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Research Article

Evaluation of Genetic Damage in Open-Cast Coal Mine Workers Using the Buccal Micronucleus Cytome Assay

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Coal is the largest fossil fuel source used for the generation of energy. However, coal extraction and its use constitute important pollution factors; thus, risk characterization and estimation are extremely important for the safety of coal workers and the environment. Candiota is located to the southeast of the state of Rio Grande do Sul and has the largest coal reserves in Brazil, and the largest thermal power complex in the state. In the open-cast mines, the coal miners are constantly exposed to coal dust. The human buccal micronucleus cytome (BMCyt) assay has been used widely to investigate biomarkers for DNA damage, cell death, and basal cell frequency in buccal cells. The aim of this study was to assess whether prolonged exposure to coal dust could lead to an increase in genomic instability, cell death, and frequency of basal cells using the BMCyt assay. In the analysis of epithelial cells, the exposed group (n = 41) presented with a significantly higher frequency of basal cells, micronuclei in basal and differentiated cells, and binucleated cells compared to the non-exposed group (n = 29). The exposed group showed a significantly lower frequency of condensed chromatin cells than the non-exposed group. However, we found no correlation between DNA damage and metal concentration in the blood of mine workers. DNA damage observed in the mine workers may be a consequence of oxidative damage resulting from exposure to coal residue mixtures. In addition, our findings confirm that the BMCyt assay can be used to identify occupational risk. Environ. Mol. Mutagen. 54:65–71, 2013.

Key words: BMCyt; open-cast coal mine; occupational exposure

INTRODUCTION

Coal is the largest fossil fuel source used for the generation of energy. However, coal extraction and its use constitute important pollution factors; thus, risk characterization and estimation are extremely important for the safety of coal workers and the environment. Candiota, which has the largest coal reserves in Brazil, is situated in the southeast of the state of Rio Grande do Sul (RS), approximately 400 km from Porto Alegre and 50 km from the Uruguayan border. The coal from the open-cast mines in this area is used locally to generate electricity at the largest thermal power complex in the state. During coal extraction, large quantities of coal dust particles are emitted, contributing to environmental pollution. Coal residues consist of a mixture of substances, including carbon, hydrogen, nitrogen, oxygen, sulfur, small mineral particles, and inorganic compounds in the ash [Chen et al., 2005].

Coal miners are constantly exposed to coal dust; this exposure increases their risk of developing several diseases, e.g., pneumoconiosis, progressive massive fibrosis,

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bronchitis, loss of lung function, emphysema, and even stomach, liver, and lung cancer [Rockette, 1977; Schins et al., 1995; Une et al., 1995; Beckman and Ames, 1997; Schins and Borm, 1999; Skowronek and Zemla, 2003]. Studies of coal miners using chromosomal aberration, micronucleus, and sister chromatid exchange assays have revealed higher levels of DNA and chromosomal damage with respect to control subjects [Sram et al., 1985; Donbak et al., 2005; Leon-Mejia et al., 2011].

A wide range of methods is currently used for the detection of the early biological effects of DNA-damaging agents in occupational settings. In recent years, the human buccal micronucleus cytome (BMCyt) assay has been used increasingly to investigate biomarkers for DNA damage, cell death, and basal cell frequency in buccal cells [Holland et al., 2008]. Its growing use may be explained by the relative technical simplicity of the assay and the variety of complementary toxicological endpoints that are evaluated [Bonassi et al., 2011].

In the present study, we used the BMCyt assay to assess whether prolonged exposure to coal dust leads to an increase in genomic instability, cell death, and the frequency of basal cells.

METHODS

Study Population and Sample Collection

This study was approved by the Committee on Research Ethics at Universidade Federal do Rio Grande do Sul (N. 2007978), and written informed consent was obtained from each individual before the research began.

Subjects from Candiota and Bagé were sampled from March 2009 to March 2012. Candiota is the city where the coal mine is situated, and Bagé is a neighboring city. This study involved a total of 70 individuals, 29 non-exposed subjects and 41 that were occupationally exposed to coal. The workers were from a Candiota open-cast coal mine, where they were involved with coal extraction and coal transport until its arrival at the storage centers; all of the workers were exposed to large quantities of coal dust. All workers reported using some kind of protection during the workday, e.g., gloves, breathing masks, glasses, waterproof boots, etc. The non-exposed group consisted of individuals from Candiota and Bagé with no known exposure to genotoxic agents, including coal, radiation, and chemicals.

