Vol. 11, 287–290, March 2002

Correlations of Blood Lead with DNA-Protein Cross-Links and Sister Chromatid Exchanges in Lead Workers[1]

Fang-Yang Wu, Pao-Wen Chang, Chin-Ching Wu, and Hsien-Wen Kuo[2]

Institute of Environmental Health [F-Y. W., P-W. C., H-W. K.] and Department of Public Health [C-C. W.], China Medical College, Taichung, and Department of Nuclear Science, National Tsing Hua University, Hsinchu [C-C. W.], Taiwan, Republic of China

Abstract

Levels of sister chromatid exchanges (SCEs), high-SCE frequency cells (HFCs), DNA-protein cross-links (DPCs), blood lead (BLL), and zinc protoporphyrin (ZPP) were measured in peripheral blood from three groups. The lead workers were divided into two groups: a high BLL group (≥15μg/dl) and a low BLL group (<15 μg/dl). The control subjects were selected from an area that had not been contaminated with lead and had normal BLL and ZPP levels. In addition, exposure to airborne lead was measured for 11 lead workers, and the time-weighted average was shown to range from 0.19 to 10.32 mg/m^3. The BLL levels of 9 of 11 workers were >15 μg/dl, of which, 3 exceeded current exposure limits (≥40 μg/dl). The BLL levels of all 11 controls were <15 μg/dl. The average SCE and DPC values for the workers were 6.1 SCEs/cell and 1.9%, which were significantly higher (P < 0.01, Wilcoxon's test) than the value of 5.2 SCEs/cell and 1.1% for the control subjects. Lead workers had significantly higher BLL and ZPP levels than did the controls. Statistically significant increases in DPCs, SCEs, and HFCs were observed for the high-BLL group compared with the control group. The results of this study suggest that DPCs, SCEs, and HFCs are reliable biomarkers for monitoring workers exposed to lead and clearly indicate health effects from occupational exposure to lead.

Introduction

Epidemiological studies have shown that human exposure to lead compounds is associated with a higher incidence of renal tumors and lung and stomach cancer (1–3). Lead(II) has been shown to be a weak mutagen and possibly a carcinogen in Chinese hamster V79 cells (4, 5). Exposure of cultured cells to lead(II) *in vitro* at a toxic dose resulted in CAs[3] and SCEs (6, 7). In several *in vivo* studies, the cytogenetic effects of lead have been reported. Some studies showed an increased CA and/or SCE frequency in lymphocytes from workers exposed to lead (6, 8–10), whereas others reported negative results (11, 12). SCEs have potential long-term genotoxic consequences. Previous studies using human cells dosed with lead *in vitro* have suggested that lead ions decrease the fidelity of DNA synthesis or repair (13, 14) and inhibit the activity of DNA polymerase β and ligase (9). 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 (10). These studies indicate the necessity of considering the influence of DNA repair processes when assessing the genotoxicity of lead compounds.

DPCs induced in treated cells may persist; their presence can be easily detected at some time after removal of the genotoxic agent (15–17). Genotoxic compounds may induce poor repair ability, which may result in the production of DNA-protein complexes during DNA replication. The apparent low capacity for repair makes this type of DNA damage a potentially important lesion to use as a biomarker of exposure. Lead compounds are cytotoxic to cultured cells at concentrations where an insoluble precipitate is formed (18). Previous *in vitro* studies have suggested that many heavy metals can produce DPCs. The concentrations required for many of these agents to cause this effect were extremely toxic to cells. Lead compounds exhibit induced DPCs at 5 mM *in vitro* (19). In lead workers, DPC formation in lymphocytes has not been identified to date.

Cytogenetic end points such as SCEs have been used in conjunction with DNA adduct analyses to monitor human exposure to harmful agents. Kriek *et al.* (20) have suggested that there is a correlation between the cytogenetic end point and DNA adduct. Therefore, in the present study, we evaluated genotoxicity, using DPCs and SCEs as a measure of DNA damage. This is the first report of the use of a DPC assay to assess lead compounds for their capacity to induce genetic damage in long-term lead workers.

