Potential Mutagenic Effect of Lead Acetate in Mouse Bone Marrow and Culture Mouse Spleen Cells

Fawzia A. E. Aly

Department of Genetics and Cytology, National Research Centre, Dokki, Cairo, Egypt

Received October 19, 2001; accepted October 31, 2001

Summary The induction of chromosome aberrations and sister chromatid exchanges (SCEs) by lead acetate was investigated in mouse bone-marrow cells *in vivo* and in culture mouse spleen cells. In the *in vivo* study, mice were treated orally by gavage with 5 dose levels of lead acetate 25, 50, 75, 100 and 150 mg/kg body wt. A repeated daily dose of 10 mg/kg body wt. was given for 15 successive days (Cumulation dose 150 mg/kg). A statistically significant difference in the percentage of chromosome aberrations was determined with the high and repeated doses. Lead acetate induced a significant and dose-dependent increase in SCEs frequency in mouse bone-marrow. The *in vitro* study, the concentrations 0.5, 1, 5, 10, 15 μ g/ml were tested for the induction of chromosome aberrations and SCEs. Lead acetate at 5, 10, 15 μ g/ml caused a high percentage of metaphases with chromosome aberrations after treatment of the cell cultures for 24 h. The frequency of SCEs/cell increased with increasing concentration of lead acetate. It reached $12.44 \pm 0.45/\text{cell}$ ($p < 0.01$) after treatment with highest dose (15 μ g/ml) compared with 5.37 ± 0.57 in the control. In conclusion, in both used models the study demonstrated that lead acetate produced genotoxic effect.

Key words Lead acetate, Genotoxicity, Mouse bone-marrow, Mouse spleen cultures.

Lead is one of the most commonly encountered and utilized metal. It is widespread industrial usage and continual release into the environment as an exhaust emission, exposure to this compound has become of major public health concern. The toxic effect of the lead compound have been well reported (Gerber *et al.* 1980, Goyer 1993), although inconsistent results have been published about their mutagenic, clastogenic and carcinogenic properties. Lead and inorganic lead compounds have been classified by IARC (1987) as possible human carcinogens on the basis of sufficient evidence for carcinogenicity in experimental animals, but inadequate evidence for carcinogenicity in human. Lead acetate, lead subacetate and lead oxide enhanced the number of tumors induced by some organic compounds, including 2-(ethylnitrosamino) ethanol (Shirai *et al.* 1984) and N-(4-fluoro-4-biphenyl) acetamide (Tanner and Lipsky 1984).

Many studies have analyzed the genotoxicity of lead compounds on human and animal cells. Culture lymphocyte cells from exposed naive cells cultured with lead salts demonstrated increased levels of chromosome aberrations and sister chromatid exchanges. (Bauchinger and Schmid 1972, Beek and Obe 1974, Deknadt *et al.* 1977, Grandjean *et al.* 1983, Montaldi *et al.* 1987). However, the results remain contradictory, which the response appears to depend on a number of factors such as a cell type, duration and route of exposure and can also be influenced by synergistic factors (Vaglenov *et al.* 1998). There are also many other studies which did not demonstrate a clastogenicity of lead compounds in human lymphocytes (Schmid *et al.* 1972, Deknadt and Deminatti 1978, Gasiorek and Bauchinger 1981, Beckman and Nordenson 1986).

The present work show the capacity of lead acetate to increase the number of chromosome aberrations and SCEs in mouse bone marrow cells studied *in vivo* as well as in cultured mouse spleen cells.

Materials and methods

In vivo study

Animals and chemical: Male Swiss mice aged 8–10 weeks and weighted 25–30 g obtained from a close random bred colony at National Research Centre were used. Pure lead acetate was purchased from Merck Chemical Company.

Doses and treatments: Lead acetate was administered by gavage at 5 different dose levels 25, 50, 75, 100 and 150 mg/kg body wt. These doses were used for both chromosome aberrations and SCEs. Mice were orally treated by gavage with one dose of 25, 50, 75, 100 and 150 mg “lead acetate”/kg body wt. and the mice killed after 24 h and also 18 and 48 h after treatment with 150 mg/kg body wt. For repeated dose treatment, mice were orally treated with 15 successive daily doses of lead acetate at the dose level 10 mg/kg body wt. and the mice killed 24 h after the last dose.