Buccal cell samples were obtained by gently rubbing the inside of the cheeks (right and left side) with a cytobrush, which was then immersed in 5 mL of cold saline (0.9% [wt/vol] aqueous NaCl) in a conical tube and transported under refrigeration to the laboratory (BMCyt assay). Blood samples were collected by venipuncture using vacutainers with Ethylenediamine tetraacetic acid (EDTA) and processed as quickly as possible; the blood samples were transported to the laboratory at or below 8°C and processed (chemical analysis).

All individuals in the study were asked to answer a Portuguese-language version of a questionnaire from the International Commission for Protection against Environmental Mutagens and Carcinogens [Carrano and Natarajan, 1988] and to participate in a face-to-face interview, which included standard demographic data (age, gender, etc.) and questions concerning medical issues (exposure to X-rays, vaccinations, medication, etc.), life style (smoking, coffee and alcohol consumption, diet, etc.), and occupation (number of working hours per day, protective measures adopted). In all groups, individuals who smoked more than 20 cigarettes per day were considered as smokers and removed from our sample [Hoffmann et al., 2005].

BMCyt Assay

Buccal cell samples in cold saline were centrifuged at 1,500 rpm for 8 min, and the sedimented buccal cells were washed twice with saline and once with Carnoy's fixative (methanol and glacial acetic acid 3:1) under the same centrifugation conditions. The cell suspensions were dropped onto slides and allowed to air dry. The slides were stained with 2% Giemsa solution for 10 min, rinsed in distilled water, and air dried. For each individual, the frequency of the various cell types in the assay is represented as the number of cells in 2,000, as suggested by Thomas et al. [2009].

Chemical Analysis

The trace element content of total blood from the coal miners and non-exposed group was analyzed by Particle-Induced X-ray Emission (PIXE) [Johansson et al., 1995; dos Santos et al., 2010], as were soil samples from Candiota (geographic coordinates: 31° 34′ 11.7″ S/53° 41′ 46.9″ W) and Bagé (31° 19′ 53″ S/54° 06′ 25″ W). This technique identifies the multi-elemental composition of a sample by the X-rays emitted when a proton beam interacts with the atoms of the sample.

The blood samples were maintained at 4°C until sample preparation, when the samples were dried at 37°C for 48 h. Soil samples at a depth of 20 cm were collected using a spade from five random locations in July 2010; gravel, stones, and plant material were removed from the samples. The subsamples were homogenized in order to form a composite sample for each sampling area. Approximately 500 g to 1 kg of surface soil was collected using stainless steel spatulas, placed in a glass container protected from light, and maintained at 4°C until sample preparation.

Briefly, the dried blood and soil samples were homogenized and pressed into thick pellets for PIXE analysis. The experiments were carried out at the Ion Implantation Laboratory of the Physics Institute, Federal University of Rio Grande do Sul. A 3 MV Tandetron accelerator provided a 2 MeV proton beam with an average current of 3 nA and 0.5 nA for the blood and soil samples irradiation, respectively. The samples were irradiated for 400 s (blood) and 600 s (soil). During the experiments, the pressure inside the reaction chamber was keep at approximately 10^{-6} mbar. The X-rays produced by the samples were detected using a Si(Li) detector with an energy resolution of approximately 150 eV at 5.9 keV. The PIXE system was calibrated using a range of reference materials, including apple leaves, bovine liver, fish muscle, and buffalo river sediment, among others. The present study used bovine liver and buffalo river sediment from the National Institute of Standards and Technology (reference materials 1577b and 8704, respectively). The standardization procedure adopted was as described by Johansson et al. [1995] and included all experimental parameters important for the PIXE spectra quantification. The spectra were fitted and the elemental concentrations were obtained using the GUPIXWIN software package [Campbell et al., 2000]. The data are expressed as parts per million (mg/kg).

