphobilinogen (U-PBG)

Figure 1: Percentage Change of blood investigations of SilverJewellery Workers with respect to Control

cells also have been suggested as a causative mechanism for lead-induced anemia (Osterode *et al.*, 1999).

However, in our study alterations of hematological parameters were not severe which might be due to slightly elevated blood lead level (Mean PbB of SJW is 23.23 μg/dl and PbB range 11.9 – 37 μg/dl). Similar results in an earlier study support our findings (ATSDR, 2007). The increased WBC count (p<0.001, 29.8%) in our study may be due to the dust particles and vapors of lead. All the silver jewellery workers were working in unhygienic/unventilated places and also were exposed to the high temperature, thereby leading to increased WBC count.

Blood lead was negatively correlated with activated δ-ALAD (-0.45, P<0.01), non-activated δ-ALAD (-0.49, P<0.001) and positively correlated with Act / N-Act δ-ALAD ratio (0.37, P<0.05), U-δ-ALA (0.38, P<0.01) and U- PBG (0.45, P<0.01) (Table 2). A significant negative correlation of PbB with Hb (-0.37, P<0.01), HCT (-0.39, P<0.01), MCV (-0.41, P<0.01), RBCs (-0.32, P<0.05) and a positive correlation with WBCs (0.46, P<0.01) were observed



Haemoglobin -Hb, Hematocrit -HCT, Mean Corpuscular Volume -MCV, Mean Corpuscular Hemoglobin-MCH, Mean Corpuscular Hemoglobin Concentration -MCHC, Red Blood Cells- RBC Count and White Blood Cells -WBC Count

Figure 2: Percentage Change of Hematological Investigations of Silver Jewellery Workers concerning Control Group

in the study group (Table 4). From these results, it appears that the impairments of hematological and heme biosynthesis-related parameters because of increased lead were consistent among 29-49% of silver jewellery workers.

Similar blood levels of lead and its adverse impact on hematological and heme biosynthesis related parameters may also be found in workers involved in many unorganized industries involved in battery recycling and production of leaded glass and pottery (ATSDR, 2017).

CONCLUSION

This study found significantly higher levels of lead among silver jewellery workers, thereby negatively impacting their hematological parameters. This finding might be due to more occupational exposure to lead fumes and vapors. Therefore, the silver jewellery manufacturing workers should be regularly screened for blood lead levels and its health hazards. Also, these workers should be motivated to use protective gear such as masks, goggles, aprons, etc during work to minimize the adverse effect of lead exposure.

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Institution and Ethics approval and informed consent

Ethics approval was obtained from Institutional Ethics Committee of Krishna Institute of Medical Sciences. The written informed consent is obtained from all the participants in this study.

Conflict of Interest

The authors declare that there is no conflict of interest for this study.

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REFERENCES

Al-Modhefer, A. J. A., Bradbury, M. W. B., Simons, T. J. B. 1979. Observations on the chemical nature of lead in human blood serum. *Clinical Science*, 81(6):823–829.

ATSDR 1998. Toxicological Profile for Cadmium. Agency for Toxic Substances and Disease Registry, Atlanta, Georgia.

ATSDR 2007. Toxicological profile for lead. Agency for Toxic Substances and Disease Registry, Atlanta, GA: US Department of Health and Human Services. CDC, Agency for Toxic Substances and Disease Registry.

ATSDR 2017. Case studies in environmental medicine. Agency for Toxic Substances and Dis-

ease Registry, Lead Toxicity. Course: WB2832. 12th June 2017;9-161.

Chisolm, J. J., Thomas, D. J., Hamill, T. G. 1985. Erythrocyte porphobilinogen synthase activity as an indicator of lead exposure in children. *Clinical Chemistry*, 31(4):601–605.

Declaration of Helsinki 1964. Amended by World Medical Assembly. *British Medical Journal*, 313(70):1448–1449. Venice, Italy.

EPA 1986. Air Quality Criteria for Lead. Research Triangle Park, NC: U. S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office. ISBN: EPA600883028F.

Magellan Diagnostics 2015. LeadCare® II Blood Lead Analyzer User's Guide. Lead Care II Blood Lead Testing System.1-9. (70-6551-Lead Care II-User's-Guide-Rev-06-USB).

Mauzerall, D., Granick, S. 1956. The occurrence and determination of delta-aminolevulinic acid and porphobilinogen in the urine. *The Journal of Biological Chemistry*, 219(1):435–446.

Osterode, W., Barnas, U., Geissler, K. 1999. Dose-dependent reduction of erythroid progenitor cells and inappropriate erythropoietin response in exposure to lead: new aspects of anaemia induced by lead. *Occupational and Environmental Medicine*, 56(2):106–109.

Patil, A., Bhagwat, V., Patil, J., Dongre, N., Ambekar, J., Jailkhani, R., Das, K. 2006a. Effect of Lead (Pb) Exposure on the Activity of Superoxide Dismutase and Catalase in Battery Manufacturing Workers (BMW) of Western Maharashtra (India) with Reference to Heme biosynthesis. *International Journal of Environmental Research and Public Health*, 3(4):329–337.

Patil, A. J., Bhagwat, V. R., Patil, J. A., Dongre, N. N., Ambekar, J. G., Das, K. K. 2006b. Biochemical Aspects of Lead Exposure in Silver Jewelry Workers in Western Maharastra (India). *Journal of Basic and Clinical Physiology and Pharmacology*, 17(4):213–229.

Rimington 1971. Quantitative Determination of Porphobilinogen and Porphyrins in Urine and Faeces. Association of Clinical Pathologists. *Broadsheet*, 70:1–9.

Stauber, J. L., Florence, T. M., Gulson, B. L., Dale, L. S. 1994. Percutaneous absorption of inorganic lead compounds. *Science of The Total Environment*, 145(1-2):55–70.

Trester, C. 1999. Modern Technology for Cellular Differentiation: Principles of Measurement in Sysmex Haematology Analyzers: Technology Update in Laboratory Haematology. Sysmex Scientific Seminar, India.

Wada, O., Toyokawa, K., Urata, G., Yano, Y., Nakao, K. 1969. A simple method for the quantitative analysis of urinary delta-aminol evulinic acid to evaluate lead absorption. *Occupational and Environmental Medicine*, 26(3):240–243.

World Health Organization 1995. Biological indices of lead exposure and body burden. In: IPCS, Inorganic lead. Environmental Health Criteria, 118. Geneva, Switzerland: WHO, 165: 114-118.