A non treated group of animals were used as control, another group was intraperitoneal injected with 1 mg “mitomycin C” and used as positive control.

Slide preparations: For studying chromosome aberrations, both lead acetate treated and control were injected with 10 mg/kg body wt. colchicine 2–3 h before killing. Bone-marrow metaphases were prepared following the method of Yosida and Amano (1965) and stained with phosphate buffer Giemsa.

The method of SCEs described by McFee *et al.* (1983). Bone-marrow cells were fixed and stained with fluorescence plus Giemsa method of Perry and Wolff (1974).

Microscopic observation and statistics: Five animals were taken for each treatment and control. Only well-spread metaphases with 40 chromosomes were analyzed. One hundred metaphases per animal were analyzed in order to determine the frequencies of chromosome aberrations. To determine the frequency of SCEs, 30 well-spread metaphases per animal were scored.

The significance of the incidence of abnormalities in experimental versus control data was calculated using the *t*-test.

In vitro study

Cell culture: Male Swiss mice 6–8 weeks old were used. The spleen was washed with RPMI 1640 medium, cut in small pieces, and the cells were centrifuged at 1000 rpm for 10 min. Spleen cells at a concentration of 2×10^5 were cultured in plastic tissue culture dishes (Falcon) containing RPMI 1640 medium plus 10% foetal calf serum, antibiotics (100 units/ml) of penicillin and 100 µg/ml of streptomycin and 2 µg/ml concanavalin A, and were maintained in 5% CO₂ incubator at 37°C.

Chromosome aberrations: Spleen cells were exposed to 5 different concentrations of lead acetate for 24 h the full culture period. The concentrations used were 0.5, 1, 5, 10 and 15 µg/ml. Colchicine was added to the cell culture 2 h prior to harvesting. The cell pellet was suspended in 0.075 M KCl at 37°C for 20 min. The cells were fixed in methanol-acetic acid (3-1), dropped onto dry slides, air-dried and stained with Giemsa in phosphate buffer (pH 6.8). Three separate experiments were conducted using each lead acetate concentration. At least 100 metaphases were analyzed for chromosome aberrations/experiment.

Sister chromatid exchanges (SCEs): The doses used were the same as those previously mentioned. Bromodeoxyuridine (BrdU, Sigma) was added at the time of culturing the cells at a concentration 10 µg/ml medium. The cell cultures were treated with lead acetate solution for 24 h and colchicine was added 2 h before harvesting. The total length of culture time was 24 h. Hypotonic treatment, fixation of the cells and chromosome preparation were done with the standard air drying technique previously described. The differential staining of sister chromatids was performed as indicated for the *in vivo* study.

A separate experiment was conducted using distilled water (negative control) and mitomycin C

at final concentration $0.15 \mu\text{M}$ (positive control). Three separate experiments were conducted using each dose. At least 30 metaphases per culture were analyzed for SCEs/cell (90 cells/dose).

The significance of differences between the experimental and control data was calculated using *t*-test.

Results

In vivo study

Table 1 presents a detailed study of the effect of lead acetate at different doses on the induction of chromosome aberrations in mouse bone-marrow. The percentage of the induced aberrations increased by increasing the dose of lead acetate. It reached 3.6 ± 0.4 and 10.0 ± 0.6 ($p < 0.01$) 24 h after oral treatment by gavage with 25 and 150 mg/kg body wt. respectively (Table 1). The mean percentage of metaphases with chromosome aberrations was found to be statistically significant after single treatment with the high doses (75, 100, 150 mg/kg body wt.) and repeated dose (10 mg/kg body wt. for 15 consecutive days).

Lead acetate induced both structure and numerical chromosome aberrations which represented by gaps, fragments and/or break and robertsonian translocations. Some metaphases with more than one type of aberrations were appeared. (Fig. 1a–c).

Lead acetate induced a significant and dose dependent increase in SCEs frequency in mouse bone-marrow cells. The frequency of SCEs/cell was found to be 9.62 ± 0.31 ($p < 0.01$) after oral treatment by gavage with the highest dose (150 mg/kg body wt.) compared with 4.33 ± 0.20 and 12.02 ± 0.52 for non-treated and mitomycin C respectively (Table 2).