Statistical Analysis

The normality of the variables was evaluated by the Kolmogorov–Smirnov test. Students' t-test was used to compare the demographic characteristics of the study populations. Statistical analysis of the differences in the parameters measured by the BMCyt assay was carried out using the non-parametric Mann–Whitney U test. Correlations between different variables were determined by Spearman's rank correlation test, when applicable. The critical level for rejection of the null hypothesis

was considered to be a 2-tailed *P*-value of 5%. The analyses were performed using GraphPad Prism version 5.00 for Windows software.

RESULTS

No significant difference in average age was detected between the non-exposed (46.69 ± 18.11 years) and exposed groups (36.86 ± 14.00 years) (t-test). The mean exposure time for the exposed group was 13.83 ± 8.12 years (length of employment in coal mines).

The results of the BMCyt assay are presented in Table I. The evaluation of epithelial cells revealed a higher frequency of basal cells in the coal dust-exposed individuals than in the non-exposed group ($P < 0.001$, Mann–Whitney U test). The exposed group presented with a significantly higher number of micronuclei in basal ($P < 0.001$) and differentiated cells ($P < 0.001$), and binucleated cells ($P < 0.05$) as compared with the non-exposed group. The exposed group had a significantly lower frequency of cells with condensed chromatin than the non-exposed group ($P < 0.001$). No correlation was found between age and exposure time in relation to the parameters tested by the BMCyt assay (data not shown).

TABLE I. BMCyt Assay for Cells Collected from the Non-Exposed and Exposed Groups (Mean ± Standard Deviation)

Parameters		Non-Exposed	Exposed
Basal cells			
	Normal	1.00 ± 1.31	14.34 ± 6.73***
	MN	0.03 ± 0.19	0.51 ± 0.78***
Differentiated cells			
DNA damage	MN	0.21 ± 0.41	3.10 ± 2.22***
	Nuclear buds	6.93 ± 3.46	7.66 ± 3.39
	Binucleated cells	10.79 ± 3.67	13.26 ± 4.90*
Cell death	Condensed chromatin	11.79 ± 3.53	5.73 ± 3.62***
	Karyorrhectic cells	10.00 ± 2.42	10.80 ± 4.98
	pyknotic cells	8.45 ± 2.98	8.46 ± 3.23
	Karyolytic cells	13.07 ± 3.00	13.12 ± 4.45

*Data significant in relation to the non-exposed group at $P < 0.05$; ***$P < 0.001$ (Mann–Whitney U test).

Table II presents the cross-correlation analysis between the biomarkers of the BMCyt assay for all individuals in this study. Basal cells showed a positive correlation with micronuclei in basal cells ($r = 0.512$, $P < 0.001$) and differentiated cells ($r = 0.722$, $P < 0.001$), binucleated cells ($r = 0.352$, $P = 0.003$), and karyorrhectic cells ($r = 0.266$, $P = 0.026$). Differentiated cells with micronuclei showed a positive correlation with micronuclei in basal cells ($r = 0.519$, $P < 0.001$), binucleated cells ($r = 0.327$, $P = 0.006$), and karyorrhectic cells ($r = 0.331$, $P < 0.001$). Karyolytic cells had a positive correlation with karyorrhectic cells ($r = 0.188$, $P = 0.024$). Condensed chromatin cells showed a negative correlation with basal cells ($r = -0.654$, $P < 0.001$) and micronuclei in basal cells ($r = -0.302$, $P = 0.011$) and differentiated cells ($r = -0.498$, $P < 0.001$).

The results of the chemical analyses performed via the PIXE technique to characterize the metal content in blood and soil samples are shown in Tables III and IV, respectively. All of the trace elements detected in the samples are listed in the tables. No significant difference was observed between the blood samples from the coal workers and the non-exposed group. Among the metals, the most common were magnesium, aluminum, copper, and zinc. Phosphorous, sulfur, and lead were significantly higher in soil from Candiota than Bagé ($P < 0.001$).

