Eur. J. Oncol., vol. 15, n. 3-4, pp. 149-156, 2010

Cancer risk assessment, primary prevention and chemoprevention in occupational health using chromosomal aberration and sister chromatid exchange (SCE) as biomarkers

Valutazione di rischio cancerogeno, prevenzione primaria e chemioprevenzione negli ambienti professionali utilizzando l'aberrazione cromosomica e lo scambio di cromatidi "fratelli" (SCE) come biomarcatori

Anna Tompa*, Mátyás G. Jakab**, Jenő Major**

* Semmelweis University, Department of Public Health, Budapest, Hungary
** National Institute of Chemical Safety, Budapest, Hungary

Summary

Genotoxicological investigations serve as tools to detect the damages caused by the environmental and occupational mutagens and carcinogens acquired by the somatic cells. These damages are well demonstrated in the course of genotoxicological monitoring by chromosomal mutation, sister chromatid exchange (SCE), and the blastic transformation activity of peripheral blood lymphocytes (PBL), among workers who were exposed to benzene and heavy metals. Workers exposed to different concentration of benzene were monitored for over 20 years. These studies showed an increase of the chromosomal aberrations in workers exposed to benzene above the 1 ppm. At the same time, parallel to the lowering of benzene levels, a decrease in the cytogenetic parameters to the level of the industrial control was observed, during the active preventional period. The main

Riassunto

Indagini genotossicologiche vengono utilizzate per identificare i danni rilevati nelle cellule somatiche causati da mutageni ed elementi cancerogenici presenti nell'ambiente e nel posto di lavoro. Questi danni sono evidenziabili attraverso il monitoraggio genotossicologico per mezzo di una mutazione cromosomica, scambio di cromatidi "fratelli" (SCE), e la trasformazione blastica, in lavoratori esposti a benzene e metalli pesanti. I lavoratori furono esposti a differenti concentrazioni di benzene per un periodo di vent'anni. Questi studi mostrano un incremento dell'aberrazione cromosomica nei soggetti esposti a concentrazione di benzene superiore ad 1 ppm. Parallelamente all'abbassamento dei livelli di benzene fu osservata una diminuzione dei parametri citogenetici a livello di controllo industriale. Il principale punto di intervento fu il miglioramento del sito di

Received/Pervenuto 4.6.2010 - Accepted/Accettato 9.7.2010
Address/Indirizzo: Professor Anna Tompa, Semmelweis University, Department of Public Health, 1089 Budapest, Nagyvárad tér4., Hungary
E-mail: tomann@net.sote.hu

point of intervention was the improvement of the work-sites including lowering the benzene exposure and convincing the workers to change their life styles avoiding confounding factors (e.g. drugs, alcohol, medication and smoking). This monitoring system was also used to determine the protective effects of some natural products with known antioxidant capacity against the *in vivo* genotoxic effects of these pollutants. In the case of heavy metal (precious metals, chromium, cadmium and nickel) exposed workers, after a chemoprevention treatment with the nutritional supplement Humetta® containing various antioxidants and chelating agents, the results showed a decrease in genotoxic effects, together with improved health status based on the clinical laboratory data. Eur. J. Oncol., 15 (3-4), 149-156, 2010

Key words: **benzene, heavy metals, genotoxicity, workplace, exposure**

lavoro, includendo l'abbassamento dell'esposizione al benzene e convincendo i lavoratori a cambiare il loro stile di vita evitando fattori confondenti (ad esempio droghe, alcool, fumo e medicinali) che potessero influenzare il risultato delle analisi. Questo sistema di monitoraggio fu anche usato per determinare gli effetti protettivi di alcuni prodotti naturali con riconosciute proprietà antiossidanti contro gli effetti genotossici di questi inquinanti. Nel caso di lavoratori esposti a metalli pesanti (metalli preziosi, cromo, cadmio, nichel), dopo un trattamento chemiopreventivo con il supplemento nutrizionale Humetta®, contenente vari antiossidanti ed agenti chelanti, i risultati mostrarono una diminuzione degli effetti genotossici, accoppiata ad un miglioramento della salute comprovato da dati clinici di laboratorio. Eur. J. Oncol., 15 (3-4), 149-156, 2010