Materials and Methods

Study Population. Each subject was interviewed by questionnaire to determine age, gender, medication, health status, occupational history, and smoking habits. All lead workers were selected from a storage battery manufacturer in central Taiwan. The workers were divided into a high-BLL group (BLL ≥ 15 μg/dl; 23 subjects) and a low-BLL group (BLL < 15 μg/dl; 34 subjects). Control subjects were selected from an area that had not been contaminated with lead, and had normal BLL and ZPP

Received 7/12/01; revised 12/26/01; accepted 1/6/02.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This study was supported by the National Science Council (Grant NSC-89-2314-B-039-035) and the China Medical College (Grant CMC 88-EM-03) in Taichung, Taiwan.
[2] To whom requests for reprints should be addressed, at Institute of Environmental Health, China Medical College, No. 91, Hsueh-Shin Road, Taichung, Taiwan, R. O. C. Phone: (886) 4-2205-4076; Fax: (886) 4-2201-9901; E-mail: hwkuo@mail.cmc.edu.tw.

[3] The abbreviations used are: CA, chromosomal aberration; SCE, sister chromatid exchange; DPC, DNA-protein cross-link; BLL, blood lead; ZPP, zinc protoporphyrin; TWA, time-weighted average; HFC, high-SCE frequency cell.

Downloaded from http://aacrjournals.org/cebp/article-pdf/11/3/287/1740872/ce0302000287.pdf by guest on 24 August 2022

Table 1 Age, smoking habits, biomarkers, and airborne lead levels among 11 lead workers and 11 controls

Subjects	Age (yrs)	Smoking (pack-years)	BLL (μg/dl)	ZPP (mg/g HB)[a]	SCEs/ cell[b]	HFCs[c]/ 30 cells	DPCs[d] (%)	Lead exposure	
								Years	TWA (μg/m^3)
Lead workers									
1	50	0	27.6	60.0	5.3	2	2.4	11	1.05
2	44	15	57.8	231.2	6.1	4	2.0	17	1.73
3	43	0	42.5	87.6	5.6	3	1.9	10	10.32
4	43	0	26.8	82.0	6.1	2	1.6	13	4.73
5	39	0	23.0	48.6	5.4	3	1.2	11	1.70
6	45	0	25.2	81.4	6.1	4	1.8	19	6.74
7	42	0	7.9	30.1	6.0	4	2.8	6	0.19
8	45	0	10.4	32.0	6.1	3	1.9	6	0.84
9	42	4	39.5	83.7	7.1	5	1.0	12	1.22
10	48	0	18.4	23.3	6.7	4	0.9	11	0.24
11	57	12.5	28.1	55.8	6.4	4	3.1	11	0.73
Controls									
12	45	0	3.3	16.5	5.1	0	1.9		
13	55	5	7.0	21.3	6.1	0	1.1		
14	48	0	1.5	13.2	4.9	0	0.8		
15	43	0	3.2	14.9	4.7	0	1.1		
16	39	0	2.8	16.1	4.7	2	0.7		
17	49	0	4.1	18.4	5.0	1	0.8		
18	44	6.7	4.3	20.6	5.3	0	1.2		
19	42	5	2.2	14.4	5.7	1	1.4		
20	46	0	4.3	15.9	5.6	0	1.0		
20	42	0	4.7	27.7	4.8	4	0.9		
22	42	0	2.8	11.6	4.9	1	1.4		

[a] HB, hemoglobin.
[b] Average of SCEs (6.1 ± 0.5) for lead group (subjects 1–11) was significantly higher than for control group (5.2 ± 0.5; Wilcoxon's test, $P < 0.01$).
[c] HFCs, 5% of cells with a high frequency of SCEs.
[d] Average of DPCs (1.9 ± 0.5) for lead group (subjects 1–11) was significantly higher than for control group (1.1 ± 0.4; Wilcoxon's test, $P < 0.01$).

levels. Exposure to airborne lead was measured for 11 lead workers, and the TWA was calculated (Table 1). Eleven control subjects were matched for age and smoking habits with lead workers.

Personal exposure monitoring for airborne lead was performed during an 8-h work shift and carried out using personal samplers (SKC Airchek Sampler Model 224-52) with battery-powered pumps operated at 2.0 liters/min. Quantitative analysis of airborne lead was performed by graphite furnace atomic absorption spectrophotometry (Model 5100 AAS; Perkin-Elmer).

Measurement of Biological Markers. Blood samples were collected from all subjects and drawn into EDTA-containing tubes. BLL was measured by graphite furnace atomic absorption spectrophotometry. ZPP was determined by hematofluorimetry (Aviv/Model 206; Biomedical Inc.).