TABLE II. Cross-Correlation Between the Biomarkers of the BMCyt Assay for the Combined Groups

		Basal cells	MN in basal cells	MN in differentiated cells	nuclear buds	Binucleated cells	Condensed chromatin	karyorrhectic cells	Pyknotic cells
MN in basal cells	Spearman correlation	0.512							
	Sig. (2-tailed)	0.000							
MN in differentiated cells	Spearman correlation	0.722	0.519						
	Sig. (2-tailed)	0.000	0.000						
Nuclear buds	Spearman correlation	NS	NS	NS					
	Sig. (2-tailed)								
Binucleated cells	Spearman correlation	0.352	NS	0.327	NS				
	Sig. (2-tailed)	0.003		0.006					
Condensed chromatin	Spearman correlation	−0.654	−0.302	−0.498	NS	NS			
	Sig. (2-tailed)	0.000	0.011	0.000					
Karyorrhectic cells	Spearman correlation	0.266	NS	0.331	NS	NS	NS		
	Sig. (2-tailed)	0.026		0.000					
Pyknotic cells	Spearman correlation	NS	NS	NS	NS	NS	NS	NS	
	Sig. (2-tailed)								
Karyolytic cells	Spearman correlation	NS	NS	NS	NS	NS	NS	0.188	NS
	Sig. (2-tailed)							0.024	

MN: micronuclei; NS: not significant.

TABLE III. Elemental Composition of Blood Samples by PIXE Analysis (Results in mg/kg)

Heavy metal	Non-Exposed	Exposed
Magnesium	144.2 ± 76.7	177.7 ± 86.3
Aluminum	110.4 ± 51.7	125.1 ± 37.3
Sulfur	5,597 ± 695.9	5,206 ± 581.4
Potassium	6,462 ± 2,920	5,910 ± 3,286
Iron	2,312 ± 504.9	2,003 ± 438.3
Copper	4.90 ± 1.81	5.20 ± 1.07
Zinc	40.34 ± 7.0	42.4 ± 9.84

TABLE IV. Elemental Composition of Soil Samples by PIXE Analysis (Results in mg/kg)

Element	Non-Exposed	Exposed
Magnesium	1,365 ± 448.1	1,623 ± 231.1
Aluminum	42,640 ± 4,841	69,650 ± 2,003
Silicon	415,000 ± 45,950	289,600 ± 4,083
Phosphorus	155.1 ± 16.26	282.0 ± 203.5***
Sulfur	104.6 ± 18.75	286.8 ± 12.87***
Potassium	12,230 ± 1,767	7,133 ± 585.8
Calcium	1,416 ± 139.6	1,049 ± 7.78
Titanium	6,080 ± 2,077	7,855 ± 284.5
Chromium	31.68 ± 14.86	57.22 ± 14.73
Manganese	753.4 ± 126.8	616.0 ± 84.31
Iron	15,450 ± 2,035	51,640 ± 6,815
Copper	55.07 ± 10.11	72.90 ± 20.07
Zinc	21.40 ± 3.92	49.95 ± 18.05
Zirconium	143.7 ± 61.41	536.0 ± 238.7
Lead	22.77 ± 10.54	61.98 ± 0.12***

***$P < 0.001$ in relation to the non-exposed group. Mann–Whitney U test.

DISCUSSION

Mineral coal production in RS, the southern state of Brazil, exceeds 32×10^9 tons, representing approximately 89% of the Brazilian reserves. This region produces 38% of the country's coal. Open-cast coal mine workers are potentially exposed to coal dust containing quartz, trace metals, inorganic minerals, and organic aromatic compounds [Leon et al., 2007]. There are only a few studies on the occupational hazard effects (genotoxicity) for coal miners; in RS, genotoxic endpoints related to open-cast coal extraction have only been evaluated in rodent populations [da Silva et al., 2000].

In this study, the BMCyt assay was used to investigate biomarkers for DNA damage, cell death, and regenerative potential in the buccal cells of non-exposed and coal dust-exposed groups. This study is the first to use the BMCyt assay to evaluate all possible endpoints in coal miners. Kumar et al. [2011] utilized buccal cells to analyze the genotoxic effects of coal dust only in relation to the frequency of micronuclei in differentiated cells.

