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The evaluation of micronucleus frequency by acridine orange fluorescent staining in peripheral blood of rats treated with lead acetate

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The data concerning the mutagenic, clastogenic and carcinogenic properties of inorganic lead compounds have been conflicting. Here, we evaluated the frequency of micronuclei in the peripheral blood of female rats treated with three different lead acetate doses. Outbred female Wistar rats were treated by gavage once per week for 10 weeks with cumulative doses of 140, 250 and 500 mg/kg body weight (body wt) of lead acetate. Mitomycin C (MMC) 2 mg/kg body wt was used as a positive control. The aim of the present study was to investigate the possible cytotoxic and genotoxic effects of lead acetate on peripheral blood reticulocytes using the micronucleus test following chronic exposure. The results show the effects of lead acetate in peripheral blood reticulocytes. These effects are both cytotoxic and genotoxic because of a decrease in the number of polychromatic erythrocytes in the peripheral blood and an increase in frequency of micronucleated reticulocytes, respectively.

Introduction

Lead can be present in the air in the vicinity of factories, and, before leaded gasoline was banned, was present on the highways. Vegetables are polluted by lead from the air and a considerable amount of lead contamination is found in cereals and broad-leafed vegetables. Because of its persistence in the environment, exposure to lead has become a major public health concern (1–3). In eukaryotic cells, this metal is usually genotoxic through a mechanism that until now has not been well characterized and possibly involves indirect damage to DNA, either by affecting the stabilization of chromatin or by interacting with repair processes (4,5).

In recent years, a great deal of effort has focused on the development of techniques for monitoring the health of organisms chronically exposed to pollutants (6,7). As a class heavy metals can either be mutagenic by themselves or can enhance the mutagenic effect of other agents (8). Actual environmental exposures typically involve multiple agents indicating a need to evaluate the toxicity of combinations of substances. Lead is a heavy metal whose widespread distribution in the environment has the potential to affect a large number of people and, thus, its genotoxicological investigation is of importance.

The ideal method for detecting genotoxic damage in sentinel species should enable detection of many classes of damage in a variety of cell types from a range of organisms, provide data concerning the individual cell, and be sensitive and rapid. The most commonly used methods for evaluating levels of DNA damage involve scoring chromosomal aberrations, micronuclei and/or sister chromatid exchanges in proliferating cell populations. Evaluation of micronuclei provides a measure of both the clastogenic and the aneugenic potential of environmental agents. Recently, micronucleus (MN) assay has been widely used in monitoring genetic damage in different tissue and cell types (9).

The mouse MN assay is the most widely used and best validated *in vivo* test for genotoxicity (10,11). The *in vivo* MN assay has become increasingly accepted as the model of choice for evaluating the genotoxic potential of chemicals and/or radiation. The earliest applications of this model focused on the frequency of micronuclei in polychromatic erythrocytes (PCEs) of mouse bone marrow (12,13). Data were eventually developed showing that mouse peripheral blood is an acceptable cell population for detecting micronucleated PCEs (MNPCEs) as long as the sampling schedule accounted for the release of newly formed micronucleated erythrocytes from bone marrow to the blood (14,15).

Although the circulating blood of the mouse is a useful tissue for measuring both the acute and cumulative DNA damage produced by environmental agents (14,15), it would be advantageous to measure genotoxicity in other animal species. For instance, the rat is the most frequently used rodent species in repeat-dose toxicology studies (16–19). Also aquatic organisms (20), plants (21,22) and mammalian species living in close proximity to man (23) have been used as sentinel organisms in environmental studies.

The purpose of the present work was to determine the genotoxic effects of lead acetate in the peripheral blood of female laboratory rats using MN formation as the genetic endpoint. Additionally, the ratio of PCEs to normochromatic erythrocytes was calculated to evaluate the cytotoxic effects of lead acetate in peripheral blood reticulocytes.

Materials and methods

Chemicals

Lead acetate 3 hydrate ($Pb[C_2H_3O_2]_2{\cdot}3H_2O$, CAS number: 301-04-2) was obtained from Riedel de Haen (Seelze, Germany). Mitomycin C (MMC $C_{15}H_{18}N_4O_5$, >98%, CAS number 50-07-7) was obtained from Serva (Heidelberg, Germany). MMC was dissolved with sterile distilled water and used as positive control for the MN assay.