In vitro study

Increasing the concentration of lead acetate from 0.5 to $15 \mu\text{g/ml}$ gave an increase in the percentage of chromosome aberrations in cell culture. This percentage reached 3.75 ± 0.25 and 11.75 ± 0.69 ($p < 0.01$) after treatment the cell culture with 0.5 and $15 \mu\text{g}$ lead acetate/ml.

Table 3 represents the percentage of the different types of chromosome aberrations. A high

Table 1. Chromosome aberrations in mouse bone-marrow after oral treatment by gavage with different doses of lead acetate

Doses (mg/kg) and treatment	Mice killed after (h)	Abnormal metaphases No.	Metaphases with aberrations (%)					Mean % of ^b aberrant cells $\pm$ S.E.
			Gaps	Frag. and/or break	R.T	Others ^a	Polyploidy	
Mitomycin C	24	151	6.0	14.8	2.4	4.6	2.4	24.2 ± 0.7
Control "Non-treated"	24	22	2.2	2.2	—	—	—	2.2 ± 0.4
Lead acetate Single treatment								
25	24	29	2.2	3.2	—	—	0.4	3.6 ± 0.4
50	24	31	1.6	4.0	0.2	—	0.4	4.6 ± 0.8
75	24	41	1.2	5.4	1.0	—	0.6	$7.0 \pm 0.5^{**}$
100	24	48	1.4	5.2	1.6	0.4	1.0	$8.2 \pm 0.4^{**}$
150	18	57	2.2	5.2	1.2	1.6	1.2	$9.2 \pm 0.4^{**}$
	24	62	2.4	6.0	1.6	1.0	1.4	$10.0 \pm 0.6^{**}$
	48	49	3.0	4.2	1.4	—	1.2	$6.8 \pm 0.6^{**}$
Repeated treatment (10 mg/kg body wt. for 15 consecutive days)	24	54	2.4	5.6	1.0	0.8	1.0	$8.4 \pm 0.6^{**}$

^a Metaphases with gaps + deletion or gaps + break. ^b Mean % excluding gaps. ^{**} Significant at 0.01 level (*t*-test).

Table 2. Frequency of sister chromatid exchanges (SCEs) in mouse bone-marrow cells after oral treatment by gavage with different doses of lead acetate

Doses (mg/kg) and treatment	Total metaphase No.	SCEs No.	SCEs/cell $\pm$ S.D. Mean
Mitomycin C	150	1803	12.02 $\pm$ 0.52
Control (Non-treated)	150	650	4.33 $\pm$ 0.20
Lead acetate			
Single treatment			
25	150	1009	6.73 $\pm$ 0.34**
50	150	1059	7.06 $\pm$ 0.28**
75	150	1156	7.71 $\pm$ 0.13**
100	150	1262	8.41 $\pm$ 0.15**
150	150	1443	9.62 $\pm$ 0.31**
Repeated treatment (10 mg/kg body wt. for 15 consecutive days)	150	1343	8.95 $\pm$ 0.59**

** Significant at 0.01 level (*t*-test).

Table 3. Chromosome aberrations in cultured mouse spleen cells treated with different concentrations of lead acetate

Doses (μ g/ml) and treatment	Examined cells No.	Abnormal metaphases No.	Metaphases with aberrations (%)					Mean % of ^b aberrant cells $\pm$ S.E.
			Gaps	Frag. and/or break	R.T	Others ^a	Polyploidy	
Mitomycin C 0.15 μ M/ml	300	102	6.33	15.33	3.33	5.66	3.33	27.67 $\pm$ 0.66
Control "Non-treated"	300	15	2.33	2.33	—	—	0.33	2.66 $\pm$ 0.57
Lead acetate								
0.5	400	23	2.00	2.25	0.75	—	0.75	3.75 $\pm$ 0.25
1	500	34	2.60	3.60	0.20	0.40	—	4.20 $\pm$ 0.49
5	400	37	3.00	4.50	0.75	1.00	—	6.25 $\pm$ 0.41**
10	300	38	3.33	5.33	1.00	1.66	1.33	9.33 $\pm$ 0.86**
15	400	61	3.50	7.75	1.25	1.75	1.00	11.75 $\pm$ 0.69**

Frag= Fragments, R.T=Robertsonian translocation. ^aMetaphases with gaps+deletion or gaps+break. ^bMean % excluding gaps. ** Significant at 0.01 level (*t*-test).