Parole chiave: **benzene, metalli pesanti, genotossicità, ambienti professionali, esposizione**

Introduction

Heavy metals and benzene exposures are ubiquitously present as industrial and environmental pollutants. Human exposure may result from occupational setting. Primary prevention of occupational diseases is the most powerful tool of health protection in occupational health. Eliminating the hazardous agents, occupational diseases may be completely preventable. The detection of early signs of the effects of occupational and environmental agents causing DNA-damages, mutations and chromosome aberrations, is useful indicator of the increased risk in the development of cancer (1-8). Current interpretation of the mechanism of cancer development attributes the appearance of tumors primarily to environmental factors by means of somatic mutations and only about 5-10% of the cases related only to the original inherent genetic changes. The combined effects of these occupational and environmental factors may increase the risk of cancer development (1, 5, 9).

A possible attempt for the prevention is the elimination of the harmful agents from the (working) environment (primary prevention), or to promote the elimination of somatic mutations (chemoprevention). Chemoprevention can promote apoptosis, an increase of the activity of DNA-repair or the elimination of mutagenic metabolites, e.g. with the help of antioxidants (10, 11).

The aim of our studies is to investigate the changes in health status and the early effects of genotoxic agents on workers' blood samples after benzene and heavy metal exposure, using genotoxic biomarkers, like chromosomal aberrations, SCE and Comet-assay.

Methods

Sample selection

All donors voluntarily took part in the study after giving their informed consent, and were interviewed by a physician in order to collect data on age, medication, life-style (smoking and drinking habits), as well as medical and work histories, exposure to

known, or suspected environmental and occupational mutagens. Active smokers were considered as "smokers", none of the individuals were dependent on alcohol, subjects were considered as "drinkers" with less than the equivalent of 80 g pure alcohol consumption daily.

Genotoxicology monitoring

The multiple end-points of genotoxicology monitoring, developed in our laboratory, includes among others the determination of the frequencies of chromosome aberrations (CA) and SCE performed in peripheral blood samples, as it was previously described (12-16). Approximately 5 ml of heparinized whole blood were collected through venipuncture from each donor. Lymphocytes were isolated after dilution 1:1 with RPMI 1640 (Gibco BRL) without serum, lymphocyte separation was made using Histopaque 1077 (Sigma Chemical Co.). After centrifugation at 1300 rpm for 45 min, the buffy coat was collected, and the cells were washed three times with RPMI 1640 without serum. For the determination of labeling index, cells were incubated at 37°C at 5% CO_2 level for 72 h in the presence of ^{3}H-thymidine (^{3}H-TdR, Amersham Biosciences UK Limited) and 0.5% phytohemagglutinin (PHA, Gibco Invitrogen Corporation). Proliferating cells labeled with tritiated thymidine were counted through autoradiography. In this study, we also studied the preparations for CA and the incidence of the SCE. Cytogenetic investigations were carried out on chromosomes in the metaphases of isolated lymphocytes of the control and the exposed volunteers. Briefly, 0.8 ml of extracted blood samples were cultured with 20% fetal calf serum supplemented by 0.5% PHA. The cultures were incubated at 37°C at 5% CO_2 level for 50 h (CA) and 72 h (SCE). 5-Bromo-2'-deoxyuridine (BrdU, Sigma-Aldrich) used in SCE analysis to identify the first and subsequent metaphases, was added at a concentration of 5 μg/ ml at 22th h of culture. The slide preparation was performed using standard methods of chromosome staining according to the Fluorescens + Giemsa method (17, 18). The results were evaluated both individually and at the group level. Determination of aberrations was carried out by two independent investigators. Each coded slides were individually scored with 100 metaphase values. In the course of the evaluations only first metaphases with 46 ± 1 chromosomes were taken into consideration. Mitoses containing only achromatic lesions (gaps) and/or aneuploidy were not considered as aberrant. For the determination of the incidence of SCE the lymphocytes were cultivated on a 72 h standard culture and 50 metaphases were evaluated by the methods of Carrano and Natarajan (19).