Animal treatment

Healthy adult female Swiss albino rats (Wistar rat) [6–8 weeks of age and average body weight (body wt) of 180–200 g] were used in this study. Rats were obtained from the Experimental Animal Center, University of Gaziantep, Turkey. The rats were randomly selected and housed in polycarbonate boxes (four rats per box) with steel wire tops and rice husk bedding. They were maintained with 12 h dark/light cycle in a controlled atmosphere of 22 ± 2°C temperature and 50–70% humidity with free access to pelleted feed and fresh tap water. The animals were allowed to acclimate for 14 days before treatment.

The rats were treated by gavage with 140, 250 and 500 mg/kg body wt lead acetate dissolved in saline once per week for 10 weeks (~70 days).

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The untreated control rats were treated identically with equal volumes of normal saline only via gavage throughout the study. Since positive controls may be administered by a different route and treatment schedule than the test agent (24) a single dose of MMC (2 mg/kg, i.p.) was administered at the 10th week dosing time.

Dose selection

The dose selection of lead acetate was based on human environmental exposures. The 140 mg/kg body wt dose was an approximate environmental daily-exposure level (25). This dose was used as the minimum dose. At the beginning of study, 1000 mg/kg body wt dose was selected as exposure level in industrial areas (26). However, animals did not tolerate this dose and so the dose was reduced to 500 mg/kg body wt. Thus, 500 mg/kg dose is determined as the maximum dose.

MN assay

Whole blood smears were collected on the day following the last lead acetate administration or 1 day after MMC treatment. Whole blood smears were prepared on clean microscope slides, air dried, fixed in methanol and stained with acridine orange (125 μg/ml in pH 6.8 phosphate buffer) for 1 min just before the evaluation with a fluoresence microscope using a 40× objective (27). The frequency of PCEs per total erythrocytes was determined using a sample size of 2000 erythrocytes per animal. The number of MNPCEs was determined using 2000 PCE per animal. Briefly, immature erythrocytes, i.e. PCEs were identified by their orange–red color, mature erythrocytes by their green color and micronuclei by their yellowish color.

Statistical analysis

Data were compared by one-way variance analysis. Statistical analysis was performed using SPSS for Windows 9.05 package program. Multiple comparisons were performed by least significant difference test. $P < 0.05$ was considered to be the level of significance.

Results

A representative fluorescence photomicrograph of MNPCE from a lead acetate-treated rat is shown in Figure 1.

The %PCE and MNPCEs in rats treated by gavage with three different doses of lead acetate are shown in Table I. Lead acetate induced a dose-related increase in MN frequency (Figure 2) with all the doses of lead acetate producing significant increases in the frequency of MNPCEs. The responses produced by the lowest dose (140 mg/kg) and medium dose (250 mg/kg), however, were not significantly different and there was no significant difference between the MNPCEs frequency induced by the 250 and 500 mg/kg doses. Although the MNPCE frequencies in rats treated with lead acetate were significantly higher than the frequency in control (0.87 ± 0.12), they were much less than the MNPCE frequency produced by the positive control, 2 mg/kg MMC (25.5 ± 0.35).

All three doses of lead acetate decreased the %PCEs when compared with the negative control (Table I and Figure 3) ($P < 0.001$). However, a significant difference was not observed between the responses produced by the 140 and 250 mg/kg body wt doses of lead acetate ($P = 0.078$).

Discussion

Industrial activity and automobile use have resulted in an increase in the burden of lead in the environment. Human ingestion of toxic metals from the environment is a matter of continuing concern. Lead finds its way into plants, animals and human beings through the consumption of water and food, and the breathing of air. Lead is a potential carcinogen and causes several toxic effects such as damage to the central nervous

Table I. The induction of micronucleated polychromatic erythrocytes (MNPCEs) and number of PCE in peripheral blood erythrocytes of rats exposed to lead acetate

Treatment groups	Number of examined animals	PCE/total erythrocytes (%) (means ± SE)	MNPCEs/ 1000 PCEs (means ± SE)
Isotonic saline (vehicle control)	4	2.075 ± 0.048	0.87 ± 0.125
Lead acetate			
140 mg/kg body wt	4	1.875 ± 0.025***	1.75 ± 0.25**
250 mg/kg body wt	4	1.762 ± 0.038***	1.91 ± 0.05**
500 mg/kg body wt	4	1.225 ± 0.052***	2.41 ± 0.16**
Positive control			
MMC (2 mg/kg body wt)	4	0.587 ± 0.043***	25.5 ± 0.35**

PCEs, polychromatic erythrocytes; MNPCEs, micronucleated polychromatic erythrocytes; MMC, mitomycin C.
**$P < 0.05$ compared with control group.
***$P < 0.001$.

