BIOMEDICAL AND ENVIRONMENTAL SCIENCES 15, 298-305 (2002)

Environmental Exposure to Lead as a Risk for Prostate Cancer

M.K.J. SIDDIQUI[*,1], S. SRIVASTAVA[+], AND P.K. MEHROTRA [#]

[*]*Industrial Toxicology Research Centre. Post Box No. 80, M.G Marg, Lucknow-226 001, India;*
[+]*Dr. RML Avadh University, Faizabad-224001, India;* [#]*Dr. SPM Civil Hospital, Lucknow-226001, India*

Objective To evaluate the possible role of environmental exposure to lead as a risk factor for prostate pathology in patients suffering from prostate cancer (PCA) and benign prostate hyperplasia (BPH). **Methods** Blood lead (BPb) level was determined in PCA and BPH cases using a graphite furnace Atomic Absorption Spectrophotometer and compared with those in a control group living in the similar socioeconomic environment. **Results** BPb was significantly higher in PCA and BPH cases than in normals ($P<0.05$). Blood levels of zinc and copper were significantly lower in PCA and BPH cases when compared with controls ($P<0.05$). In all the three groups, a statistically significant positive correlation between lead and thiobarbituric acid reactive substances (TBARS) measured as malondialdehyde, and negative correlation between blood lead and antioxidant GSH level, indicative of possible generation of reactive oxygen species, were also observed after adjusting for age as a possible confounders. However, positive association between blood lead and TBARS was relatively higher in PCA patients ($r=0.77$, $P<0.05$) than in BPH ($r=0.32$, $P<0.05$) and normal ($r=0.30$, $P<0.05$). **Conclusion** These results with limited power seem to suggest for the first time that environmental exposure of aging males to lead may be a risk factor for prostate cancer and/or benign prostate hyperplasia possibly through generation of reactive oxygen species and/or reducing the level of zinc which acts as a cellular growth protector.

Key words: Benign prostate hyperplasia; Prostate cancer; Blood lead; Atomic Absorption Spectrophotometer

INTRODUCTION

Abnormal growth of the prostate gland in aging males is a common pathology, as reflected in the incidence of benign prostate hyperplasia (BPH) and prostate carcinoma (PCA). Prostate cancer is a second leading cause of cancer related death (after lung cancer) in men in USA[1], but it ranks 5th in incidence and 4th in cancer mortality for men in Mumbai, India[2]. The incidence is reported to be increasing by 1% every year[2]. Although, a variety of growth factors, steroidal hormones, proteases and other factors are involved in normal prostatic morphogenesis and function, but their role in BPH and PCA remains poorly understood[3,4]. Metallic lead and its compounds have been studied for their carcinogenicity in several animal tissues like prostate, kidney and lung[5-7]. Based on occupational and

[1]Correspondence should be addressed: Dr. M.K.J. Siddiqui, Industrial Toxicology Research Centre, Post Box No. 80, M.G. Marg, Lucknow-226001, India. Telephone: 0522-227586, Fax: 91522 228227, E-mail: itrc@sancharnet.in

Biographical note of the first author: Dr. Siddiqui is a senior scientist with extensive research experience in Human and Environmental Health and contributed significantly to biochemical, analytical and regulatory toxicology.

0895-3988/2002
CN 11-2816

environmental studies, investigators[8] suggested a potential role of lead in the etiology of human cancer. A number of studies have been carried out on genotoxic effects of lead exposure[9] but the evidence for direct toxicity to the genetic material could not be observed. Nevertheless, some supportive evidences for indirect mechanisms were available in these studies. Lead induced inhibition of DNA repair[10], interactions with calcium[11], and the ability to act as a promoter in the production of cancer[12] are thought to be the most important indirect mechanisms for genotoxic potential of lead compounds.