Results obtained from buccal cells revealed significant increases in micronuclei and binucleated cells in differentiated cells and in micronuclei in basal cells among the coal workers. In our study, there was no correlation between age and time of service in relation to the BMCyt parameters. Biomonitoring studies of the peripheral lymphocytes of coal workers demonstrated increased adduct formation and increases of non-cellular and cellular sources of reactive oxygen species (ROS), which can induce oxidative DNA damage [Schins et al., 1995; Schoket et al., 1999; Donbak et al., 2005; Celik et al., 2007; Leon-Mejia et al., 2011]. Other studies using different assays have also demonstrated higher levels of DNA damage in individuals exposed to coal. In buccal cells, Kumar et al. [2011] observed higher frequencies of micronuclei in individuals exposed to coal. Micronuclei are formed mainly from chromatids or chromosome fragments, which remain excluded from the main cell nucleus following mitosis [Holland et al., 2008], indicating an increased risk of cancer [He et al., 2000]. Buccal cells are in constant contact with the environment, which suggests that the oral epithelium is an important target site for the toxicants in coal dust; therefore, our results revealed higher genotoxicity in coal workers (higher frequency of micronuclei in differentiated and basal cells) compared with the unexposed group due to the coal workers' exposure to coal dust.

Binucleated cells were also found to be more frequent in the exposed group. The precise significance of these cells is not known, but they may be indicative of the failure of cytokinesis. As shown in a study using the BMCyt assay to study Down syndrome [Thomas et al., 2008], the binucleated/mononucleated cell ratio could be an important biomarker for identifying individuals with cytokinesis failure caused by higher than normal rates of aneuploidy. Aneuploidy stands out as a consistent marker of malignancy [Sanchez-Siles et al., 2011].

The cell death analyses revealed a lower frequency of cells with condensed chromatin in the exposed group. An increased percentage of cells with condensed chromatin cells occurs in the pre-keratinization process [Pindborg et al., 1980] and represents cytotoxicity [Thomas et al., 2008]. Thus, it is believed that the increase of this cell type in unexposed individuals must have occurred at random.

In this study, the exposed group demonstrated higher frequencies of basal cells compared with the non-exposed group. The proportion of basal cells in buccal cells is an indication of the regenerative capacity of this tissue [Thomas et al., 2009]. It has been postulated that repeated exposure to cytotoxic agents can result in chronic cell injury, compensatory cell proliferation (regenerative capacity), hyperplasia, and ultimately tumor development [Swenberg, 1993; Mally and Chipman, 2002]. In concordance, the results of the cross-correlation study suggest a significant positive relationship between basal cells with micronuclei and differentiated cells with micronuclei, binucleated cells, and karyorrhectic cells. The positive

correlation between basal cells and karyorrhectic cells suggests that the latter are derived directly from basal cells, similar to the observations of Thomas et al. [2008]. These cytogenetic alterations in coal mine workers cells may be due to oxidative damage.

The primary target cells of inhaled coal dust particles are macrophages and epithelial cells. Activated macrophages (phagocytosis toxicity) produce excessive amounts of ROS and cytokines. ROS are believed to play a major role in the primary genotoxicity of particles, which may be derived from their surface properties, the presence of transition metals, intracellular iron mobilization, and/or lipid peroxidation [Schins, 2002]. In vitro studies have led to the identification of the primary genotoxic properties of particles, whereas recent in vivo studies provide further support for the correlation between particle-induced lung inflammation and secondary genotoxicity. The formation of ROS from inflammatory cells is considered to be a biomarker for the genotoxicity of non-fibrous particles (coal dust). Previously, some authors observed the increased formation of 8-OHdG in peripheral blood lymphocytes and altered antioxidant capacity in the serum and red blood cells of coal miners [Schins et al., 1995; Schins and Borm, 1999]. When there is excessive production of ROS, or when there are insufficient in vivo defense mechanisms, oxidative stress may occur. This stress may result in DNA damage, lipid peroxidation, protein modification, membrane disruption, and mitochondrial damage [Zhai et al., 2002]; all of these modifications are capable of inducing cytogenetic damage.