Altogether 18 subjects, all men, from a benzene producing facility in an oil refinery were involved in the 20 years follow-up genotoxicological investigations and 22 subjects (14 men and 8 women) occupationally exposed to heavy metals took part in this study.

Characterization of Humetta® treatment

HUMETTA® is registered by the National Institute for Food Safety and Nutrition. The basic ingredient in the products is Humifulvate, a chemically identifiable and distinct humate/polyphenolic complex consisting of humic, fulvic and phenolic acids, derived from a peat found only in Hungary, on the Northern shore of Lake Balaton, also containing calcium huminate, the degradation product of lignans, shell remnants, calcareous materials, sand and other minerals. Humifulvate is processed into a concentrate for inclusion in dietary supplement products for oral consumption, either in liquid or in solid form. The chemical and biological properties of this peat bog have been studied for more than 40 years.

Clinical laboratory tests

Blood samples of donors were taken to clinical laboratory test for qualitative and quantitative analysis. The red blood cells count and hemoglobin content showed the value and the types of anemia which were detected through complete blood count and analysis of blood smears including white blood cell count too. The blood sugar, iron and cholesterol level was routinely measured with automatic laboratory techniques in fasting condition.

Results

In Table 1, the main demographic data of the investigated groups of 18 benzene exposed workers (all men) followed by the cytogenetic monitoring and 22 metal workers (14 men and 8 women) exposed to heavy metals, like precious metals, chromium, cadmium and nickel are shown. The mean age of the benzene exposed workers at the beginning of the examination was 32.3 years, and 43.7 in the last year, since some individuals in the cohorts have been changed during the 20 years follow-up. Eight workers were smokers at the beginning of the examination, and only five remained as a smoker at the end of it. The moderate alcohol consumption was reduced from 16 to 13 at the end of this study. The health education and life style advices were regular parts of the interview of each meeting. Three of the donors quitted smoking and stopped alcohol consumption and the Metabolic X Syndrome improved in two cases of benzene exposed group (Table 2).

The participants to this study presented no health problems, or complains during the observed period. In the fig. 1, the average chromosomal aberrations of this benzene exposed group was compared to industrial controls parallel to the benzene average concentration in the ambient air in working places during the follow up study taking samples annually from 1990 to 2010. The industrial control group showed only 1.7% CA in average, but in the case of benzene exposure this increased to a value up to 5.2% in 1990, and decreased below the industrial control between 2001 to 2003, parallel to dropping the benzene concentration down to zero level. In 2004 an increased benzene concentrations in the working area was measured and CA level increased again up to 2.2%, and to 3.6% in 2006. In 2010, CA value dropped back to 2004 level. The fluctuations in CA values were attributable to the fluctuations in benzene exposures.

Table 1 - Main demographic data of the investigated subjects

Groups	Gender	Number of subjects	Mean age (years ± SE)	Smokers	Alcohol consumption
Industrial control	Men	53	38.5 ± 1.5	24	33
	Women	34	38.9 ± 1.7	12	11
Benzene exposed	Men	18	1st: 32.3 ± 1.6	8	16
			Last: 43.7 ± 2.4	5	13
Metal exposed	Men	14	49.3 ± 2.1	7	10
	Women	8	52.0 ± 1.3	1	2

Table 2 - Prevalence of hematology alterations and Metabolic X Syndrome among industrial controls, benzene and metal exposed subjects

Groups	Number of subjects	Gender	Metabolic X Syndrome %	Hematology alterations %
Industrial controls	87	Men: 53	7.5	5.7
		Women: 34	2.1	23.5
Benzene exposed	18	Men	First investigation: 38.8	61.0
			Last investigation: 27.8	16.7
Metal exposed	14	Men	Before treatment: 50.0	71.4
			After treatment: 35.7	42.9
	8	Women	Before treatment: 12.5	100.0
			After treatment: 0	50.0