In some recent studies, generation of reactive oxygen species (ROSs) has also been evaluated as an indirect mechanism of lead induced DNA damage[13]. Since lead ions have no ability to generate highly reactive hydroxyl radical ($OH^{\bullet}$) from superoxide radical ($O\dot{2}$) or H_2O_2[13], attention has been paid to the accumulation of δ-aminolevulinic acid (ALA) in lead induced DNA damage. ALA, formed in the rate limiting step of heme biosynthetic pathway, has been proposed to act as an endogenous pro-oxidant in lead poisoning[14]. The inhibitory effect of both inorganic and organic lead compounds on δ-aminolevulinic acid dehydrogenase (ALAD)[15,16] accounts for an accumulation of ALA that can be fastly oxidized to generate ROS leading to an oxidative DNA damage[17]. According to the assumptions mentioned above, it is reasonable to investigate the environmental exposure of aging males to lead as a possible risk factor for prostate enlargement.

Since, oxidative stress damages the cellular structure and is linked to many diseases including cancer, the present study was to determine the blood level in PCA, BPH and normal males and to evaluate some possible relations of malondialdehyde and glutathione status with lead exposure as an indirect measurement of lead induced generation of ROS that enhances TBARS and reduces glutathione level causing oxidative stress. Blood zinc and copper levels were also measured as part of their interaction with lead as possible way of abnormal growth and development of the prostate.

MATERIALS AND METHODS

Subjects

Blood (five mL) from 40 BPH and 17 PCA patients, admitted to a local hospital in Lucknow for undergoing transurethal resection of the prostate, were collected in a preheparinized vials for this study. Twenty normal men without any bladder outflow obstructive (BOO) symptoms/signs and living in the same socioeconomic environment were also taken for the collection of their blood. The report on pathological evaluation was obtained for each subject in BPH and PCA group. Detailed case histories of the subjects as given in Table 1, were recorded and blood samples, as coded numbers, were transported under ice cold conditions to the Department of Analytical Toxicology, Industrial Toxicology Research Centre, Lucknow for metals analysis and other biochemical assays.

Analysis of Lead

Blood lead was determined using a graphite furnace atomic absorption spectrometer (Varian SpectrAA 250+, Varian Australia Pty Ltd., Victoria, Australia)[18]. The instrument was calibrated using aquous standards of 10, 20, 30 and 40 µg/L. Detection limit was 3 µg/L. Fifty microlitres of blood were diluted 1:10 in the diluent in a 1.0 mL polystyrene autosampler tube. The diluent (Triton X-100 0.1% w/v); $NH_4H_2PO_4$ 0.2% (w/v); NH_3 *0.14* mol/L) was prepared in deionized water. The calibration blank used was 0.2% nitric acid,

0.2% $NH_4H_2PO_4$ solution and reagent blank was diluent solution. Accuracy and precision of the method were checked by spiking the samples with known amounts of standard. The coefficients of variation were 6% and 4% at 10 and 40 µg/L.

TABLE 1

Characteristic of Cases

Variables	Normal ($(\bar{x}\pm s)$/ Percentage)	BPH ($(\bar{x}\pm s)$/ Percentage)	PCA ($(\bar{x}\pm s)$/ Percentage)
Age	53.1±6.4 (20)	70±10.0 (41)	71.0±7.5 (17)
Smoker	75% (15)	48.8% (20)	47.0% (8)
Non-smoker	25% (5)	51.2% (21)	52.9% (9)
Vegetarian	55% (11)	70.7% (29)	47% (8)
Non-vegetarian	45% (9)	29.3% (12)	53% (9)
Rural	15% (3)	46.0% (19)	47.0% (8)
Urban	85% (17)	54.0% (22)	53.0% (9)

Note. Data in parentheses indicates number of cases in the group.