Our analyses of blood samples revealed no significant difference in metal concentrations between the exposed and non-exposed groups using PIXE. However, higher concentrations of magnesium, aluminum, copper, and zinc were observed in the cells from coal miners than in those from non-exposed individuals. In addition, soil samples from Candiota demonstrated significant increases of phosphorous, sulfur, and lead. In general, metal genotoxicity is caused by indirect mechanisms. Recently, Beyersmann and Hartwig [2008] described three predominant mechanisms of metal genotoxicity: (1) interference with cellular redox regulation and induction of oxidative stress, which may cause oxidative DNA damage or trigger signaling cascades that lead to the stimulation of cell growth; (2) inhibition of major DNA repair systems that results in genomic instability and the accumulation of critical mutations; and (3) deregulation of cell proliferation by the induction of signaling pathways or the inactivation of growth controls, e.g., tumor suppressor genes. Our results from the BMCyt assay provided support for the occurrence of all of these mechanisms.

Despite the significant differences in metal concentrations in soil samples, we found no significant differences in metals between exposed and non-exposed blood measurements. This result can be due to synergistic and antagonistic effects that can be expected from inorganic and organic contaminants in the organism, leading to different unpredicted biological effects. A very important point to be considered when discussing metal presence in blood samples is the interaction among inorganic elements in biological organisms. Shi and Wang [2004] suggested that previous exposure to one element may affect the bioaccumulation of other elements. This kind of interaction might have influenced the absorption of inorganic elements by the mine workers.

Finally, DNA damage in the mine workers may be a consequence of oxidative damage resulting from exposure to coal residue mixtures containing metals and polycyclic aromatic hydrocarbons (PAH) [Pahlich, 1975]. Da Silva et al. [2000] and Dallarosa et al. [2005] demonstrated that environmental samples from Candiota had a high concentration of hydrocarbons. All technological processes associated with an open fire or temperatures between 400°C and 600°C, which may lead to the production of PAH, should be considered potentially hazardous [Sram et al., 1985]. PAH can induce DNA lesions as single-strand breaks via DNA repair mechanisms [Brescia et al., 1999; Pavanello et al., 2005]; electrophilic metabolites that covalently interact with DNA [Singh et al., 2007] can form adducts with purines, especially with guanine, after metabolic activation by the P450 enzymatic complex [Baird et al., 2005].

Occupational hazard effects in coal miners, in relation to DNA damage, were observed when compared with a non-exposed group. Because of the complex nature of coal mining environments, it is difficult to relate genotoxic effects to a specific agent or compound. The DNA damage observed in the mine workers may be a consequence of oxidative damage resulting from their exposure to coal residue mixtures, including metals. Our results also confirmed that the BMCyt assay could be used to assess biomarkers for occupational exposure.

AUTHOR CONTRIBUTIONS

Drs. Paula Rohr, Juliana Da Silva and Kátia Kvitko designed the study and applied for Research Ethics Board approval. Dr. Paula Rohr contacted the coal miners. Drs. Paula Rohr and Fernanda Rabaioli da Silva collected the samples. Drs. Paula Rohr and Fernanda Rabaioli da Silva did buccal micronucleus cytome assay. Drs. Carla E. I. dos Santos, Johnny F. Dias and MSc Rafaela Debastiani measured metal levels. BSc Merielen Sarmento, Carem Porto scored MN slides. Drs. Paula Rohr and Juliana da Silva analyzed the results. Dr. Paula Rohr, Juliana da Silva, and Kátia Kvitko prepared draft figures and tables. Dr. Paula Rohr prepared the manuscript draft with important intellectual input from Drs. Juliana Da Silva and Kátia Kvitko. All authors approved the final manuscript.

Drs. Paula Rohr, Juliana Da Silva, and Kátia Kvitko had complete access to the study data.

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REFERENCES

Baird WM, Hooven LA, Mahadevan B. 2005. Carcinogenic polycyclic aromatic hydrocarbon-DNA adducts and mechanism of action. Environ Mol Mutagen 45:106–114.

Beckman KB, Ames BN. 1997. Oxidative decay of DNA. J Biol Chem 272:19633–19636.

Beyersmann D, Hartwig A. 2008. Carcinogenic metal compounds: Recent insight into molecular and cellular mechanisms. Arch Toxicol 82:493–512.