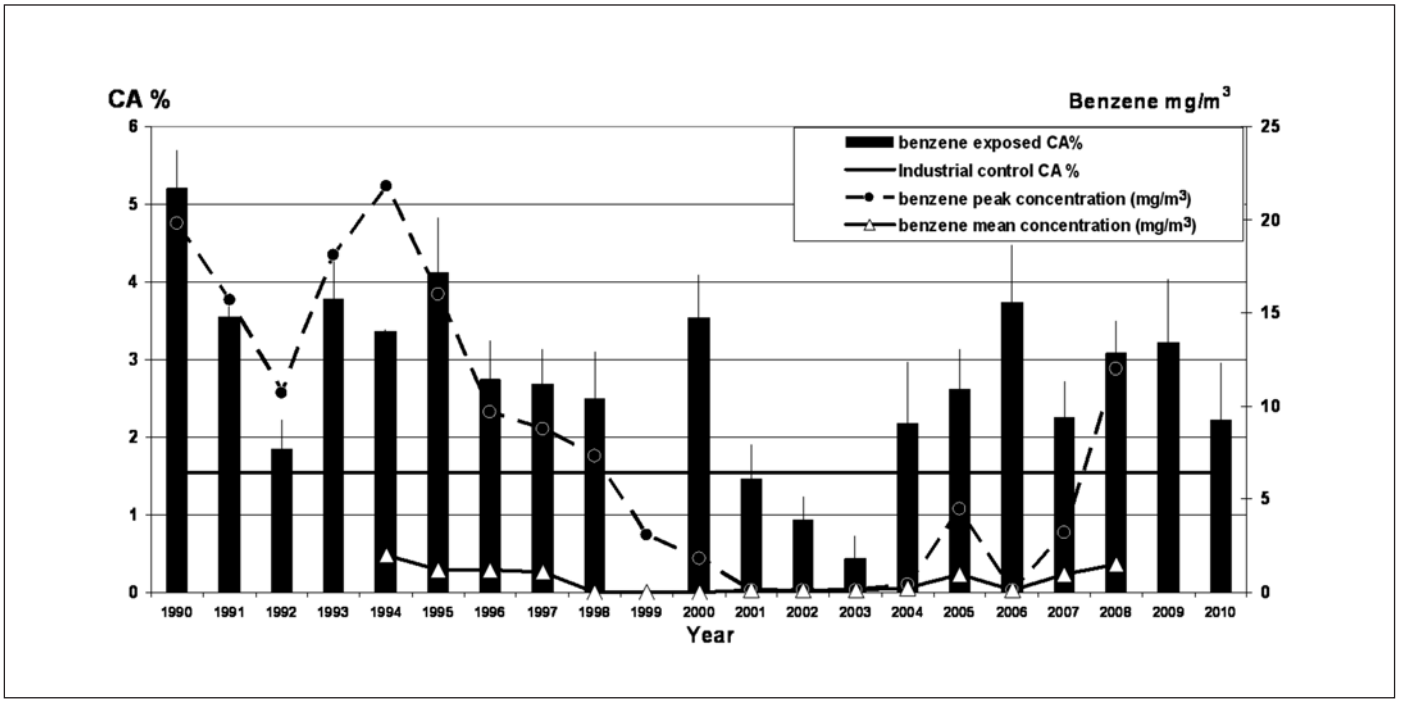

Fig. 1. Follow up examination of benzene exposed workers parallel to the benzene in the ambient.
CA: chromosome aberrations

Table 2 shows the incidence of two main types of diseases which were characterized by clinical laboratory tests. Both groups of exposed workers presented mild hematological and metabolic disorders, including anemia, related to the iron deficiency, and insulin resistance (increased blood sugar content), obesity (BMI over 30), high blood pressure and cholesterol level. When these symptoms were present together, it was called Metabolic X Syndrome. At the first examination, hematological alterations were present in 11 cases out of 18 donors of the benzene exposed donors, and at the time of the last examination only 3 out of 18 donors presented anemia. During the follow up study subjects with iron deficiency were treated with oral iron supplementation. In two out of seven donors with Metabolic X Syndrome the symptoms improved in the end of the follow up.