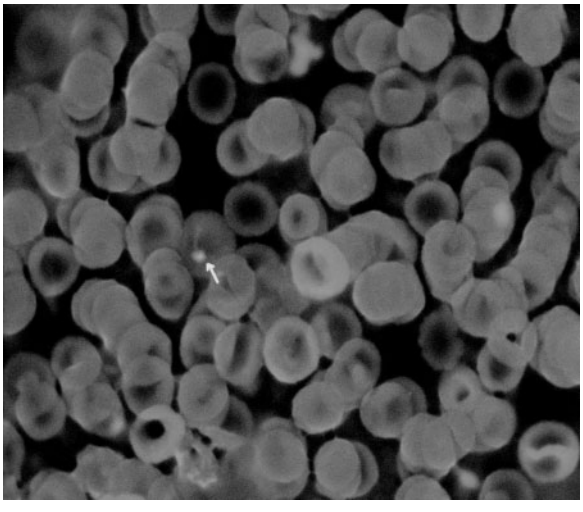

Fig. 1. Acridine orange stained peripheral blood erythrocytes of rat treated with lead acetate (250 mg/kg). Arrow indicates micronucleus in immature (polychromatic) erythrocyte.

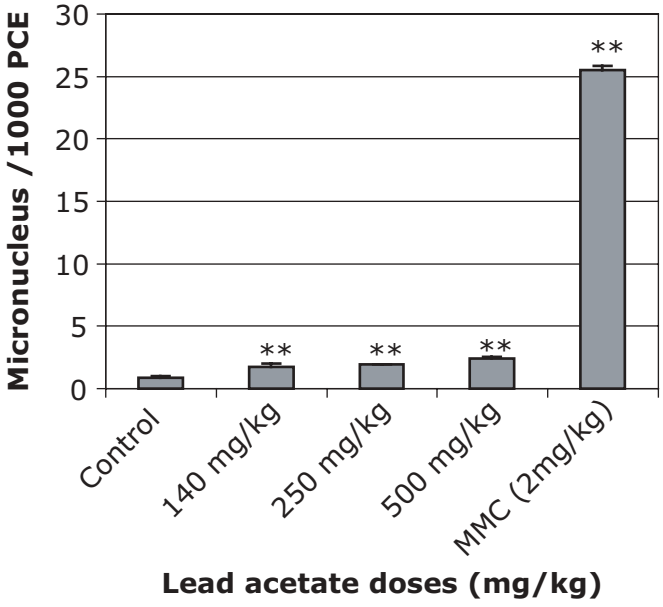

Fig. 2. Frequency of micronuclei in the peripheral blood of rat treated with lead acetate. MMC, Mitomycin C (positive control) (2 mg/kg); PCE, polychromatic erythrocytes. **$P < 0.05$.

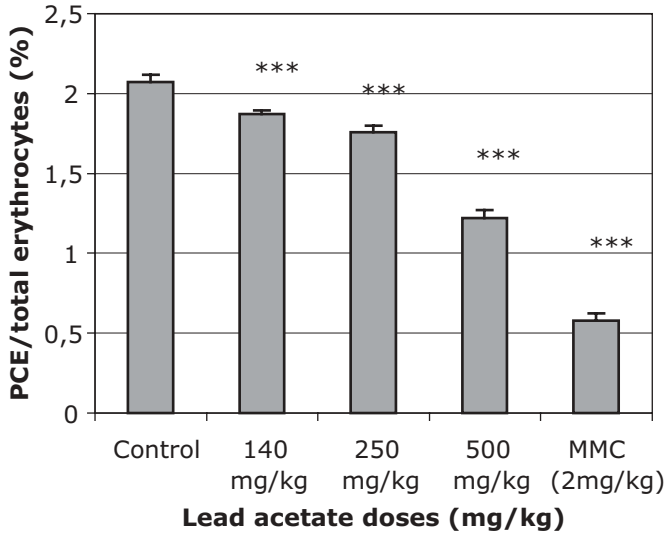

Fig. 3. Frequency of PCEs in the peripheral blood of rat treated with lead acetate. MMC, Mitomycin C (positive control) (2 mg/kg); PCE, polychromatic erythrocytes. ***$P < 0.001$.