Bonassi S, Coskun E, Ceppi M, Lando C, Bolognesi C, Burgaz S, Holland N, Kirsh-Volders M, Knasmueller S, Zeiger E, Carnesoltas D, Cavallo D, da Silva J, de Andrade VM, Demircigil GC, Dominguez Odio A, Donmez-Altuntas H, Gattas G, Giri A, Giri S, Gomez-Meda B, Gomez-Arroyo S, Hadjidekova V, Haveric A, Kamboj M, Kurteshi K, Martino-Roth MG, Montero Montoya R, Nersesyan A, Pastor-Benito S, Favero Salvadori DM, Shaposhnikova A, Stopper H, Thomas P, Torres-Bugarin O, Yadav AS, Zuniga Gonzalez G, Fenech M. 2011. The HUman MicroNucleus project on eXfoLiated buccal cells (HUMN(XL)): The role of life-style, host factors, occupational exposures, health status, and assay protocol. Mutat Res 728:88–97.

Brescia G, Celotti L, Clonfero E, Neumann GH, Forni A, Foa V, Pisoni M, Ferri GM, Assennato G. 1999. The influence of cytochrome P450 1A1 and glutathione S-transferase M1 genotypes on biomarker levels in coke-oven workers. Arch Toxicol 73:431–439.

Campbell JL, Hopman TL, Maxwell JA, Nejedly Z. 2000. The Guelph PIXE software package III: Alternative proton database. Nucl Instr Meth Phys Res B 170:193–204.

Carrano AV, Natarajan AT. 1988. International Commission for Protection Against Environmental Mutagens and Carcinogens. ICPEMC publication no. 14. Considerations for population monitoring using cytogenetic techniques. Mutat Res 204:379–406.

Celik M, Donbak L, Unal F, Yuzbasioglu D, Aksoy H, Yilmaz S. 2007. Cytogenetic damage in workers from a coal-fired power plant. Mutat Res 627:158–163.

Chen Y, Shah N, Huggins FE, Huffman GP. 2005. Transmission electron microscopy investigation of ultrafine coal fly ash particles. Environ Sci Technol 39:1144–1151.

da Silva J, de Freitas TR, Heuser V, Marinho JR, Erdtmann B. 2000. Genotoxicity biomonitoring in coal regions using wild rodent *Ctenomys torquatus* by Comet assay and micronucleus test. Environ Mol Mutagen 35:270–278.

Dallarosa JB, Teixeira EC, Pires M, Fachel J. 2005. Study of the profile of polycyclic aromatic hydrocarbons in atmospheric particles (PM10) using multivariate methods. Atmos Environ 39:6587–6596.

Donbak L, Rencuzogullari E, Yavuz A, Topaktas M. 2005. The genotoxic risk of underground coal miners from Turkey. Mutat Res 588:82–87.

dos Santos CEI, da Silva LRM, Boufleur LA, Debastiani R, Stefenon CA, Amaral L, Yoneama ML, Dias JF. 2010. Elemental characterisation of Cabernet Sauvignon wines using Particle-Induced X-ray Emission (PIXE). Food Chem 121:244–250.

He JL, Jin HY, Jin LF, Gao SY. 2000. Monitoring of human exposure to radiation with the binucleated lymphocyte micronucleus assay. Biomed Environ Sci 13:32–36.

Hoffmann H, Hogel J, Speit G. 2005. The effect of smoking on DNA effects in the comet assay: A meta-analysis. Mutagenesis 20:455–466.

Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, Knasmueller S, Fenech M. 2008. The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: The HUMN project perspective on current status and knowledge gaps. Mutat Res 659:93–108.

Johansson SAE,Campbell JL,Malmqvist KG, editors. 1995. Particle-Induced X-ray Emission Spectrometry (PIXE). New York:John Wiley.

Kumar A, Yadav A, Giri SK, Dev K, Gautam SK, Gupta R, Aggarwal N. 2011. Effect of genetic polymorphism of GSTM1 and GSTT1 genotypes on cytogenetic biomarkers among coaltar workers. Environ Toxicol Pharmacol 32:128–135.

Leon G, Perez LE, Linares JC, Hartmann A, Quintana M. 2007. Genotoxic effects in wild rodents (*Rattus rattus* and *Mus musculus*) in an open coal mining area. Mutat Res 630:42–49.