Analysis of Zinc and Copper

Zinc and copper were analysed in blood by flame atomic absorption spectrometer (Varian Spectr AA 250+) using hollow cathode lamps (213.9 nm, 324.7 nm for zinc and copper respectively). The instrument was calibrated using aquous standards of 0.2, 0.4, 0.8, and 1.2 µg/mL for zinc 0.5, 1.0, 1.5, and 2.0 µg/mL for copper. Detection limit was 0.01 µg/mL for zinc and 0.05 µg/mL for copper. One millilitre of blood in 50 mL Erlenmayor flask was added five mL deionized water, glass beads and two mL of 1:1 mixture of concentrated HNO_3 and $HClO_4$. The samples were digested at 120-150℃ till a clear solution was obtained, approximately in two hours, quantitatively transferred to a 10 mL volumetric flask and made up to volume with deionized water. A sample blank was always prepared with each set of samples in order to control the possible metal contamination by external sources.

Estimation of Lipid Peroxidation

The product of lipid peroxidation malondialdehyde was estimated in the blood by the method of Stocks and Dormandy[19] by adding phosphate buffer (pH 7.4; 0.1 mol/L) in 0.5 mL blood and incubated for 30 min at 37℃ and centrifuged to collect 3.0 mL supernatant and added 1.0 mL 1% TBA and then placed in boiling water bath for 15 min. Contents were cooled in ice and centrifuged for 15 min at 2500 rpm. O.D. of the supernatant was taken against a suitable blank at 532 nm and was converted to equivalent of MDA (nmol/mL) using molar extinction coefficient of 1.56×10^5 mol/L^{-1}cm^{-1}.

Estimation of Glutathione

For estimation of GSH[20], 0.4 mL of blood mixed with 1.6 mL of water was added in 2 mL of 10% TCA and centrifuged at 2 000 rpm for 15 min. 1 mL of supernatant was added to 1.5 mL of 0.3 mol/L Na_2HPO_4 solution and 0.25 mL of 0.04% DTNB. Colour was read at 412 nm.

Statistical Analysis

Student's *t* test was used to compare the mean values of metals, GSH and MDA in the blood of normal, BPH and PCA cases. Partial regression coefficient between lead and GSH/MDA was determined, after adjusting for age, in all the three groups i.e. normal men, BPH and PCA cases. The probability level determining significance was $P<0.05$. The correlation cofficient between blood lead and GSH level in PCA cases was significant (r=-0.32, $P<0.05$) (Fig. 1(A)). The correlation cofficient between blood lead and MDA level in PCA cases was also significant (r=0.77, $P<0.05$) (Fig. 1(B)).