system, kidneys and cardiovascular system. Once the lead reaches the body, it is retained in the hard tissue which can be metabolized during pregnancy and other pathological conditions (28,29).

The carcinogenicity of lead in humans has been suggested on the basis of epidemiological and experimental data (4,30,31). Inorganic lead and lead compounds have been classified by IARC (32) as possibly carcinogenic to humans (group 2B). Studies on the clastogenic effects of lead, however, are contradictory (33). Muro and Goyer (34) detected chromosome gaps and breaks by 1% lead acetate in cultured mouse leucocytes. An increase in the frequency of chromosomal aberrations was reported in human lymphocytes treated *in vitro* with lead acetate (35,36). Similarly, an increase in the frequency of sister chromatid exchange has been reported in human lymphocytes after lead sulphate treatment (37). In other studies, however, no significant increase was found in the frequency of chromosomal aberration and micronuclei in lead-exposed cultured peripheral blood lymphocytes (38). Bauchinger and Schmid (39) failed to observe any significant change in the chromosomal aberrations in CHO cells exposed to different concentrations of lead acetate. In our study we have observed that lead acetate significantly increased the MN frequency in peripheral blood of rats. Nehez *et al*. (40) reported that lead acetate administration induced the numerical chromosome aberrations and lead was attributed the effects of some other genotoxic agents, such as pesticides, on bone marrow of rats in subchronic exposure.

Most of the studies on lead-exposed humans failed to detect chromosomal damage, but when other metals like zinc or cadmium were also present the frequency of chromosomal aberrations increased considerably (41). Pelclova *et al*. (42) could not detect a significant increase in structural aberrations (chromosome and chromatid exchanges, and breaks) in 22 workers from a lead battery plant. In contrast, Vaglenov *et al*. (43) found a significant increase in MN frequency among the lead-exposed staff (millers and assemblers) of a battery plant. Yang *et al*. (44) investigated the effects of lead acetate and found that lead may induce DNA damage through a fenton reaction by interacting proteins.

Occupational exposure to high levels of lead is known to pose health risks including effects on the blood (anaemia), gastrointestinal tract and nervous system (encephalopathy and a reduced peripheral nerve function), nephropathy, hypertension and other cardiovascular effects, and a decreased sperm count. An increased risk of miscarriage and stillbirth has also been attributed to lead (45,46).

Taking into account the inconclusive results obtained in most of the different experiments carried out, lead genotoxicity has been the subject of extensive discussions. Although the genotoxicity and carcinogenity of lead are unclear, the toxicity of lead has been extensively studied on different biological systems and functions (47,48) and general patterns have emerged. Thus, while positive results were obtained for the induction of lambda prophage in *Escherichia coli* (49), lead is generally non-mutagenic in prokaryotic assays (50,51). Also, while inconsistent responses have been reported on the ability of lead to induce chromosome damage, lead often produces positive genotoxic responses in eukaryotic cells (4,52). For instance, Zelikof *et al*. (4) reported lead induced mutation in the *Hprt* gene of Chinese hamster cells V79. The inconsistent results on the genotoxicity and carcinogenicity of lead may be the result of a number of factors such as cell type, and duration and route of exposure, and the responses may be influenced by synergistic factors.

