Association of blood lead, zinc protoporphyrin and ä-aminolevulinic acid dehydratase genotype in workers with long-term lead exposure - SCEs as an indicator

Fang-Yang Wu[a*], Pao-Wen Chang[a], Chin-Ching Wu[b], Hsien-Wen Kuo[a], Chiu-Shong Liu[c], Jim-Shoung Lai[a]

[a] Institute of Environmental Health,
China Medical College, Taichung, Taiwan, R.O.C.
[b] Department of Public Health,
China Medical College, Taichung, Taiwan, R.O.C.
[c] Department of Family Medicine,
China Medical College Hospital, Taichung, Taiwan, R.O.C.

[*]All further correspondence should be addressed to:
Fang-Yang Wu, Dr.
Institute of Environmental Health,
China Medical College,
No 91, Hsueh-Shin RD,
Taichung, Taiwan, R.O.C.
Telephone number: (886) 4- 2054076
Fax number: (886) 4- 201-9901
e-mail: fywu@mail.cmc.edu.tw

Abstract

Genetic polymorphism of genotypes ä-Aminolevulinic acid dehydratase (ALAD), blood lead, zinc protoporphyrin (ZPP), sister chromatid exchanges (SCEs), and high SCE frequency cells (HFCs) were evaluated in 26 workers exposed to high levels of lead and 31 employees exposed to low levels of lead. 30 controls were matched for age, gender and smoking habits. Results showed that occupationally exposed workers had significantly higher blood lead, ZPP and hemoglobin levels than the controls. ALAD1-1 and ALAD1-2 genotype proportions were as follows: high exposure group, 65.4% vs. 34.6%; low exposure group, 80.7% vs. 19.4%; control group, 80% vs. 20%. The ALAD 2-2 genotype was not detected. There was no significant difference among the three groups with regard to ALAD genotype. Subjects with ALAD 1-2 genotypes did not have higher levels of blood lead, ZPP or hemoglobin than those with the ALAD 1-1 genotypes. Average SCE values in the high exposure group were significantly greater than those in the control group (6.2 v.s. 5.2 SCEs/cell, $p<0.05$). In HFC analysis, there was a significantly higher HFC rate (53.9%) in the high exposure group compared to the low exposure group (16.1%) and control group (10%). High exposure workers who smoked showed a higher level of HFC (80%) than the control subjects (18.2%), and also had a higher odds ratio (18). In conclusion, there was a significant association of both SCE and HFC levels with exposure to lead. However, different ALAD genotypes were not shown to be associated with levels of blood lead and ZPP among the three groups.

Key words: lead worker; ALAD genotype; SCE; HFC

1. Introduction

Exposure of workers to high levels of lead oxide dust occurs in manufacturing processes, which seems to be the most common hazard in storage battery plants. The most important routes of exposure are via inhalation and/or ingestion [1,2]. Blood lead levels (BLL) have been used as an indicator of lead exposure and its effects on health. BLL is affected by recent exposure and often does not reflect long-term exposure; therefore, earlier exposure is more accurately reflected by zinc protoporphyrin (ZPP) concentration. The toxic effects of lead may be the consequence of both recent and earlier exposure. Lead workers with similar exposure intensities differ in BLL and are affected differently with regard to health [3]. Therefore, there are considerable interindividual differences in the toxicokinetics of lead. Individual variations are due, in part, to genetic factors, such as polymorphisms in ä-Aminolevulinic acid dehydratase (ä-ALAD). ä-ALAD is present in erythrocytes, which has been found to be inhibited by lead [4]. The degree of inhibition has been correlated with BLL.

ä-ALAD is a polymorphic enzyme encoded by a single gene with two common alleles (ALAD^{1} and ALAD^{2}), and has three isoforms (ALAD1-1, ALAD1-2 and ALAD2-2) [5,6]. Some studies have suggested a relationship between the ALAD isozyme phenotypes and BLL. Individuals who have at least one copy of ALAD^{2} , have higher BLL [7,8]. Other studies have indicated that no association was found between ALAD genotype and BLL or bone lead levels [9]. In contrast, urinary calcium and creatinine concentrations were lower in ALAD^{2} carriers than in ALAD^{1} carriers [9].