The heavy metal exposed donors were also repeatedly investigated and in their cases the chemoprevention was also introduced and the effect of Humetta® treatment was controlled three months later (Table 2). Complex clinical laboratory testing were performed before and after administration of chemopreventive agent. After chemoprevention a significant improvement was observed in anemia: 10 cases dropped to 6 among males and among females, the number of 8 cases decreased to 4 cases. The metabolic disorders showed less marked improvement, although 3 cases normalized from 8 cases (7 from men and 1 case from women), after Humetta® treatment.

The mean values of the genotoxicological parameters are shown in fig. 2. Twenty-two metal exposed subjects voluntarily took part in the chemoprevention, and the cumulated data from industrial workers without known occupational exposure served as controls. The PHA stimulation rate (labeling index, LI) of metal exposed donors was only 7.5%, which is very low and half of the industrial controls (17.5%). As a consequence of chemoprevention 3 months later, Humetta® treatment increased PHA stimulation rate up to 13.7%. The obtained results suggest that chemoprevention with chelating agents such as Humetta® may help in the prevention of immune suppression, metabolic disorders as well as the iron deficiency caused anemia of occupational exposures to heavy metals. As fig. 2 shows, the chemoprevention within this short period of treatment showed no significant effect on the level of chromosomal aberrations and SCE.

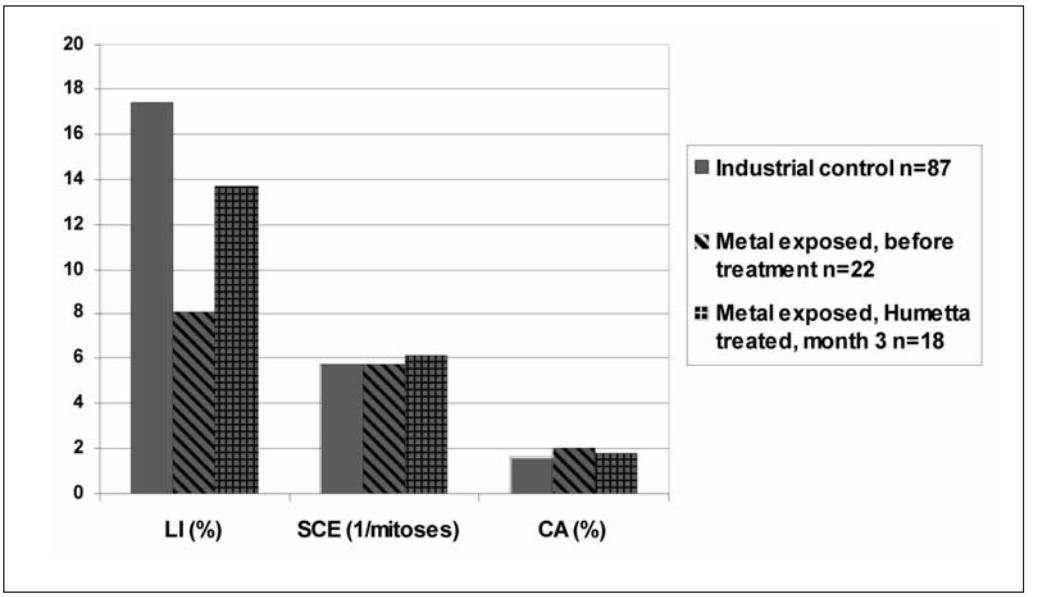

Fig. 2. Humetta® treatment-related changes in the mean values of genotoxicological parameters among workers exposed to metals.
LI: labeling index; SCE: sister chromatid exchanges; CA: chromosome aberrations

Discussion

Primary prevention of occupational diseases is of major importance in public health. The detection of early signs of the effects of occupational and environmental carcinogens causing DNA-damages, mutations and chromosome aberrations, are indications of the increase in the risk of the development of cancer. A possible attempt of prevention is the elimination of the harmful agents from the (working) environment (primary prevention), or to promote the elimination of somatic mutations (chemoprevention). Chemoprevention may promote apoptosis, an increase of the activity of DNA-repair or the elimination of mutagenic metabolites, e.g. with the help of antioxidants. The aims of our studies were to investigate the changes in health status and in the genotoxicology parameters of workers from different work places with increased cancer risk.