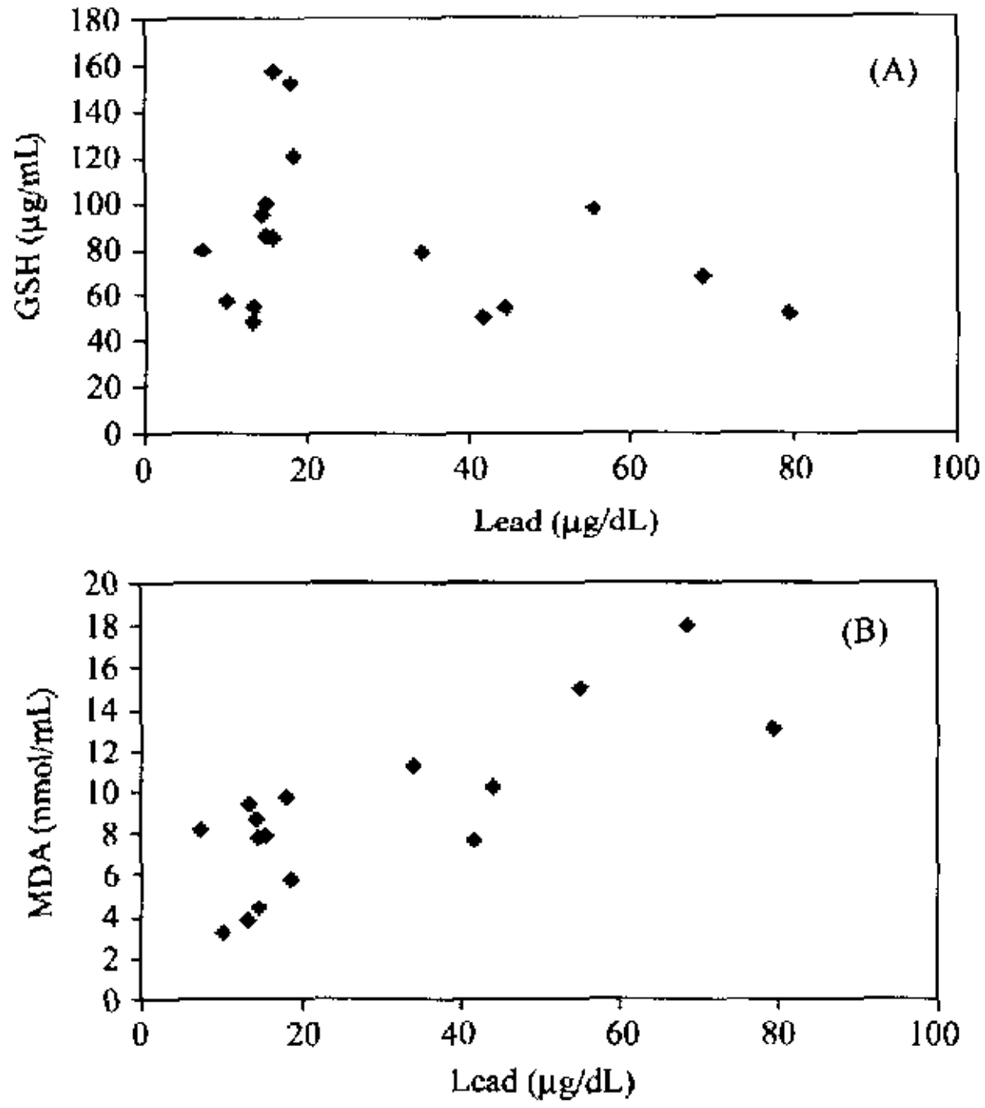

FIG. 1. Blood lead levels plotted against blood GSH (A) and blood MDA (malondialdehyde) levels (B) in prostate cancer cases indicating the condition of oxidative stress.

RESULTS

BPb levels in normal, BPH and PCA groups are given in Table 2, indicating a boundary between three groups. It is clear that BPb was about three times higher ($P<0.05$) in PCA group (28.24 µg/dL) than in normals (10.19 µg/dL). Similarly, the level was twice as higher in BPH group as in normals (23.39 µg/dL vs 10.19 µg/dL) with statistically significant differences ($P<0.05$). Interestingly, both zinc and copper levels in the blood were lower in BPH and PCA groups when compared with those in normal group, as controls ($P<0.05$) (Table 2).

Antioxidant GSH status showed a statistically significant decline in the blood of PCA group in comparison with controls ($P<0.05$). However, GSH status in BPH group was not very different from controls (Table 2). Similarly, lipid peroxides in the blood, as determined

by malondialehyde levels, were significantly higher only in PCA group (P <0.05) and not in BPH group when compared with those of normals (Table 2).

TABLE 2

Blood Levels of Lead, Zinc, Copper GSH and Malondialdehyde (MDA) in Normal Men, BPH and PCA Patients