An in vitro study has shown an increase in chromosome aberration (CA) frequency in human lymphocytes treated with lead compounds compared with control cells [10]. In contrast, another study, on cultured Chinese hamster cells, showed no difference between control and lead compound treated cells [11]. A number of studies on the

cytogenetic effects of lead in vivo have been reports. Some studies have shown an increase in CAs and/or SCE frequency in lymphocytes from workers exposed to lead [12-15], whereas others have reported negative results [16,17]. Any damage induced in cellular DNA may persist for some time until circulating blood cells are stimulated to divide in culture. Published data using human cells dosed with lead in vitro have suggested that lead ions decrease the fidelity of DNA synthesis or repair [18,19], and inhibit the activity of DNA polymerase β and ligase [20]. Hartwig et al. reported indirect mechanism of lead-induced genotoxicity in Chinese hamster V79 cells [21]. These studies indicate the necessity to consider the influence of DNA-repair processes when assessing the genotoxicity of lead compounds. Therefore, the purpose of this study was to investigate the relationship between BLL and ZPP levels as well as ALAD genotypes in lead workers and to evaluate SCE induction among battery manufacturing workers exposed to high levels of lead.

2. Materials and methods

2.1. Study subjects

Two groups of lead-exposed workers were examined a storage battery manufacturer in central Taiwan. The high exposure group consisted of 26 workers in direct, long-term contact with lead oxides. The low exposure group was selected from the welding area of the same factory as the high exposure group, and consisted of 31 employees. The 30 controls were selected according to age, gender and smoking habits to match the lead-exposed groups. Each subject was interviewed using a construct questionnaire.

2.2 Lead analysis

Venous blood was obtained from study subjects and drawn into EDTA-containing tubes. Blood lead was measured by graphite furnace atomic absorption spectrophotometry (GFAAS) (Perkin Elmer 5100 AAS). ZPP was determined using hematofluorimetry (Aviv/Model 206, Biomedical Inc.).

2.3. Genotypes of ALAD

ALAD genotype was determined for each subject for which a residual blood specimen was available. Genomic DNA was prepared from human blood lymphocytes by standard procedures. The ALAD genotypes were determined by a modification of the method described by Wetmur et al. [21] and Schwartz et al. 〔22〕. A commercial PCR kit containing Taq DNA polymerase (Wako Corp. Tokyo) was 1 μg DNA. Blood samples were preheated for 3 cycles of 3 min. at 94℃ and then 3 min. at 55℃. Taq polymerase was then added. Reactions were heated for 10 sec at 55 ℃, 30 sec at 71℃ and 30 sec at 94℃ for 35 cycles in a Thermal Cycler. PCR

products was a 916 base pair fragment, which was electrophoresed on a 3% agarose gel. This fragment was cleaved at the *Msp1*restriction site. Digestion with *Msp1* restriction enzyme yielded 582 bp (ALAD^1) or 511 bp (ALAD^2) fragments.

2.4. SCE assay

Whole blood was drawn into heparinized tubes. The cultures were set up by adding 0.3 ml of blood to 5.0 ml of RPMI 1640 medium with 15% fetal bovine serum, 3% phytohemagglutinin (Gibco), and 20μM bromodeoxyuridine (Merck). The cells were incubated in the dark for 70h at 37℃, and then 2h before fixation, 2×10^{-7} M colcemid was added. To detect SCE, the slides were stained using the fluorescence-plus-Giemsa method 〔23〕. For the observation of SCE per cell, 30 second-division metaphases were scored per sample. Exchanges at the centromere were not included in the count. The percentage of high frequency cells (HFC) was also determined; HFC was defined as the percentage of lymphocytes exhibiting an SCE score over the median 95% value of the results from the 19 non-smoking control subjects. The cut-off value was 9 SCE/cell.

2.5. Statistical analysis

SAS/pc+ 6.04 statistics software (SAS/STAT) was used for all data analysis. χ^2-test and t-test were used to compare the basic characteristics, blood lead and ZPP levels, and SCE frequency between lead workers and controls. HFC analysis used Mantel-Haenszel χ^2-test. The regression model was also used.

3. Results

The characteristics of the high and low exposure groups and control group are summarized in Table1. For the ALAD genotypes, there was no significant difference among the three groups. The high exposure, low exposure and control groups had ALAD1-1 vs. ALAD1-2 genotype proportions of 65.4% vs. 34.6%, 80.7% vs. 19.4%, and 80% vs. 20%, respectively. No subjects were homozygous for the ALAD^{2} allele (ALAD 2-2 genotype). For BLL, ZPP and hemoglobin, there was a significant difference between each of the three groups. BLL and ZPP levels were highest in the high exposure group (28.4 and 102.8, respectively), and hemoglobin concentration was highest in the low exposure group (16.9). Seven workers (12%) in the high exposure group had BLL that exceeded the current regulation levels (40ug/dl for males and 30 ug/dl for females) (data not shown).