The mechanisms for these genotoxic responses may involve indirect damage to DNA affecting the stabilization of chromatin (53) or interacting with repair processes (5). Lead is believed to covalently interact with tertiary phosphate ions in nucleic acids and proteins (54–56). Lead is reported to affect the fidelity of DNA synthesis *in vitro* (57). In a study performed by Jagetia and Aruna (58), it was reported that lead nitrate treatment increased erythropoiesis in mouse bone marrow as evidenced by an increase in the %PCEs. Similarly, in another study, lead acetate administration has been reported to increase the cell proliferation in rat kidney by 40-fold (59). The increase in cell population in some organs/tissues of rats indicates that lead led the tumor formation in some tissues, particularly kidney tissue. In contrast, Sata *et al*. (60) reported a decrease in cells of the immune system in workers exposed to high lead levels. This result is consistent with our findings of reduced %PCEs in rats treated with lead acetate and with previous reports indicating that lead compounds are cytotoxic.

Genotoxicity can be modulated by different factors. In particular, it is accepted that many dietary constituents markedly influence or alter the adverse effects of genotoxic agents (61,62). In addition, it has recently been reported that diets rich in antioxidant nutrients may reduce the risk of certain types of human cancer (63) and that multivitamin dietary supplements can significantly decrease the frequency of chromosome aberrations in workers exposed to chemicals (64–66).

In conclusion, although the studies regarding the toxicity and clastogenic effects of lead are contradictory, our results clearly demonstrated that lead acetate administration significantly increased the frequency of MNPCEs and decreased the %PCEs in peripheral blood of rats. An advantage of animal studies is that they provide a complete biological system which can evaluate the overall effect of subtle changes observed in cell systems. Carefully controlled animal studies are an essential step in the extrapolation of biological effects to human health safety. The fundamental similarities in cell structure and biochemistry between animals and humans provide a general valid basis for prediction of likely effects of chemicals on human

populations. These data allow an estimate of the genetic risk of lead exposure by using biomarkers such as MN.