Blood	Lead Range ($\bar{x}\pm s$)	Zinc Range ($\bar{x}\pm s$)	Copper Range ($\bar{x}\pm s$)	GSH Range ($\bar{x}\pm s$)	MDA Range ($\bar{x}\pm s$)
Normal (n=20)	1.2-43.1 10.2±10.0	3.5-10.4 6.9±1.7	0.2-5.1 1.0±1.1	50.2-255.3 121.8±56.1	3.08-11.77 6.7±2.9
BPH (n=41)	1.47-96.12 23.4±19.6**	0.5-16.8 4.6±3.2*	0.1-1.0 0.5±0.2*	36.4-307.7 116.7±58.0ª	2.3±20.5 7.9±4.8
PCA (n=17)	7.2-79.3 28.2±22.0**	0.8-9.2 4.4±2.5*	0.1-1.2 0.5±0.3	48.2-156.9 84.5±33.5*	3.3-18.0 8.7±4.0**

Note. Level of lead in µg/dL; Levels of zinc copper and GSH in µg/mL; Levels of MDA in nmol/mL; *Significantly lower in blood of BPH/PCA than normal men (P<0.05) but differences between BPH and PCA not significant; **Significantly higher in blood of BPH/PCA than normal men (P<0.05) but differences between BPH and PCA not significant; ªSignificantly higher in blood of BPH than PCA (P<0.05) however, difference between BPH and normal men not significant.

Partial regression coefficients as determined between lead and GSH and between lead and malondialdehyde levels in the blood (after adjusting for age) showed a negative association between lead and GSH status and a positive association between lead and malondialdehyde levels in all the three groups (Table 3). However, positive association between blood lead and malondialdehyde levels was much higher in PCA group than in other two groups (Table 3).

TABLE 3

Partial Regression Coefficient Between Blood Lead and GSH and Blood Lead MDA Levels in Normal Men, BPH and PCA (After Adjusting for Age)

	Normal Men Lead	BPH Lead	PCA Lead
GSH	−0.38*	−0.20*	−0.32*
MDA	0.30*	0.32	0.77*

Note. * Statistically significant (P<0.05).

The correlation between blood lead and GSH levels in PCA group, as shown in Fig. 1(A), indicated a negative relationship between the two variables. However, a positive relationship was noticed between blood lead and malondialdehyde levels (Fig. 1(B)). These relationships suggested a state of oxidative stress caused by lead.

DISCUSSION

The deleterious effects of lead are associated with either acute exposure to high levels of the element or chronic exposure to low levels of the metal. The present study points out the chronic exposure of aging males to lead was through environmental contamination as none of the subjects reported any occupational or accidental exposure to lead during their

examination by the doctor. The etiology and pathogenesis of BPH still present unresolved questions and are regarded as the result of hormonal imbalance (altered oestrogen/testosterone ratio) or testosterone, dihydrotestosterone or estrogen stimulation either perinatal or presenescent[21]. Prostate cancer appears androgen dependent during the early stages of oncogenesis as initial stimulation of prostatic growth is mediated by androgens[4].

Several lines of evidence suggest that cellular damage mediated by oxidants may be involved in some of the pathogenesis associated with lead intoxication[14, 22]. Blood levels of malondialdehyde, a product of lipid peroxidation were strongly correlated with blood lead concentrations in lead exposed workers[23]. In the present study, a three times higher blood lead level in PCA group and two times higher in BPH group than in controls (Table 2) might be expected to generate ROS leading to lipid peroxidation as statistically significant higher levels of malondialdehyde in the blood of PCA group was observed in the present study (Table 2). Malondialdehyde level was higher than in controls, but in the BPH group it was not statistically significant (Table 2). Apparently, quantitatively significant generation of ROS/lipid peroxide in PCA may differentiate it from that of BPH. Our findings are further supported by the recent report of Dursun *et al.*[24] who found significantly higher differences in the blood levels of lead exposed workers, with 15±10 μg/dL as compared to controls, 2.37±6.28 μg/dL plasma (2.67±0.69 μmol/L) and erythrocyte (27.53±6.28 nmol/gHb); lipid peroxide levels in workers with occupational exposure to lead were significantly higher than in controls, with 1.23±0.61 μmol/L and 14.35±2.08 nmol/gHb, respectively. Lead could bind to the membrane and act to stimulate lipid oxidation by causing change in membrane physical properties. Through this mechanism, lead would favour the propagation of lipid oxidation causing lateral phase separation, and by increasing lipid oxidation rates, it could be cytotoxic by membrane related processes[25].