Table 2 compares BLL, ZPP levels and hemoglobin concentration for ALAD1-1 and ALAD1-2 genotypes. The mean BLL among workers in the high exposure group who were homozygous for the ALAD^{1} allele was 27.7 ug/dl, while the mean value for the ALAD^{2} allele was 29.7 ug/dl. The mean ZPP level of the high exposure workers who were homozygous for the ALAD^{1} allele was 109.4 ug/dl, while the mean for those who were heterozygous for the ALAD^{2} allele was 88.4 ug/dl. Using the *t* test, exposed and control subjects who carried the ALAD^{2} allele, and in whom the ALAD^{1} allele was homozygous, did not differ significantly with respect to BLL, ZPP and hemoglobin levels.

Table 3 shows that average SCE values in the high exposure group was significantly greater than those in the control group (6.2 v.s. 5.2 SCEs/cell, $p<0.05$). Smokers in the high exposure group and control groups differed by 6.5 and 5.7 SCEs/cell ($p<0.01$), respectively. In both work duration groups, SCE frequency was

higher in the high exposure group compared to the low exposure group, but there was no significant difference between the work duration groups in both exposure groups.

Table 4 reveals a significantly higher HFC percentage (53.9%) in the high exposure group compared to the low exposure group (16.1%) and control group (10%). Smokers and non-smokers exposed to high levels of lead had higher HFC percentages (80% and 47.6%) than the low exposure (14.3% and 16.7%) and control groups (18.2% and 5.3%). High exposure subjects with work duration of 15 or more years had significantly higher HFC percentages (54.6%) than the low exposure group (19.1%).

Multiple linear regression analysis was used to examine SCEs frequency for smoking, work duration, ALAD genotypes, BLL and ZPP levels (Table 5). Analyses of lead workers, smoking and BLL were significantly associated with SCE values, but the other variables were not. A significant difference between the observed incidence of SCEs was revealed when smokers and non-smokers of all three groups were compared.

4. Discussion

ALAD polymorphism has been shown to vary among different racial groups. The proportion of ALAD1-2 allele among Koreans is 11% 〔24〕, which is similar to German (11%) 〔25〕, and Italian (11%) 〔26〕 but higher than Japanese (6%) 〔25〕. The proportion among Taiwanese in a previous study was found to be 4.4% 〔27〕. The current study showed a prevalence of 19.4% and 20% among low exposure and control groups, but the prevalence among high exposure workers was 34.6%. This proportion was higher than the estimated range of 10~20% among Caucasian populations 〔22,25,26,28〕. In the current study, the ALAD 2-2 genotype was not detected. Differences in the proportion of $ALAD^2$ allele may be due to ethnic, individual factors and/or variation resulting from small sample sizes. However, previous studies have shown that the $ALAD^2$ allele may be associated with an increased risk of lead poisoning 〔7〕. Therefore, it is essential to explore further the factors that effect an increased proportion of $ALAD^2$ allele among workers exposed to high lead levels.

Interindividual differences of BLL were found to be influenced by the type and route of exposure, age, gender, and genetic component (such as ALAD genotype). The ALAD polymorphism appears to influence the toxicokinetics of lead. The ä-ALAD activity in erythrocytes is known to be inhibited by lead 〔29〕. In previous studies of lead workers, it has been shown that there were higher BLL among subjects of the $ALAD^2$ allele than among homozygotes for the $ALAD^1$ allele 〔8,28,30〕. The data presented here show that mean BLL of subjects with the ALAD1-2 genotype were similar to those with ALAD1-1 under the same conditions. The ALAD genotype was not found to be associated with BLL and ZPP levels. Similar observations have

been made of occupationally lead-exposed subjects. However, there was no difference in ALAD activity between the ALAD 2-2 genotype and ALAD 1-1 genotype, matched for blood lead levels, possibly due in part to a small sample size〔28,31〕.

Wulf (1980) and Andersen et al. (1982) observed an increase in SCE frequency in human lymphocytes exposed to different lead-ion concentrations 〔32,33〕. In the current study, the significantly increased SCE frequencies in workers' lymphocytes were consistent with the results reported by Grandjean et al. (1983) in a similar study on healthy individuals occupationally exposed to lead 〔13〕. Furthermore, SCE values were directly proportional to BLL. In the current study, it has been demonstrated that lead has a genotoxic effect as indicated by the significant increase in SCE frequency among long-term lead workers. However, some authors have not found any effect of lead exposure on SCEs in vivo and in vitro〔16,34,35〕.