References

1. Kertész,M. (1998) Air quality monitoring data April–September 1997 and October 1997–March 1998. *Egészségtudomány*, **42**, 206–287 (in Hungarian).
2. Vasköví,B., Gyöngyös,E., Várkonyi,T. and Kertész,M. (1998) Study of air pollution in Tatabánya on the basis of development conception for national air quality monitoring network. *Egészségtudomány*, **42**, 102–115 (in Hungarian).
3. Vaglenov,A., Creus,A., Laltchev,S., Petkova,V., Pavlova,S. and Marcos,R. (2001) Occupational exposure to lead and induction of genetic damage. *Environ. Health Perspect.*, **109**, 205–298.
4. Zelikof,J.T., Li,J., Hartvig,A., Wang,X., Costa,M. and Rossman,T. (1988) Genetic toxicology of lead compounds. *Carcinogenesis*, **9**, 1727–1732.
5. Hartwig,A., Schlepegrell,R. and Beyersmann,D. (1990) Indirect mechanism of lead induced genotoxicity in cultured mammalian cells. *Mutat. Res.*, **241**, 75–82.
6. Silva,J., Freitas,T.R.O., Heuser,V., Marinho,J.R. and Erdtmann,B. (2000) Genotox biomonitoring in coal regions using wild rodent *Ctenomys torquatus* by comet assay and micronucleus test. *Environ. Mol. Mutagen.*, **35**, 270–278.
7. Silva,J., Freitas,T.R.O., Marinho,J.R., Speit,G. and Erdtmann,G.B. (2000) Alkaline single-cell gel electrophoresis (Comet) assay for environmental *in vivo* biomonitoring with native rodents. *Genet. Mol. Biol.*, **23**, 241–245.
8. Minissi,S. and Lombi,E. (1997) Heavy metal content and mutagenic activity, evaluated by *Vicia faba* micronucleus test, of Tiber river sediments. *Mutat. Res.*, **393**, 17–21.
9. Çelik,A., Mazmanci,B., Çamlıca,Y., Aşkın,A. and Çömelekoğlu,Ü. (2005) Induction of micronuclei by lambda-cyhalothrin in Wistar rat bone marrow and gut epithelial cells. *Mutagenesis*, **20**, 125–129.
10. Mavournin,K.H., Blakey,D.H., Cimino,M.C., Salamone,M.F. and Heddle,J.A. (1990) The *in vivo* micronucleus assay in mammalian bone marrow and peripheral blood. A report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res.*, **239**, 29–80.
11. Morita,T., Asano,N., Awogi,T. *et al.* (1997) Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens Group 1, 2A and 2B. The summary report of the 6th collaborative study by CSGMT/JEMS MMS. *Mutat. Res.*, **389**, 3–122.
12. Heddle,J.A. (1973) A rapid *in vivo* test for chromosomal damage. *Mutat. Res.*, **18**, 187–190.
13. Schmidt,W. (1976) The micronucleus test for cytogenetic analysis. In Hollaender,A. (ed.), *Chemical Mutagens*, Vol. 4. Plenum Press, New York, pp. 31–53.
14. MacGregor,J.T., Wehr,C.M. and Gould,D.H. (1980) Clastogen-induced micronuclei in peripheral blood erythrocytes: the basis of an improved micronucleus test. *Environ. Mol. Mutagen.*, **2**, 509–514.
15. Schlegel,R. and MacGregor,J.T. (1982) The persistence of micronuclei in peripheral blood erythrocytes; detection of chronic chromosome breakage in mice. *Mutat. Res.*, **104**, 367–369.
16. Albanase,R. and Middleton,B.J. (1987) The assessment of micronucleated polychromatic erythrocytes in rat bone marrow; technical and statistical consideration. *Mutat. Res.*, **182**, 323–332.
17. Garriot,M.L., Brumy,J.D., Kindig,D.E., Patron,J.W. and Schwier,L.S. (1995) The *in vivo* rat micronucleus test; integration with a 14-day study. *Mutat. Res.*, **342**, 71–76.
18. MacGregor,J.T., Tucker,J.D., Easmond,D.A. and Wyrobek,A.J. (1995) Integration of cytogenetic assays with toxicology studies. *Environ. Mol. Mutagen.*, **25**, 328–337.
19. Shi,X.-C., Krisma,G. and Ong,T.M. (1992) Induction of micronuclei in rat bone marrow by four model compounds. *Teratog. Carcinog. Mutagen.*, **11**, 251–258.
20. Petras,M., Vrzoc,M., Pandrangi,R., Ralph,S. and Perry,K. (1995) Biological monitoring of environmental genotoxicity in Southwesterrn Ontario. In Butterworth,F.M., Corkum,L.D. and Guzmán-Rincón,J. (eds). *Biomonitors and Biomarkers as Indicators of Environmental Change*. Plenum Press, New York, pp. 115–137.
21. Ma,T.H. (1995) Trandescantia (Spiderwort) plants as biomonitors of the genotoxicity of environmental pollutants. In Sandhu,S and Waters,M.D. (eds), *Application of Short-Term Bioassays in the Analysis of Complex Environmental Mixtures*. Plenum Publishing Co., New York, pp. 207–216.
22. Gomez-Arroyo,S. and Villalobos-Pietrini,R. (1995) Chromosomal aberration and sister chromatid exchanges in *Vicia faba* as genetic monitor of environmental pollutants. In Butterworth,F.M., Corkum,L.D. and Guzmán-Rincón,J. (Eds), *Biomonitors and Biomarkers as Indicators of Environmental Change*. Plenum Press, New York, NY, pp. 95–113.