Leon-Mejia G, Espitia-Perez L, Hoyos-Giraldo LS, Da Silva J, Hartmann A, Henriques JA, Quintana M. 2011. Assessment of DNA damage in coal open-cast mining workers using the cytokinesis-blocked micronucleus test and the comet assay. Sci Total Environ 409:686–691.

Mally A, Chipman JK. 2002. Non-genotoxic carcinogens: early effects on gap junctions, cell proliferation and apoptosis in the rat. Toxicology 180:233–248.

Pahlich E. 1975. Effect of SO-2-pollution on cellular regulation. A general concept of the mode of action of gaseous air contamination. Atmos Environ 9:261–263.

Pavanello S, Pulliero A, Siwinska E, Mielzynska D, Clonfero E. 2005. Reduced nucleotide excision repair and GSTM1-null genotypes influence anti-B[a]PDE-DNA adduct levels in mononuclear white blood cells of highly PAH-exposed coke oven workers. Carcinogenesis 26:169–175.

Pindborg JJ, Reibel J, Roed-Peterson B, Mehta FS. 1980. Tobacco-induced changes in oral leukoplakic epithelium. Cancer 45:2330–2336.

Rockette HE. 1977. Cause specific mortality of coal miners. J Occup Med 19:795–801.

Sanchez-Siles M, Ros-Llor I, Camacho-Alonso F, Lopez-Jornet P. 2011. A novel application of the buccal micronucleus cytome assay in oral lichen planus: A pilot study. Arch Oral Biol 56:1148–1153.

Schins RP. 2002. Mechanisms of genotoxicity of particles and fibers. Inhal Toxicol 14:57–78.

Schins RP, Borm PJ. 1999. Mechanisms and mediators in coal dust induced toxicity: A review. Ann Occup Hyg 43:7–33.

Schins RP, Schilderman PA, Borm PJ. 1995. Oxidative DNA damage in peripheral blood lymphocytes of coal workers. Int Arch Occup Environ Health 67:153–157.

Schoket B, Poirier MC, Mayer G, Torok G, Kolozsi-Ringelhann A, Bognar G, Bigbee WL, Vincze I. 1999. Biomonitoring of human genotoxicity induced by complex occupational exposures. Mutat Res 445:193–203.

Shi D, Wang W-X. 2004. Modification of trace metal accumulation in the green mussel *Perna viridis* by exposure to Ag, Cu, and Zn. Environ Pollut 132:265–277.

Singh R, Sram RJ, Binkova B, Kalina I, Popov TA, Georgieva T, Garte S, Taioli E, Farmer PB. 2007. The relationship between biomarkers of oxidative DNA damage, polycyclic aromatic hydrocarbon DNA adducts, antioxidant status and genetic susceptibility following exposure to environmental air pollution in humans. Mutat Res 620:83–92.

Skowronek J, Zemla B. 2003. Epidemiology of lung and larynx cancers in coal mines in Upper Silesia—Preliminary results. Health Phys 85:365–370.

Sram RJ, Hola N, Kotesovec F, Novakova A. 1985. Cytogenetic analysis of peripheral blood lymphocytes in glass workers occupationally exposed to mineral oils. Mutat Res 144:277–280.

Swenberg JA. 1993. Cell proliferation and chemical carcinogenesis: Summary and future directions. Environ Health Perspect 101 (Suppl 5):153–158.

Thomas P, Harvey S, Gruner T, Fenech M. 2008. The buccal cytome and micronucleus frequency is substantially altered in Down's syndrome and normal ageing compared to young healthy controls. Mutat Res 638:37–47.

Thomas P, Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, Knasmueller S, Fenech M. 2009. Buccal micronucleus cytome assay. Nat Protoc 4:825–837.

Une H, Esaki H, Osajima K, Ikui H, Kodama K, Hatada K. 1995. A prospective study on mortality among Japanese coal miners. Ind Health 33:67–76.

Zhai R, Liu G, Ge X, Yang C, Huang C, Wu C, Christiani DC. 2002. Genetic polymorphisms of MnSOD, GSTM1, GSTT1, and OGG1 in coal workers' pneumoconiosis. J Occup Environ Med 44:372–377.

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