DPCs were detected by methods described in previous studies (21–23) and are described briefly below. WBCs (2×10^6) were lysed in a 0.5% SDS-20 mM Tris-HCl (pH 7.5) solution and stored at −70°C until analysis. Fluorescence was measured at 450 nm with excitation at 360 nm on a Horfer Model Fluorescence Spectrophotometer.

SCE levels were determined according to the procedures described in previous studies (24–26). Whole blood from the three groups was drawn into heparinized tubes, and analyses were completed within 1 week. The cells were incubated in the dark for 70 h at 37°C in the presence of 5.0 ml of RPMI 1640 with 15% fetal bovine serum, 3% phytohemagglutinin (Life Technologies, Inc.), and 20 μM bromodeoxyuridine (Merck). Two h before fixation, 2×10^{-7} M Colcemid was added. SCEs were prepared according to conventional protocols and stained using the fluorescence-Giemsa method. For the observation of SCEs per cell, 30 second-division metaphases were scored per sample. Exchanges at the centromere were not included in the count. Analysis of the HFCs was performed according to Carrano and Moore (27). A HFC was defined as a cell with a percentage of lymphocytes exhibiting an SCE score over the median 95% value of the results from the 19 nonsmoking control subjects. The cutoff value was 9 SCEs/cell.

Statistical Methods. SAS/pc+ 6.04 statistics software (SAS/STAT) was used for all data analyses. Mean values for BLL and ZPP in exposed workers and controls were assessed by parametric tests. A *t* test was used to compare SCE levels and percentages of DPCs and HFCs among lead workers and controls. Multiple linear regression models were used to compare lead workers and controls with regard to the percentage of DPCs and SCE levels after adjustment for age, gender, and smoking.

Results

The ages, smoking habits, biomarkers, and airborne lead levels among 11 lead workers and 11 controls are shown in Table 1. The TWA of exposure to lead ranged from 0.19 to 10.32 mg/m^3. Some of these values exceed current exposure limits in Taiwan. The average SCE and DPC value for workers was 6.08 SCEs/cell and 1.9%, which were significantly higher ($P < 0.01$, Wilcoxon's test) than the value of 5.16 SCEs/cell and 1.1% for the control subjects. Three lead workers who smoked had significantly increased BLL (57.8, 39.5, and 28.1 μg/dl, respectively) and ZPP levels (231.2, 83.7, and 55.8 μg/dl, respectively). The BLL levels for 9 of 11 workers were >15 μg/dl, of which 3 exceeded the permissible limit in Taiwan (≥40 μg/dl). The BLL levels for all 11 controls were <15 μg/dl.

Downloaded from http://aacrjournals.org/cebp/article-pdf/11/3/287/1740872/ce0302000287.pdf by guest on 24 August 2022

Table 2 Demographic data, biomarkers, and smoking habits among the three groups

	Lead workers		Control group
	High BLL (BLL ≥15 μg/dl)	Low BLL (BLL <15 μg/dl)	
Number of subjects	23	34	30
Age (years)	46.9 ±9.9[a]	42.41 ±9.11	40.23 ± 5.43
Work duration (years)	14.7 ± 6.4	16.28 ± 9.27	
Gender			
M (%)	16 (69.6)	21 (61.8)	19 (63.3)
F (%)	7 (30.4)	13 (38.2)	11 (36.7)
Smoking (%)	6 (26.1)	6 (17.7)	11 (36.7)
Blood lead (± SD), μg/dl	32.5 ± 14.5[b]	9.3 ± 2.9[d]	4.2 ± 1.4
ZPP (± SD), μg/dl	111.4 ± 101.2[b]	36.6 ± 14.6[e]	19.4 ± 5.2
SCEs (± SD)	6.1 ± 0.7[a]	5.5 ± 0.7	5.2 ± 0.5
Smoking	6.4 ± 0.5[a]	5.8 ± 0.4	5.7 ± 0.3
Nonsmoking	5.9 ± 0.7[a]	5.5 ± 0.7	4.9 ± 0.4
HFCs (%)	11 (47.8)[a]	8 (23.5)	3 (10.0)
Smoking	4 (66.7)[c]	1 (16.7)	2 (18.2)
Nonsmoking	7 (41.2)[a]	7 (25.0)	1 (5.3)
DPCs (%)	1.5 ± 0.6[a]	1.2 ± 0.5	1.0 ± 0.3
Smoking	1.8 ± 0.7[c]	1.2 ± 0.4	1.0 ± 0.2
Nonsmoking	1.4 ± 0.5[a]	1.1 ± 0.5	1.0 ± 0.3

[a–c] High-BLL *vs.* control group: [a] $P < 0.05$; [b] $P < 0.001$; [c] $P < 0.01$.
[d,e] Low-BLL *vs.* control group: [d] $P < 0.001$; [e] $P < 0.01$.