23. Tice,R.R. (1995) Applications of the single cell gel assay to environmental biomonitoring for genotoxic pollutants. In Butterworth,B.E., Corkum,L.D. and Guzmán-Rincón,J. (eds), *Biomonitors and Biomarkers as Indicators of Environmental Change*. Plenum Press, New York, pp. 69–79.
24. Hayashi,M., Tice,R.R., Macgregor,J.T. *et al.* (1994) *In vivo* rodent erythrocyte micronucleus assay. *Mutat. Res.*, **312**, 293–304.
25. Escribano,A., Revilla,M., Hernández,E.R., Seco,C., González-Riola,J., Villa,L.F. and Rico,H. (1997) Effect of lead on bone development and bone mass: a morphometric, densitometric, and histomorphometric study in growing rats. *Calcif. Tissue Int.*, **60**, 200–203.
26. Gruber,H.E., Gonick,H.C., Khalil-Manesh,F., Sanchez,T.V., Motsinger,S., Meyer,M. and Sharp,C.F. (1997) Osteopenia induced by long-term, low- and high-level exposure of the adult rat to lead. *Miner. Electrolyte Metab.*, **23**, 65–73.
27. Holden,H.E., Majeska,J.B. and Studwell,D. (1997) A direct comparison of mouse and rat marrow and blood as target tissues in the micronucleus assay. *Mutat. Res.*, **391**, 87–89.
28. Silbergeld,E.K., Schwartz,J. and Mahaffey,K.R. (1988) Lead and osteoporosis mobilization of lead from bone in post menopausal women. *Environ. Res.*, **47**, 79–94.
29. Silbergeld,E.K. (1991) Lead in bone: implications for toxicology during pregnancy and lactation. *Environ. Health Perspect.*, **91**, 63–70.
30. Cooper,W., Wong,O. and Kheifets,L. (1985) Mortality among employees of lead battrery plants and lead producing plants, 1947–1980. *Scand. J. Work. Environ. Health*, **11**, 331–450.
31. Stoner,G., Shimkin,M., Troxell,M., Thompson,T. and Terry,L. (1976) Test for carcinogenicity of metallic compounds by the pulmonary tumor response in strain A mice. *Cancer Res.*, **36**, 1744–1747.
32. IARC (1987) *Monograph on the evaluation of carcinogenic risks to Humans. An updating of IARC Monographs 1 to 42, Supplement 7*. IARC, Lyon, France, pp. 230–231.
33. IPCS (1995) *Environmental Health Criteria 165. Inorganic Lead.* WHO, Geneva, pp. 208–210.
34. Muro,L.A. and Goyer,R.A. (1969) Chromosome damage in experimental lead poisoning. *Arch. Pathol.*, **87**, 660–663.
35. Beek,B. and Obe,G. (1974) Effect of lead acetate on human leukocyte chromosomes *in vitro*. *Experientia*, **30**, 1006–1007.
36. Obe,G., Beek,B. and Dudin,G. (1975) Some experiments on the action of lead acetate on human leucocytes *in vitro*. *Mutat. Res.*, **29**, 283.
37. Wulf,H.C. (1980) Sister chromatid exchanges in human lymphocytes exposed to nickel and lead. *Dan. Med. Bull.*, **27**, 40–42.
38. Hoffmann,M., Hagberg,S., Karlsson,A., Nilsson,R., Ranstam,J. and Hogstedt,B. (1984) Inorganic lead exposure does not effect lymphocyte micronuclei in car radiator repair workers. *Hereditas*, **101**, 223–226.
39. Bauchinger,M. and Schmid,E. (1972) Chromosome analysis of cultures of Chinese hamster cells after treatment with lead acetate. *Mutat. Res.*, **14**, 95–100.
40. Nehez,M., Lorencz,R. and Desi I. (2000) Simultaneous action of cypermethrin and two environmental pollutant metals, cadmium and lead, on bone marrow cell chromosomes of rats in subchronic administration. *Ecotoxicol. Environ. Saf.*, **45**, 55–60.
41. IPCS (1977) *Environmental Health Criteria 3. Lead.* WHO, Geneva, pp. 94–123.
42. Pelclova,D., Picková,J. and Patzelova,V. (1997) Chromosomal aberration, hormone levels and oxidative phenotype (P450 2D6) in low occupational lead exposure. *Central Eur. J. Environ. Med.*, **3**, 314–322.
43. Vaglenov,A.K., Laltchev,S.G., Nosko,M.S., Pavlova,S.P., Petkova,V.V. and Karadjov,A.D. (1997) Cytogenetic monitoring of workers exposed to lead. *Central Eur. J. Occup. Environ. Med.*, **3**, 298–308.
44. Yang,J.L., Wang,L.C., Chang,C.Y. and Liu,T.Y. (1999) Singlet oxygen is the major species participating in the induction of DNA strand breakage and 8-hydroxydeoxyguanosine adduct by lead acetate. *Environ. Mol. Mutagen.*, **33**, 194–201.
45. World Health Organization (2000) *Air Quality Guidelines for Europe*, 2nd edn. European Series No 91, WHO, Geneva.
46. Wixson,B.G. and Davies,B.E. (1994) Lead in soil guidelines. Environmental Geochemistry and Health **14** Suppl. 145–168 (Trace Substances in Environmental health – XXV).
47. Gerber,G.B., Leonard,A. and Jacquet,P. (1980) Toxicity, mutagenicity and teratogenicity of lead. *Mutat. Res.*, **76**, 115–141.