Lead can lower cellular concentrations of hematoproteins and glutathione, thus reducing the redox buffering capacity of cells[26]. Under such conditions, ROS generated by other events may not be neutralized and thereby the likelihood of oxidative damage to DNA may be increased[26]. δ-aminolevulinic acid (ALA) whose levels are increased by Pb can generate ROS and cause oxidative damage to DNA in Chinese hamster ovary cells *in vitro*[26]. In the present study, glutathione level in blood of the PCA group was significantly lower than in controls (84.5±33 vs 122±56). Again GSH blood level in BPH was not significantly different from that of controls (117±58 vs 122±56). It is therefore, interesting to note that both reduction in blood GSH and increase in malondialdehyde level, i.e. state of oxidative stress were statistically significant in PCA group when compared with controls. However, it was not different in case of BPH. Thus, it may be possible that environmental exposure to lead (only in PCA) is toxicologically significant to generate ROS leading to oxidative damage, which may increase the risk of prostate cancer. Oxidative stress in the present study could be due to direct participation of lead in free radical mediated reactions, or to an associated accumulation of δ-ALA, a metabolite that can undergo iron-catalysed oxidation with the generation of ROS. Douki *et al.*[27] reported some mutagenic properties of ALA, and 4,5-dioxovaleric acid (the final oxidation product of ALA) as an efficient alkylating agent of the guanine moieties in DNA. In PCA group blood lead of 28 μg/dL might be expected to cause elevation of blood ALA and thereby 4,5-dioxovaleric acid as ALAD activity was reported to be markedly inhibited in lead exposed persons, with a negligible effect level perhaps as low as 10 μg/dL[28]. Antilla *et al.*[29] in a nested case control study of 16 male glioma cases found an odd ratio of 11.0 (1.0-6.3) for those with blood lead >28 μg/dL vs those with less than 14 μg/dL (*P* for trend=0.03). Fleshner and Klotz[30] reported that oxidative stress was higher in benign epithelium of prostate cancer patients than in men

without the disease.

There were statistically significant correlations between blood lead and blood GSH and malondialdehyde levels in normal men, BPH and PCA (Table 3). However, correlation between blood lead and GSH and malondialdehyde levels were relatively higher in PCA than in other two groups. This higher correlation might be evaluated as a supportive evidence of an indirect mechanism for lead induced carcinogenecity.

Zn acts as a cellular growth protector, including growth of neoplastic cells, and its deficiency was demonstrated to be involved in several stages of malignant transformations[31]. It is also suggested that Zn protects against Cd induced prostate cancer, the mechanism of which is not clear[32]. Results of this study indicated the possibility of Pb-Zn interaction, both in PCA and BPH, as the blood Zn levels were brought down from 6.9±1.7 to 4.4±2.5 μg/mL in PCA and to 4.6±3.2 μg/mL in BPH. Bush *et al.*[33] reported a reduction in Zn level to about one third of normal in malignant prostate and an inverse relationship between Zn concentration and prostate pathology. The reduced level of blood Zn and Cu in BPH and PCA than in normals in this study may also be related to some pathology of prostate.

There are potential confounders which may play a role in prostate cancer, such as diet, age and ethnicity. We also found age as a potential confounder and therefore, adjusted the data for age in partial regression coefficient determination. Still, a strong positive association between blood lead and malondialdehyde level in PCA (Table 3) was detected. However, this case-control study could only provide evidences on correlations between lead exposure and the occurrence of prostate cancer and BPH, but not on causal relationship.

ACKNOWLEDGEMENT

Authors express their sincere thanks to the Director, Industrial Toxicology Research Centre, Lucknow for his keen interest in the work. Thanks are also due to Ms. Poonam Saxena and Mr. Pramod Kumar Srivastava for technical assistance in lead analysis and word processing respectively.