Tobacco smoking is the most important cause of lung cancer. There is strong evidence of a relationship between smoking habits and SCE frequencies 〔36-39〕. Our data also showed the influence of smoking on SCE frequencies; the difference between smokers and non-smokers was statistically significant in all three groups (Table 5). In addition, since HFC percentage was used as a sensitive indicator of occupational exposure to a similar heavy metal (chromiun) in our previous study on chromium workers,〔38,39〕it was used as a biological effect marker for lead in the current study. In the current study, the considerable increase in the percentage of cells with ≥ 9 SCEs per cell suggests the existence of a subgroup of lymphocytes in which genotoxic lesions persist. The difference in the HFC percentage among the high exposure group and the low exposure or control groups was about 37.8% and 43.9% for three studies. The smoking groups who were exposed to high levels of lead had

higher HFC percentages (exceeded 47.6%) than the non-smoking subjects; this finding may mean that the effect of the lead exposure on HFC is similar to the effect of smoking.

Previous epidemiological studies have shown an association of high exposure to lead among battery industry workers and the incidence of renal tumor, lung and stomach cancer 〔40-43〕. Other data has revealed that after adjustment for smoking, ethnic, and socioeconomic factors, there remains a high risk of lung cancer for workers exposed to lead 〔42〕. Lead is a weak mutagen in mammalian cells. Some studies have reported that lead has a direct effect on the reproductive system 〔44,45〕, and an indirect effect on the DNA repair system 〔18-21〕. Lead has been demonstrated to disrupt DNA synthesis and repair 〔18,19〕. Several papers have shown that the interaction of lead with polymarases or ligases involved in DNA excision repair step and inhibition of DNA repair may constitute one mechanism of genotoxicity 〔18-20〕. DNA impairment and genotoxic effects may be magnified when repair is inhibited, as shown in a study on UV-induced SCEs in cells exposed to different lead-ion levels 〔21〕. In conclusion, there is a significant association between BLL and ZPP with SCE frequency among lead-exposed workers. However, there was no significance between lead exposure groups with regard to ALAD genotypes. As such, it is important for reseachers to consider indirectly induced SCE formation when measuring the genotoxicity of lead oxides among battery manufacturing plant workers.

Acknowledgements

This study was supported by Grants NSC-89-2314-B-039-035 from National Science Council.

References

〔1〕 S. Skerfving, Biological monitoring of exposure to inorganic lead, In: Clarksun TW, Friberg L, Nordberg GF, Sager PR ed. Biological monitoring of toxic mentals. New York, NY: Plenum press, 1988, pp. 169-198.

〔2〕 J. Zelikoff, H. Li J. H. Hartwig A. Wang X. W. Costa M. and Rossman. T. G. Genetic toxicology of lead compound. Carcinogenesis 9 (1988) 1727-1732.

〔3〕 A. Schütz, S. Skerfving, J. Ranstan, J-O, Christoffersson, Kinetics of lead in blood after the end of occupational exposure, Scand. J. Work Envion. Health 13 (1987) 221-231.

〔4〕 Ingvar A. Bergdahl. Anders Grubb. Andrejs Schutz. Robert J. Desnick. James G. Wetmur. Shigeru Sassa. and Staffan Skerfving. Lead binding to delta-aminolevulinic acid dehydratase in human erythrocytes. Pharmacology & Toxicology 81 (1997) 153-158.

〔5〕 J.G. Wetmur, D. F. Bishop, C. Cantelmo, R. J. Desnick, Human ä-aminolevulinic acid dehydratase : Nucleotide sequence of a full-length cDNA clone, Proc. Natl. Acad. SCI. USA 83 (1986) 7703-7707.

〔6〕 V. R. Potluri, K. H. Astrin, J. G. Wetmur, D. F. Bishop, R. J. Desnick, Human ä-aminolevulinate dehydratase: Chromosomal localization to 9q34 by in situ hybridization, Hum. Genet. 76 (1987) 236-239.

〔7〕 K. H. Astrin, D. F. Bishop, J. G. Wetmur, B. Kaul, B. Davidow, R. J. Desnick, ä-aminolevulinic acid dehydratase isozymes and lead toxicity, In: Mechanism of chemically-induced porphyrinopathies. Eds. E. K. Silbergeld, B. A. Fowler, Ann. N. Y. Acad. Sci. New York, vol. 514, 1987, pp. 23-39.