Table 4. Frequency of sister chromatid exchanges (SCEs) in cultured mouse spleen cells treated with different concentrations of lead acetate

Doses (μ g/ml)	Total metaphase No.	SCEs No.	SCEs/cell $\pm$ S.E. Mean
Mitomycin C (0.15 μ M/ml)	120	1785	14.78 $\pm$ 0.86
Control (Non-treated)	120	645	5.37 $\pm$ 0.57
Lead acetate			
0.5	90	625	6.94 $\pm$ 0.25**
1	90	696	7.73 $\pm$ 0.41**
5	90	845	9.39 $\pm$ 0.37**
10	90	906	10.06 $\pm$ 0.31**
15	90	1120	12.44 $\pm$ 0.45**

** Significant at 0.01 level (*t*-test).

Fig. 1. a) Metaphases with Robertsonian translocation, b) chromatid break, c) chromatid fragment from mouse bone marrow cells after oral treatment by gavage with lead acetate. d-f) Metaphases from cultured mouse spleen cells showing SCE's induced after treatment with different concentrations of lead acetate.

percentage of fragment and/or breaks were observed. Numerical aberrations in the form tetraploid were appeared in a low percentage.

The induced increase in SCEs frequency after treatment of mouse spleen cell cultures with the test concentrations of lead acetate was dose-dependent (Table 4, Fig. 1d-f).

Discussion

The *in vivo* chromosome aberrations assay is one of the important bioassays for monitoring the genotoxicity of environmental chemical (Ad Hoc Committee 1972, Dulout *et al.* 1983) and it may be more useful in predicting possible health risks. For this reasons, the *in vivo* test used to study the genotoxic effect of the heavy metal "lead acetate".

The present study indicates that lead acetate caused a significant increase in chromosome aberrations and SCEs in mouse bone-marrow after oral treatment by gavage with different doses and in primary culture of mouse spleen cells. The frequency of abnormal metaphases was nearly 18, 24 h after treatment with the highest dose of lead acetate (150 mg/kg body wt.). It has been argued that a single fixation time of 18 h can be used to identify clastogenicity in bone-marrow cells (Preston *et al.* 1987). Repeated treatment with 10 mg/kg body wt. for 15 consecutive days (cumulation dose 150 mg/kg body wt.) induced a statistically significant in percentage of chromosome aberrations. This may be due to the accumulation effect of lead acetate.

The inability of lead ions to induce significant number of chromosome aberrations and SCEs have been reported earlier (Beek and Obe 1975, Willems *et al.* 1982, Hartwing *et al.* 1990). On the other hand, positive induction has been reported in sperm morphology (Eyden *et al.* 1978, Wyrobek and Bruce 1978), micronucleus test in mouse (Watanabe *et al.* 1985), *hprt* locus in Chinese hamster cell V79 (Zelikoff *et al.* 1988) at the *gpt* locus of G12 cells (Roy and Rossman 1992) and in rat bone-marrow (Abul-Nasr and Mahrous 2001).

The high variability of results has conducted different authors to consider the way that lead exposure can induce genotoxic effects. Thus, lead compounds do not appear to damage DNA directly, but apparently can take part in a Fenton reaction to generate hydroxyl radicals in the presence of H_2O_2 , because lead compounds in the presence of H_2O_2 can cause DNA strand breaks (Roy and

Rossman 1992) furthermore, lead ions were shown to decrease the fidelity of DNA synthesis (Sirover and Loeb 1979) and to inhibit the DNA polymerase β , one of the enzymes involved in DNA repair (Popeno and Schmaeler 1979).

The single break which includes fragments and chromatid deletions represented the most common type of aberrations *in vivo* and *in vitro* tests. A number of studies show a positive correlation between exposure to lead and chromosome damage *e.g.* chromatid breaks (Schwantiz *et al.* 1970), chromosome gaps and pulverisation of chromosomes (Beek and Obe 1974, Obe *et al.* 1975, Nordenson *et al.* 1978).

The induced increase in SCEs frequency after treatment the spleen cell cultures with the different test concentrations of lead acetate was significant and dose-dependent and was lower than that produced by the positive control (mitomycin C). The frequency of SCEs/cell reached 12.44 ± 0.45 after treatment with the highest test concentration. Such value was 2.5 fold that of the control. The higher effect of lead acetate in primary culture of mouse spleen cells may lie in the fact that the lead acetate forms an insoluble precipitate which may be phagocytized by the cell. Phagocytosis of some insoluble metal compounds by human polymorphonuclear leukocytes causes the production of H_2O_2 (Zhong *et al.* 1990).