Our studies, in accordance with the available literature data, confirmed the genotoxic effects of occupational exposure to benzene (1, 20) and heavy metals (21-24). CA frequencies fluctuate in parallel to the level of ambient benzene concentration varying between 1 and 23 ppm during the whole 20 years long follow-up. The automation of the technology and a strict personal protection are the only available means for the prevention of occupational exposure to benzene. The hazardous effects of benzene exposure appeared both in the genotoxic results (10) and the different observed health consequences, like iron deficiency, anemia, and the Metabolic X Syndrome (11, 25). The risk management was introduced during the medical interviews where all the necessary information were provided to the workers in order to have a better compliance. In case of positive cytogenetic findings it was easier to convince them to change their lifestyles, i.e. stop smoking, reduce drinking, and to use the protecting devices consciously. As a result 6 out of the 18 smokers quitted and 3 out of 18 light drinkers became abstinent, as well as they optimalized their physical activities and nutrition.

Although subjects exposed to heavy metals under the occupational limit had no complaints, the clinical laboratory findings showed hematological alteration and Metabolic X Syndrome in 70% and 50% of males, and 100% and 10% in females, respectively. This indicated the importance of application of an active chemoprevention using a 3-months long treatment with Humetta® capsules, in order to bind heavy metals. The obtained clinical and cytogenetic results indicated an improvement of health status, i.e. the prevalence of hematological alterations were reduced to half in females and to 43% in males. Similar reduction was also observed in case of Metabolic X Syndrome. Among the genotoxicology biomarkers the blastic transformation of lymphocytes (LI) was doubled. Significant changes in the cytogenetic parameters (CA and SCE) were not

observed due to the relatively short duration of the study. The present results are unique compared to relevant literature (21-23, 26, 27) since we attempted to compare the data obtained from the genotoxicology monitor with the clinical findings. The results of the applied chemoprevention alone suggest a possible means of intervention in case of exposure to heavy metals even under the occupational health limits, on the other hand chemoprevention may not replace a proper risk management.

Acknowledgements

This work was supported by the Hungarian grants NKFP 1/016/2001 and NKFP 1/B-047/2004.