〔8〕 J. G. Wetmur, G. Lehnert, R. J. Desnick, The δ-aminolevulinate dehydratase polymorphism: high blood lead levels in lead workers and environmentally

exposed children with the 1-2 and 2-2 isozyme. Environ. Res. 56 (1991) 109-119.

〔9〕 I.A. Bergdalh, L. Gerhardsson, A. Schuts, R.J. Desnick, J.G. Wetmur, S. Skerfving, Delta-aminolevulinate acid dehydratase polymorphism: influence on lead levels and kidney function in humans, Arch. Environ. Health 52 (2) (1997) 91-96.

〔10〕 A. Forni, G. Cambiaghi, G.C. Secchi, Initial exposure to lead: Chromosome and biochemical findings, Arch. Environ. Health 31 (1976) 73-78.

〔11〕 M. Bauchinger, E. Schmid, Chromosome analysis in cell cultures of the Chinese hamster after application of lead acetate, Mutat. Res. 14 (1972) 95-100.

〔12〕 G. Deknudt, Y. Manuel, G.B. Gerber, Chromosomal aberrations in workers professionally exposed to lead, J. Toxicol, Environ. Health 3 (1977) 885-891.

〔13〕 P. Grandjean, H.C. Wulf, E. Niebuhr, Sister Chromatid exchange in response to variations in occupational lead exposure, Environ. Res. 32 (1983) 199-204.

〔14〕 X.F. Huang, Z.Y. Feng, W.L. Zhai, J.H. Xu, Chromosomal aberrations and sister-chromatid exchanges in workers exposed to lead, Biomed. Environ. Sci. 1 (1988) 382-387.

〔15〕 F. Sarto, M. Stella, A. Acqua, Cytogenetic studies in 20 workers occupationally exposed to lead, Med. Lav. 69 (1978) 172-180.

〔16〕 E. Schmid, M. Bauchinger, S. Pietruck, G. Hall, The cytogenetic effect of lead in human peripheral lymphocytes in vitro and in vivo, Mutat. Res. 16 (1972) 401-406.

〔17〕 L. Dalpra, G. Tibilletti, G. Nocera, L. Giulotto, V. Auriti, V. Carnelli, G. Simoni, SCE analysis in children exposed to lead emission from a smelting

plant, Mutat. Res. 120 (1983) 249-256.

〔18〕 M.A. Sirover, L.A. Loeb, Infidelity of DNA synthesis in vitro: screening for potential mutagens or carcinogens, Science 194 (1976) 1434-1436.

〔19〕 Y. Skreb, V. Habazin-Novak, Reversible inhibition of DNA, RNA, and protein synthesis in human cells by lead chloride, Toxicology 5 (1975) 167-174.

〔20〕 E.A. Popenoe, M.A. Schmaeler, Interaction of human polymerase ß with ions of copper, lead, and cadmium, Arch. Biochem. Biophys. 196 (1979) 109-120.

〔21〕 A. Hartwig, R. Schlepegrell, D. Beyersmann, Indirect mechanism of lead-induced genotoxicity in cultured mammalian cells. Mutat. Res. 241 (1990) 75-82.

〔22〕 J.G. Wetmur, A.H. Kaya, M. Plewinska, R.J. Desnick, Molecular characterization of the human δ-aminolevulinate dehydratase 2 ($ALAD^2$) allele: implications for molecular screening of individuals for genetic susceptibility to lead poisonong, Am. J. Hum. Genet. 49 (1991) 757-763.

〔23〕 K. Goto, S. Maeda, Y. Kano and T. Sugiyama, Factors involved in differential giemsa-staining of sister chromatids, Chromosoma. 66 (1978) 351-359.

〔24〕 B.S. Schwartz, B-K. Lee, W. Stewart, Ahn, K. Springer, K. Kelsey, Association of δ-aminolevulinate acid dehydratase genotype with plant, exposure duration, and blood lead and zinc protoporphyrin levels in Korean lead workers, Am.J. Epidemiol. 142 (7) (1995) 738-745.

〔25〕 H.G. Benkmann, P. Bogdanski, W. Goedde, Polymorphism of delta-aminolevulinate acid dehydratase in various populations, Hum. Hered. 33 (1983) 62-64.

〔26〕 G. Battistuzzi, R. Petrucci, L. Silagni, F.R. Urbani, S. Caiola, δ-aminolevulinate dehydratase: a new genetic polymorphism in man, Ann. Hum.

Genet. 45 (1981) 223-229.