Table 3 Factors affecting the DPC percentages and SCE levels using multiple linear regression models

Variables	DPCs (%)			SCE level		
	β	SE	*P*	β	SE	*P*
Intercept	0.74	0.25	<0.01	5.41	0.32	<0.001
Lead exposure						
High-BLL/control group	0.37	0.23	<0.01	1.15	0.29	<0.01
Low-BLL/control group	0.24	0.12	<0.05	0.49	0.15	<0.01
Age (years)	0.01	0.01	0.16	−0.01	0.01	0.39
Gender (female = 0)	−0.27	0.11	0.12	−0.22	0.14	0.13
Smoking/nonsmoking	0.12	0.12	<0.01	0.68	0.15	<0.01
BLL (μg/dl)	0.01	0.01	<0.05	0.01	0.01	<0.05

The demographic data, biological markers, and smoking habits of all subjects are shown in Table 2. The high-BLL group was significantly older than the control group. BLL and ZPP levels were significantly higher among high-BLL workers compared with low-BLL workers and controls. SCE levels and percentages of DPCs and HFCs were significantly higher in the high-BLL group for both smokers and nonsmokers compared with the control group. SCE levels and percentages of DPCs and HFCs in the low-BLL group were higher than in the control group, but this was not significant. The percentage of HFCs was highest in the high-BLL group (47.8%), followed by the low-BLL group (23.5%) and controls (10%). There was a similar trend for smokers and nonsmokers. Smokers had higher levels of SCE and percentages of DPCs and HFCs than did nonsmokers.

Multiple linear regression models were used to determine the percentage of DPCs and SCE levels adjusted for age, smoking habits, and BLL (Table 3). When the high- and low-BLL groups were compared with the control group, smoking habits and BLL were significantly associated with the percentage of DPCs and SCE levels. Age and gender did not correlate with the percentage of DPCs and SCE levels. When smokers and nonsmokers from all three groups were compared simultaneously, there were significant differences in SCE levels among the groups. There was also a significant positive relationship between both the percentage of DPCs and SCE and BLL levels.

Discussion

A number of studies have shown a positive correlation between exposure to lead and chromosome lesions (11, 12). Popenoe and Schmaeler (9) argued that low lead ion concentrations (10^{-5} M) could inhibit the function of human DNA polymerase. In an *in vitro* study, Loeb and Mildvan (28) added lead chlorine into the fluid with cells. They found that lead oxide disrupted DNA synthesis by increasing the frequency of DNA synthesis error, thus undermining the accuracy of RNA synthesis. Montaldi (7) asserted that the covalent binding of lead ions and DNA, chromosomal protein, DNA polymerase, or substrate nucleotide precursors might jeopardize DNA replication and repair or expression of genes. Hartwig *et al.* (10) suggested that lead may inhibit DNA repair; they postulated that the mechanism might involve the interaction of lead and repair enzymes (*e.g.,* polymerase or ligase) or calcium-regulated interference and that these effects were related to DNA synthesis and repair. In an animal study on rat livers and kidneys, Morimura *et al.* (29) found that lead could influence the transcription of glutathione *S*-transferase P, and the activation of glutathione *S*-transferase P increased risk of liver and kidney cancers. Their data indicated that exposure to low concentrations

Downloaded from http://aacrjournals.org/cebp/article-pdf/11/3/287/1740872/ce0302000287.pdf by guest on 24 August 2022

of lead could induce DNA damage and increase incidences of malignant tumors.