REFERENCES

1. Wingo, P.A., Landis, S., and Ries, L.A. (1997). An adjustment to the 1997 estimate for new prostate cancer cases. *Cancer* **80**, 1810-1813.
2. Yeole, B.B. and Jussawala, D.J. (1997). Descriptive epidemiology of the cancers of male genital organs in greater Bombay. *Indian J. Cancer* **34**, 30-34.
3. Wilson, M.J. (1995). Proteases in prostate development, function and pathology. *Microse. Res. Tech.* **30**, 305-318.
4. Anumuller, G, Scitz, J., and Riva, A. (1994). Functional Morphplogy of Prostate Gland, in Ultrastructre of Male Urogeniatal Glands: Prostate Seminal Vesicles, Urethral, and Bulbourethral Glands. (A. Riva, F. Testa Riva and P. M. Motta, Eds.), pp. 61-112, Kluwer Academic Publishers, Hingham.
5. Nogueira, E. (1987). Rat renal carcinogenesis after chronic simultaneous exposure to lead acetate and N-nitrosodiethylamine. *Virch. Arch.* **B 53**, 365-374.
6. Zawirska, B. and Medras, K. (1972). The role of the kidneys in disorders of porphyrin metabolism during carcinogenesis induced with lead acetate. *Arch. Immunol. Ther. Exp.* **20**, 257-272.
7. Blakley, B.R. (1987). The effect of lead on chemical- and viral-induced tumor production in mice. *J. Appl. Toxicol.* **7**, 167-172.
8. Steenland, K. and Boffetta, P. (2000). Lead and cancer in humans: Where are we Now? *Am. J. Indus. Med.* **38**, 295-299.
9. Winder, C. and Bonin, T. (1993). The genotoxicity of lead. *Mutation Res.* **285**, 117-124.
10. Hartwig, A.R., Schlepegrell, and Beyersmann, D. (1990). Indirect mechanism of lead induced genotoxicity in cultured mammalian cells. *Mutation Res.* **241**, 75-82.
11. Deknudt, G.H. and Gerber, G.B. (1979). Chromosome aberrations in bone marrow cells of mice given a normal or calcium deficient diet supplemented with various heavy metals. *Mutation Res.* **68**, 163-168.
12. Tanner, D.C. and Lipsky, M.M. (1984). Effect of lead acetate on N-(4'-fluoro-4-biphenyl) acetamide induced renal carcinogenesis in the rat. *Carcinogenesis* **5**, 1109-1113.