Several metals, including lead, have been consider as aneugenic compounds that interfere with spindle apparatus *via* inhibition of tubulin polymerization and include disorders in chromosome distribution resulting in aneuploidy cells (Leonard *et al.* 1984). On the other hand, Vaglenov *et al.* (1998) found that very significant increase in frequency of binucleated cells with micronuclei in group of workers highly exposed to lead, whereas, micronucleus assay detects both clastogenic and aneugenic effects. Thus, it shows clearly the *in vivo* genotoxicity of lead.

The positive results obtained from this study indicate that lead acetate is genotoxic in mouse bone-marrow and mouse spleen cell cultures under the conditions tested.

References

- Abul-Nasr, S. M. and Mahrous, K. F. 2001. Cytogenetical studies of garlic and its significance for the protection against lead toxicity in albino rats. *Bull. NCR. Egypt* **26**: 111–122.
- Ad Hoc Committee of the Environmental Mutagen Society and the Institute for Medical Research, 1972. Chromosome methodologies in mutation testing. *Toxicol. Appl. Pharmacol.* **22**: 269–275.
- Bauchinger, M. and Schmid, E. 1972. Chromosome analysis in cell cultures of the Chinese hamster after application of lead acetate. *Mut. Res.* **14**: 95–100.
- Beckman, L. and Nordenson, I. 1986. Interactions between some common genotoxic agents. *Hum. Hered.* **36**: 397–401.
- Beek, B. and Obe, G. 1974. Effect of lead acetate on human leukocyte chromosomes *in vitro*. *Experientia* **30**: 1007–1008.
- and — 1975. The human leukocyte test system. VI. The use of sister chromatid exchanges as possible indicators for mutagenic activities. *Humangenetik* **29**: 127–134.
- Deknadt, G. H. and Deminatti, M. 1978. Chromosome studies in human lymphocytes after *in vitro* exposure to metal salts. *Toxicology* **10**: 67–75.
- , Colle, A. and Gerber, G. B. 1977. Chromosome abnormalities in lymphocytes from monkeys poised with lead. *Mut. Res.* **45**: 77–83.
- Dulout, F. N., Pastori, M. C. and Olivero, O. A. 1983. Malation induced chromosome aberrations in bone-marrow cells of mice: dose-response relationship. *Mut. Res.* **122**: 163–167.
- Eyden, B. P., Maisin, J. R. and Mattelin, G. 1978. Long-term effects of dietary lead acetate on survival, body weight and semen cytology in mice. *Bull. Environ. Contam. Toxicol.* **19**: 266–272.
- Gasiorek, K. and Bauchinger 1981. Chromosome changes in human lymphocytes after separate and combined treatment with divalent salts of lead, cadmium and zinc. *Environ. Mol. Mutagen.* **3**: 513–518.
- Gerber, G. B., Leonard, A. and Jacquet, P. 1980. Toxicity, mutagenicity and teratogenicity of lead. *Mut. Res.* **76**: 115–141.
- Goyer, R. A. 1993. Lead toxicity: Current concern. *Environ. Health. Perspec.* **100**: 177–187.
- Grandjean, P., Wulf, H. C. and Niebuhr, E. 1983. Sister chromatid exchange in response to variations in occupational lead exposure. *Environ. Res.* **32**: 199–204.
- Hartwing, A., Schlepegrell, R. and Beyersmann, D. 1990. Indirect mechanism of lead-induced genotoxicity in cultured mammalian cells. *Mut. Res.* **241**: 75–82.