In this study, the SCE method was used as a biomarker of cytogenetic damage because of its sensitivity to low levels of genotoxic agents (30). DPCs also represent potentially important lesions induced by certain genotoxic agents, including heavy metals (19), chemicals (23), UV light, and X-rays (31). When these lesions are induced at noncytotoxic levels that lead to mutations, they are relatively persistent in cells and not readily repaired (16, 17, 31). Therefore, a detection method for DPCs may be more sensitive when combined with SCE detection. Previous studies have revealed wide variations in terms of SCE levels and CA levels in workers exposed to lead at the workplace. Workers exposed to lead compounds showed increases in SCE and CA levels (6, 8–10). The present study showed that average SCE values in high-BLL workers were significantly higher than those in the control group. Some studies did not reveal any effect of lead exposure on chromosome aberrations or SCE levels in workers (11, 12). However, the results of the present study showed that there was an association. Smoking has been shown to cause a significant increase in SCE levels (25, 26, 32); our data were consistent with these findings. The percentage of HFCs was also used as a sensitive biomarker for genotoxicity. HFCs were defined as cells that displayed a proportion of SCEs/cell above the 95th percentile of the distribution of SCEs/cell in nonsmoking control subjects. Our previous studies revealed a significantly higher HFC rate among smoking chromium workers than among controls (26). The differences in the percentage of HFCs among high-BLL workers, low-BLL workers, and the control group were ~37.8% and 13.5% in this study. There was a similar trend for DPCs in that the highest percentage of DPCs was in the high-BLL group, followed by the low-BLL group and the control group.

Most studies on the genetic effects of occupational or environmental exposure to lead have emphasized CA levels in peripheral lymphocytes of subjects with BLL levels >20 μg/dl (33). In the present study, high-BLL workers had mean BLL levels of 32.5 μg/dl; these levels have been shown to produce cytogenetic damage in lymphocytes, detectable as SCEs and DNA-protein cross-linking. The increase in SCE levels and percentages of DPCs appeared to be dependent on BLL level. Moreover, lead compounds are capable of inducing chromosomal impairment in a number of cells and appear to influence the percentage of DPCs and SCE levels.

In conclusion, cytogenetic end points (such as SCEs and DPCs) may be used as indicators of chromosome damage and have been shown to be suitable biomarkers for exposure to lead. It is necessary to periodically monitor the exposure of long-term lead workers to lead by assessing their biomarkers, and it is important to reduce worker exposure to lead in the workplace.

References

1. Cooper, W. C. Cancer mortality patterns in the lead industry. Ann. NY Acad. Sci., *27:* 150–176, 1976.

2. Siemiatycki, J. Risk Factors for Cancer in the Workplace. London: CRC Press, 1991.

3. Fu, H., and Boffetta, P. Cancer and occupational exposure to inorganic lead compounds: a meta-analysis of published data. Occup. Environ. Med., *52:* 73–81, 1995.

4. Zelikoff, J. T., Li, J. H., Hartwig, A., Wang, X. W., Costa, M., and Rossman, T. G. Genetic toxicology of lead compounds. Carcinogenesis (Lond.), *9:* 1727–1732, 1988.

5. Roy, N. K., and Rossman, T. G. Mutagenesis and comutagenesis by lead compounds. Mutat. Res., *298:* 97–103, 1992.

6. Forni, A., Cambiaghi, G., and Secchi, G. C. Initial exposure to lead: Chromosome and biochemical findings. Arch. Environ. Health, *31:* 73–78, 1976.

7. Montaldi, A. Interaction of nitrilotriacetic acid with heavy metals in the induction of sister chromatid exchanges in cultured mammalian cells. Environ. Mutagen., *7:* 381–390, 1985.

8. Bauchinger, M., and Schmid, E. Chromosome analysis in cell cultures of the Chinese hamster after application of lead acetate. Mutat. Res., *14:* 95–100, 1972.

9. Popenoe, E. A., and Schmaeler, M. A. Interaction of human polymerase β with ions of copper, lead, and cadmium. Arch. Biochem. Biophys., *196:* 109–120, 1979.

10. Hartwig, A., Schlepegrell, R., and Beyersmann, D. Indirect mechanism of lead-induced genotoxicity in cultured mammalian cells. Mutat. Res., *241:* 75–82, 1990.

11. Deknudt, G., Manuel, Y., and Gerber, G. B. Chromosomal aberrations in workers professionally exposed to lead. J. Toxicol. Environ. Health, *3:* 885–891, 1977.

12. Grandjean, P., Wulf, H. C., and Niebuhr, E. Sister chromatic exchange in response to variations in occupational lead exposure. Environ. Res., *32:* 199–204, 1983.

13. Skreb, Y., and Habazin-Novak, V. Reversible inhibition of DNA, RNA, and protein synthesis in human cells by lead chloride. Toxicology, *5:* 167–174, 1975.

14. Sirover, M. A., and Loeb, L. A. Infidelity of DNA synthesis *in vitro*: screening for potential mutagens or carcinogens. Science (Wash. DC)., *194:* 1434–1436, 1976.