References

1. Watanabe T, Endo A, Kato Y, *et al.* Cytogenetics and cytokinetics of cultured lymphocytes from benzene-exposed workers. Int Arch Occ Env Health 1980; 46: 31-41.
2. Aksoy M, Erdem S. Follow up study on the mortality and the development of leukemia in 44 pancytopenic patients with chronic exposure to benzene. Blood 1978; 52: 285-92.
3. Morimoto K, Wolff S. Increase of sister chromatid exchange and perturbations of cell division kinetics in human lymphocytes by benzene metabolites. Cancer Res 1980; 40: 1189-93.
4. Paci E, Buiatti E, Costantini AS, *et al.* Aplastic anemia, leukemia and other cancer mortality in a cohort of shoe workers exposed to benzene. Scand J Work Env Health 1989; 15: 313-8.
5. Snyder R. Recent developments in the understanding of benzene toxicity and leukemogenesis. Drug Chem Toxicol 2000; 23 (1): 13-25.
6. Albertini R, Clewell H, Himmelstein MW, *et al.* The use of non-tumor data in cancer risk assessment: reflections on butadiene, vinyl chloride, and benzene. Regul Toxicol Pharm 2003; 37: 105-32.
7. Bloemen LJ, Youk A, Bradley TD, *et al.* Lympho-haematopoietic cancer risk among chemical workers exposed to benzene. Occup Environ Med 2004; 61: 270-4.
8. Sorahan T, Kinlen LJ, Doll R. Cancer risks in a historical UK cohort of benzene exposed workers. Occup Environ Med 2005; 62: 231-6.
9. Rothman N, Smith MT, Hayes RB, *et al*. Benzene poisoning, a risk factor for hematological malignancy, is associated with NQO1 ^{609}C®T mutation and rapid fractional excretion of chlorzoxazone. Cancer Res 1997; 57: 2839-42.
10. Tompa A, Jakab MG, Major J. Risk management among benzene-exposed oil refinery workers. Int J Hyg Env Health 2005; 208: 509-16.
11. Major J, Jakab M, Biró A, *et al.* Chemoprevention by a chelating agent among galvanisers and goldsmiths occupationally exposed to non-ferrous, precious and heavy metals. In: Cser MA, Sziklai László I, Étienne JC, *et al*. Metal Ions in Biology and Medicine, John Libbey, Paris: 2004; 8: 548-51.
12. Major J, Jakab MG, Tompa A. Genotoxicological investigation of hospital nurses occupationally exposed to ethylene-oxide: I. Chromosome aberrations, sister-chromatid exchanges, cell cycle kinetics, and UV-induced DNA synthesis in peripheral blood lymphocytes. Environ Mol Mutagen 1996; 27: 84-92.
13. Major J, Jakab MG, Tompa A. Working condition-related improvement in genotoxicological parameters of Hungarian road pavers. J Tox Environ Health 2001; 62: 319-31.
14. Tompa A, Jakab MG, Biró A, *et al.* Chemical safety and health conditions among Hungarian hospital nurses. Ann NY Acad Sci 2006; 1076: 635-48.
15. Tompa A, Jakab MG, Biró A, *et al*. Health, genotoxicology, and immune status of road pavers in Hungary. J Occup Environ Hyg 2007; 4: 154-62.
16. Jakab MG, Klupp T, Besenyei K *et al.* Formaldehyde-induced chromosomal aberrations and apoptosis in peripheral blood lymphocytes of personnel working in pathology departments. Mutat Res 2010; 698: 11-7.
17. Moorhead PS, Nowell PC, Mellman WJ, *et al.* Chromosome preparation of leukocytes cultured from human peripheral blood. Exp Cell Res 1960; 20: 613-6.
18. Perry P, Wolff S. New Giemsa method for the differential staining of sister chromatids. Nature 1974; 251: 156-8.
19. Carrano AV, Natarajan AT. Considerations on population monitoring using cytogenetic techniques. ICPEMC Publ.No.14. Mutat Res 1988; 204: 379-406.
20. Kasuba V, Rozgaj R, Sentija K. Cytogenetic changes in subjects occupationally exposed to benzene. Chemosphere 2000; 40: 307-10.
21. Bérces J, Otos M, Szirmai S, *et al.* Using the micronucleus assay to detect genotoxic effects of metal ions. Environ Health Persp 1993; 101 suppl 3: 11-3.
22. Katsifis SP, Shamy M, Kinney LP, *et al.* Interaction of nickel with UV-light in the induction of cytogenetic effects in human peripheral lymphocytes. Mutat Res – Fund Mol M 1998; 422: 331-7.
23. Hüttner E, Götze A, Nikolova T. Chromosomal aberrations in humans as genetic endpoints to assess the impact of pollution. Mutat Res 1999; 445: 251-7.

24. Rozgaj R, Kasuba V, Fucic A. Genotoxicity of cadmium chloride in human lymphocytes evaluated by the comet assay and cytogenetic tests. J Trace Elem Med Bio 2002; 16: 187-92.
25. Aksoy M, Dincol K, Akgün T, *et al.* Haematological effects of chronic benzene poisoning in 217 workers. Brit J Ind Med 1971; 28: 296-302.
26. Mabille V, Roels H, Jacquet P, *et al.* Cytogenetic examination of leucocytes of workers exposed to mercury vapour. Int Arch Occ Env Health 1984; 53: 257-60.
27. Leonard A, Bernard A. Biomonitoring exposure to metal compounds with carcinogenic properties. Environ Health Persp 1993; 101 suppl 3: 127-33.