48. Goyer,R.A. (1993) Lead toxicity: current concerns. *Environ. Health. Perspect*., **100**, 177–187.
49. Rossman,T.G., Molina,M. and Mayer,L.W. (1984) The genetic toxicology of metal compounds: I. Induction of lambda prophage in *E.coli* Wp22 l. *Environ. Mutagen*., **6**, 59–69.
50. Dunkel,V.C., Zeiger,E., Brusick,D., McCoy,E., McGregor,D., Mortelmans,K., Rosenkrantz,H.S. and Simmon,V.F. (1984) Reproducibility of microbial mutagenicity assays: I. tests with *Salmonella typhimurium* and *Escherichia coli* using a standardized protocol. *Environ. Mutagen*., **6** (Supp1 2), 1–254.
51. Kanematsu,N., Hara,M. and Kada,T. (1980) Rec assay and mutagenicity studies on metal compounds. *Mutat. Res*., **77**, 109–116.
52. Winder,C. and Bonin,T. (1993) The genotoxicity of lead. *Mutat. Res*., **285**, 117–124.
53. Johansson,L. and Pellicciari,C.E. (1988) Lead induced changes in the stabilization of the mouse sperm chromatin. *Toxicology*, **51**, 11–24.
54. Bremer I. (1974) Heavy metal toxicities. *Rev. Biophys*., **7**, 75–124.
55. Goyer,R.A., May,P., Cates,M.M. and Krigman,M.R. (1970) Lead and protein content of isolated intranuclear inclusion bodies from kidneys of lead-poisoned rats. *Lab. Invest*., **22**, 245–251.
56. Holtzman,D., Devries,C., Nguyen,H., Oslon,J. and Bensch,K. (1984) Maturation of resistance to lead encephalopathy; cellular and sub-cellular mechanisms. *Neurotoxicology*, **5**, 97–124.
57. Sirover,M.A. and Loeb,L.A. (1976) Metal activation of DNA synthesis. *Biochem. Biophys. Res. Commun*., **70**, 812–817.
58. Jagetia,G.C. and Aruna,R. (1998) Effect of various concentrations of lead nitrate on the induction of micronuclei in mouse bone marrow. *Mutat. Res*., **415**, 131–137.
59. Choie,D.D. and Richter,G.W. (1972) Cell proliferation in rat kidney induced by lead acetate and effects of uninephrectomy on cell proliferationn. *Am. J. Pathol*., **66**, 265–276.
60. Sata,F., Araki,S., Tanigawa,T., Morita,Y., Sakurai,S. and Katsuno,N. (1997) Changes in natural killer cell subpopulations in lead workers. *Int. Arch. Occup. Environ. Health*, **69**, 306–310.
61. Hayatsu,H., Arimoto,S. and Negeshi,T. (1988) Dietary inhibitors of mutagenesis and carcinogenesis. *Mutat. Res*., **202**, 429–446.
62. Fenech,M. and Rinaldy,J. (1994) The relationship between micronuclei in human lymphocytes and plasma of vitamin C, vitamin E, vitamin B and folic acid. *Carcinogenesis*, **15**, 1405–1411.
63. Byers,T. and Perry,G. (1992) Dietary carotenes, vitamin C, and vitamin E as protective antioxidants in human cancers. *Annu. Rev. Nutr*., **12**, 139–159.
64. Lazutka,J.R. (1996) Chromosome aberrations and rogue cells in lymphocytes of Chernobyl clean-up workers. *Mutat. Res*., **350**, 315–329.
65. Emerit,I., Oganesian,N., Arutyunian,R., Pogossian,A., Sarkisian,T., Cernjavski,L., Levy,A. and Feingold,J. (1997) Oxidative stress-related clastogenic factors in plasma from Chernobyl liquidators: protective effects of antioxidant plant phenols, vitamins and oligoelements. *Mutat. Res*., **377**, 239–246.
66. Oganesian,N., Emerit,I., Pogosian,A., Abramiam,A., Arutyunian,R., Agrian,K., Sarkisian,T. and Esyan,A. (1997) Antioxidant treatment of radiation injuries. *Antioxid. Radiat. Injuries*, **3**, 125–131.

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