15. Tsapakos, M. J., Hampton, T. H., and Wetterhahn, K. E. Chromium-induced DNA lesions and chromium distribution in rat kidney, liver and lung. Cancer Res., *43:* 5662–5667, 1983.

16. Sugiyama, M., Wang, X. Y., and Costa, M. Comparison of DNA lesions and cytotoxicity induced by calcium chromate in human, mouse, and hamster cell lines. Cancer Res., *46:* 4547–4551, 1986.

17. Sugiyama, M., Patierno, S. R., Cantoni, O., and Costa, M. Characterization of DNA lesions induced by $CaCrO_4$ in synchronous and asynchronous cultured mammalian cells. Mol. Pharmacol., *29:* 606–613, 1986.

18. Rossman, T. G., Zelikoff, J. T., Agarwal, S., and Kneip, T. J. Genetic toxicology of metal compounds: an examination of appropriate cellular models. Toxicol. Environ. Chem., *14:* 251–262, 1987.

19. Wedrychowski, A., Schmidt, W. N., and Hnilica, L. S. The *in vivo* cross-linking of proteins and DNA by heavy metals. J. Biol. Chem., *261:* 3370–3376, 1986.

20. KrieK, E., Rojas, M., Alexandrov, K., and Bartsch, H. Polycyclic aromatic hydrocarbon-DNA adducts in humans: relevance as biomarkers for exposure and cancer risk. Mutat. Res., *400:* 215–231, 1998.

21. Costa, M., Zhitkovich, A., and Toniolo, P. DNA-protein cross-links in welders: molecular implications. Cancer Res., *53:* 460–463, 1993.

22. Zhitkovich, A., and Costa, M. A simple, sensitive assay to detect DNA-protein-crosslinks in intact cells and *in vivo*. Carcinogenesis (Lond.), *13:* 1485–1489, 1992.

23. Costa, M., and Zhitkovich, A. DNA-protein cross-links produced by various chemicals in cultured human lymphoma cells. J. Toxicol. Environ. Health, *50:* 433–449, 1997.

24. Goto, K., Maeda, S., Kano, Y., and Sugiyama, T. Factors involved in differential Giemsa-staining of sister chromatids. Chromosoma (Berl.), *66:* 351–359, 1978.

25. Lai, J. S., Kuo, H. W., Liao, F. C., and Lien, C. H. Sister chromatid exchange induced by chromium compounds in human lymphocytes. Int. Arch. Occup. Environ. Health, *71:* 550–553, 1998.

26. Wu, F-Y., Tsai, F-J., Kuo, H. W., Tsai, C. H., Wu, W. Y., Wang, R. Y., and Lai, J. S. Cytogenetic study of workers exposed to chromium compounds. Mutat. Res., *464:* 289–296, 2000.

27. Carrano, A. V., and Moore, D. H. The rationale methodology for quantifying sister-chromatid exchanges in humans. *In:* J. A. Heddie (ed.), New Horizons in Genetic Toxicology, Vol. 99, pp. 267–304. New York: Academic Press, 1982.

28. Loeb, L., and Mildvan, A. S. The role of metal ions in the fidelity of DNA and RNA synthesis. *In:* G. L. Eichorn and L. G. Marzili (eds.), Metal Ions in Genetic Information Transfer, Vol. 3, pp. 126–142. New York: Elsevier/North-Holland, 1981.

29. Morimura, S., Suzuki, T., and Hochi, S. *Trans*-activation of glutathione transferase *P* gene during chemical hepatocarcinogenesis of the rat. Proc. Natl. Acad. Sci. USA, *90:* 2065–2068, 1993.

30. Schmid, E., Bauchinger, M., Pietruck, S., and Hall, G. The cytogenetic effect of lead in human peripheral lymphocytes *in vitro* and *in vivo*. Mutat. Res., *16:* 401–406, 1972.

31. Oleinick, L. N., Chiu, S. M., Ramakrishnan, N., and Xue, L. The formation, identification, and significance of DNA-protein cross-links in mammalian cells. Br. J. Cancer, *55:* 135–140, 1987.

32. Chin, K. L. Comparettes genotoxicity testing of mainstream whole smoke form cigarettes which burn or heat tobacco. Mutat. Res., *242:* 37–45, 1990.

33. Winder, C., and Bonin, T. The genotoxicity of lead. Mutat. Res., *285:* 117–124, 1993.

Downloaded from http://aacrjournals.org/cebp/article-pdf/11/3/287/1740872/ce0302000287.pdf by guest on 24 August 2022