〔27〕 L.L. Hsieh, S.H. Liou, Y.H. Chen, L.C. Tsai, T. Yang, T.N. Wu, Association between aminolevulinate dehydratase genotype and blood lead levels in Taiwan, JOEM 42 (2) (2000) 151-155.

〔28〕 B. Ziemsen, J. Angerer, G. Lehnert, H.G. Benkmann, H.W. Goedde, Polymorphism of delta-aminolevulinate acid dehydratase in lead exposed workers, Int. Arch. Occup. Environ. Health 58 (1986) 245-247.

〔29〕 P.L. Goering, B.A. Fowler, Regulatory roles of high-affinity metal-binding proteins in mediating lead effects on δ-aminolevulinate acid dehydratase, Ann. N.Y. Acad. Sci. 514 (1987) 235-247.

〔30〕 K.H. Astrin, D.F. Bishop, J.G. Wetmur, B. Kaul, B. Davidow, R.J. Desnick, δ-aminolevulinate acid dehydratase isozymes and lead toxicity, In: Mechanisms of chemically-induced porphyrinopathies, Ann. N.Y. Acad. Sci. 514 (1987) 23-39.

〔31〕 C.M. Smith, X. Wang, H.Hu, K.T. Kelsey, A polymorphism in the δ-aminolevulinate acid dehydratase gene may modify the pharmacokinetics and toxicity of lead, Envirom. Health Perspect. 103 (1995) 248-253.

〔32〕 H.C. Wulf, Sister chromatid exchanges in human lymphocytes exposed to nickel and lead, Dan. Med. Bull. 27 (1980) 40-42.

〔33〕 O.Andersen, H.C. Wulf, M. Ronne, G.F. Nordberg, Effects of metals on sister chromatid exchanges in human lymphocytes and Chinese hamster V79-E cells, In ‘Prevention of Occupational Cancer,” ILO Occupational Safety and Health Series, vol. 46, 1982, pp. 491-450.

〔34〕 B. Beek, G. Obe, The human leukocyte test system, VI. The use of sister chromatid exchanges as possible indicators for mutagenic activities,

Humangenetik. 29 (1975) 127-134.

〔35〕 G.R. Douglas, R.D.L. Bell, C.E. Grant, J.M. Wytsma, K.C. Bora, Effect of lead chromate on chromosome aberration sister-chromatid exchanges and DNA damage in mammalian cells in vitro, Mutat. Res. 77 (1980) 157-163.

〔36〕 F.P. Perera, R.M. Santella, D. Brenner, M.C. Poirier, A.A. Munshi, H.K. Fishman, J. Van Ryzin, DNA adducts, proteins adducts and sister-chromatid exchange in cigarette smokers and non-smokers, J. Natl. Cancer Inst. 79 (1987) 449-456.

〔37〕 M.A. Bender, Chromosomal aberration and sister chromatid exchange frequencies in peripheral blood lymocytes of a large human population sample, Mutat. Res. 212 (1989) 149-154.

〔38〕 J.S. Lai, H.W. Kuo, F.C. Liao, C.H. Lien, Sister chromoatid exchange induced by chromium compounds in human lymphocytes, Int. Arch. Occup. Environ. Health. 71 (1998) 550-553.

〔39〕 F.Y. Wu, F.J. Tsai, H.W. Kuo, C.H. Tsai, W.Y. Wu, R.Y, Wang, J.S. Lai, Cytogenetic study of workers exposed to chromium compounds, Mutat. Res. 464 (2000) 289-296.

〔40〕 W.C. Cooper, Cancer mortality patterns in the lead industry, Ann. N.Y. Acad. Sci. 27 (1976) 150-176.

〔41〕 IARC, Monograph on the evaluation of the carcinogenic risk of chemicals to human, 1980.

〔42〕 J. Siemiatycki, Risk factors for cancer in the workplace, London: CRC Press, 1991.

〔43〕 H. Fu, P. Boffetta, Cancer and occupational exposure to inorganic lead compounds: a meta-analysis of pulished data, Occup. Environ. Med. 52 (1995)

73-81.

〔44〕 M.R. Cullen, R.D. Kayne, J.M. Robins, Endocrine and reproductive dysfunction in man associated occupational inorganic lead intoxication, Arch. Environ. Health 39 (1984) 431-440.

〔45〕 H.W. Kuo, C.S. Wang, J.S. Lai, Semen quality in workers with long-term lead exposure: a preliminary study in Taiwan, Sci. Total Environ. 204 (1997) 289-292.