13. Hiraku, Y. and Kawanishi, S. (1996). Mechanism of oxidative DNA damage induced by aminolevulinic acid in the presence of copper ion. *Cancer Res.* **56**, 1786-1793.
14. Hermes–Lima, M., Pereira B., and Bechara, E.J. (1991). Are free radicals involved in lead poisoning? *Xenobiotica* **21**, 1085-1090.
15. Duydu, Y. and Vural, N. (1998). Urinary excretion of lead and delta aminolevulinic acid in workers occupationally exposed to tetraethyl lead. *Biol. Trace Elem. Res.* **63**, 185-194.
16. Duydu, Y., Suzen, S., Aydin, A., Sayal, A., Ismier, A., and Vural, N. (1998). Urinary excretion of total and inorganic lead in tetraethyl lead exposed workers. *Bull. Env. Contam. Toxicol.* **60**, 395-401.
17. Costa, C.A., Trivelato, G.C., Pinto, A.M., and Bechara, E.J.H. (1997). Correlation between plasma 5- aminolevulinic acid concentrations and indicators of oxidative stress in lead exposed workers. *Clin. Chem.* **43**, 1196-1202.
18. Khullar, K.D. (1999). Determination of lead inwhole blood by electrochemical atomic absorption spectrometry. *Labnews* **4**, 3-4.
19. Stocks, J. and Dormandy, T.L. (1971). Auto-oxidation of red cell lipids induced by hydrogen peroxide. *Br. J. Haematol.* **20**, 95-111.
20. Kuo, C.H., Steight, S.D., and Hook, J.B. (1983). Lipid peroxidation: A possible mechanism of cephaloridine induced nephrotoxicity. *Toxicol. Appl. Pharmacol.* **67**, 78-88.
21. Brys, M., Nawrocka, A.D., Miekos, E., Zydex, C., Fousinski, M., Barecki, A., and Krajewrka, W.M. (1997). Zinc and cadmium analysis in human prostate neoplasms. *Biol. Trace Elem. Res.* **59**,145-152.
22. Bechara, E.J.H., Medeiros, M.H.G., Monteiro, H.P., Hermes-Lima, M., Percira, B., Demasi, M., Costa, C.A., Abdalla, D.S.P., Onuki, T., Wendel, C.M.A., and Di Mascio, P. (1993). A free radical hypothesis of lead poisoning and inborn porphyries associated with 5-aminolevulinic acid overload. *Quim. Nova.* **16**, 385-392.
23. Jiun, Y.S. and Hsien, L.T. (1994). Lipid peroxidation in workers exposed to lead. *Arch. Environ. Health* **49**, 256-259.
24. Dursun, N., Dogan, P., and Donmez, H. (2001). Plasma and erythrocyte lipid peroxide levels in workers with occupational exposure to lead. *Biol. Trace Elem. Res.* **82**, 29-34.
25. Adonaylo, V.N. and Oteiza, P.I. (1999). Pb^{2+} promotes lipid oxidation and alterations in membrane physical properties. *Toxicology* **132**, 19-32.
26. Silbergeld, E.K., Waalkes, M. and Rice, J.M. (2000). Lead as a carcinogen, Experimental evidence and mechanism of action. *Am. J. Indus. Med.* **38**, 316-323.
27. Douki, T., Onuki, J., Medeiros, M.H.G., Bechara, E.J.H., Cadet, J., and Masico, P.D. (1998). DNA alkylation by 4,5-dioxyvaleric, the final oxidation product of 5- aminolevulinic acid. *Chem. Res. Toxicol.* **11**, 150-157.
28. Sakai, T. and Mortia, Y. (1996). Delta-aminolevulinic acid in plasma or whole blood as a sensitive indicator of lead effects, and its relation to other heme-related parameters. *Int. Arch. Occup. Environ. Health* **68**, 126-132.
29. Antilla, A., Heikkila, P., Mydri, E., Kauppinen, T., Pukkala, E., Hemberg, S. and Hemminki, K. (1996). Risk of nervous system cancer among workers exposed to lead. *J. Occup. Env. Med.* **38**, 131-136.
30. Fleshner, N.E. and Klotz, L.H. (1998). Diet, androgens, oxidative stress and prostate cancer susceptibility. *Cancer Metastasis Rev.* **17**, 325-330.
31. Zowczk, M., Iskra, M., Torniski, L., and Coffa, S. (2001). Analysis of serum Cu and Zn concentration in cancer patients. *Biol. Trace elem. Res.* **82**, 1-8.
32. Waalkes, M.P., Rehm, S., Riggs, C.W., Bare, R.M., Devor, D., E.Poirier, I.A., Wenk, M.L. and Hennemax, J. K. (1989). Cadmium carcinogenesis in male wistar rats: doseresponse analysis of effects of zinc on tumor induction in the prostate, in the testes and at the injection site. *Cancer Res.* **49**, 4282-4288.
33. Bush, I.M., Berman, E., Nourkayhan, S. *et al.* (1974). American Urological Association, 69th Annual Meeting, St. Louis, Missouri, 110.

(*Received October* 12, 2001 *Accepted June* 30, 2002)