- International Agency for Research on Cancer, Overall Evaluation of Carcinogenic and Updating of IARC Monographs 1987. Vol. 1 to 42, IARC Monogr. Eval. Carcinog. Risks Hum., 1–41.
- Leonard, A., Gerber, G. B., Jacquet, P. and Lauways, R. 1984. Carcinogenicity, Mutagenicity and Teratogenicity of Industrial Used Metals. In: Kirsch-Volders, M. (ed.). *Mutagenicity, Carcinogenicity and Teratogenicity of Industrial Pollutants*. Plenum, New York. pp. 59–126.
- McFee, A. F., Lowe, K. and San Sebastian, J. R. 1983. Improved sister chromatid differentiation using paraffin coated bromodeoxyuridine tablets in mice. *Mut. Res.* **119**: 83–88.
- Montaldi, A., Zentilin, L., Pagliani, S. and Levis, A. G. 1987. Solubilisation by nitriloacetic acid (NTA) of genetically active Cr (VI) and Pb (II) from insoluble metal compounds. *J. Toxicol. Environ. Health* **21**: 387–394.
- Nordenson, T., Beckman, G., Beckman, L. and Nordstrom, S. 1978. Occupational and environmental risks in an around a smelter in Northern Sweden, IV. Chromosome aberrations in workers exposed to lead. *Hereditas* **88**: 263–267.
- Obe, G., Beek, B. and Dudin, G. 1975. Some experiments on the action of lead acetate on human leukocytes *in vitro*. *Mut. Res.* **29**: 283.
- Perry, P. and Wolff, S. 1974. New Giemsa method for the differential staining of sister chromatids. *Nature* **261**: 156–158.
- Popeno, E. A. and Schmaeler, M. A. 1979. Interaction of human DNA polymerase *B* with ions of copper, lead and cadmium. *Arch. Biochem. Biophys.* **196**: 109–120.
- Preston, R. J., Dean, B. J., Galloway, S., Holden, H., McFee, A. E. and Shelby, M. 1987. Mammalian *in vivo* cytogenetic assays. Analysis of chromosome aberrations in bone marrow cells. *Mut. Res.* **189**: 157–165.
- Roy, N. K. and Rossman, T. G. 1992. Mutagensis and comutagenesis by lead compounds. *Mut. Res.* **298**: 97–103.
- Schmid, E., Bauchinger, M., Pietruck, S. and Hall, G. 1972. The cytogenetic effect of lead in human peripheral lymphocytes *in vitro* and *in vivo*. *Mut. Res.* **16**: 401–406.
- Schwantiz, G., Lehnert, G. and Gebhart, E. 1970. Chromosomenschaden bei beruflicher Bleibelastung. *Dtsch. Med. Wochschr.* **95**: 1636–1641.
- Shirai, T., Ohshima, M., Masuda, A., Tomano, S. and Ito, N. 1984. Promotion of 2 (ethylnitrosamino) ethanol induced renal carcinogenesis in the rat by nephrotoxic compounds. Positive responses with folic acid. *J. Natl. Cancer. Inst.* **72**: 477–480.
- Sirover, M. A. and Loeb, L. A. 1979. Infidelity of DNA synthesis *in vitro*: Screening for potential mutagens or carcinogens. *Science* **194**: 1434–1436.
- 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.
- Vaglenov, A., Carbonell, E. and Marcos, R. 1998. Biomonitoring of workers exposed to lead. Genotoxic effects, its modulation by polyvitamin treatment and evaluation of the induced radioresistance. *Mut. Res.* **418**: 79–92.
- Watanabe, M., Takayama, Y. I., Masahiko, K. and Yamamoto, M. 1985. *In vivo* clastogenicity of lead chromate in mice. *Tohoku J. Exp. Med.* **146**: 373–374.
- Willems, M. I., de Schepper, G. G., Wibowo, A. A. E., Immel, H. R., Dietrich, A. J. J. and Zielhuis, R. L. 1982. Absence of an effect of lead acetate on sperm morphology, sister chromatid exchange or on micronuclei formation in rabbits. *Arch. Toxicol.* **50**: 149–157.
- Wyrobek, A. J. and Bruce, W. R. 1978. The induction of sperm-shape abnormalities in mice and humans. *Chem. Mutagen.* **5**: 257–285.
- Yosida, T. H. and Amano, K. 1965. Autosomal polymorphism in laboratory bred and wild norway rats, *Rattus norvegicus*. *Misima Chromosoma* **16**: 658–667.
- Zelikoff, J. T., Li, J. H., Ahartwing, A., Wang, X. W., Costa, M. and Rossman, T. G. 1988. Genetic toxicology of lead compounds. *Carcinogenesis* **9**: 1727–1732.
- Zhong, Z., Troll, W., Koenig, K. L. and Frenkel, K. 1990. Carcinogenic sulfide salts of nickel and cadmium induce H₂O₂ formation by human polymorphonuclear leukocytes. *Cancer Res.* **50**: 7564–7570.
-