Micronucleus frequencies in lymphocytes and buccal cells in formaldehyde exposed workers

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Abstract

Formaldehyde (FA) is a high-volume production chemical produced worldwide with a large range of industrial and medical uses. Listed, since 2004, by IARC as a human carcinogen, FA status was recently revised by the US government who reclassified this compound as known to be a human carcinogen. Both reclassifications are based on sufficient evidence of carcinogenicity from epidemiologic studies, supporting data on mechanisms of carcinogenesis and experimental evidence in animals. The highest level of human exposure to FA occurs in occupational settings. Consistent findings of increased risks of certain types of rare cancers were found among workers with higher measures of exposure to FA (exposure level or duration). The aim of the present study was to assess the genotoxic effects of occupational exposure to FA. A group of pathology and anatomy workers was evaluated for micronuclei in lymphocytes and in exfoliated buccal cells. Genotoxic endpoints are of great interest in the risk assessment of occupational carcinogens because they precede by a long time the potential health effects, thus offering a greater potential for preventive measures. Micronuclei in lymphocytes and in exfoliated buccal cells were significantly higher in the exposed subjects when compared with controls. Air sampling was performed in the workers' breathing zone for representative working periods and an 8h-time weighted average was assessed. Results

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obtained confirm an association between genetic damage and occupational exposure to FA. Such results along with the recent implications of human carcinogenicity, point out the need for close monitoring of FA exposures. Implementation of effective control measures along with hazard prevention campaigns may be crucial to decrease the risk.
Keywords: formaldehyde, genotoxicity, micronucleus test, lymphocytes, exfoliated buccal cells.

1 Introduction

Over the years several epidemiological studies have revealed an increased risk of cancer development among workers exposed to chemicals [1]. Human monitoring is a frequently used approach to provide early warning signals for excessive exposure to toxic substances and for prediction of health risk [2].

Establishing the health outcomes of various activities and exposures requires information about the levels of exposure and the biological effects resulting from the interaction between the exposed organism and the chemical agent. Genomic damage is probably the primary basic cause of developmental and degenerative disease. It is well established that genomic damage may result from lifestyle factors, medical procedures (e.g., chemotherapy, radiotherapy), diet, individual susceptibility and environmental/occupational exposure to genotoxic compounds.

A wide range of bioindicators are currently used for the detection of early biological effects of genotoxic agents, namely cytogenetic alterations. The relevance of increased frequency of cytogenetic alterations as a cancer risk biomarker is further supported by epidemiological studies suggesting that a high frequency of chromosomal aberrations or micronucleus are predictive of an increase risk of cancer [3].

Micronucleus (MN) test is a sensitive and well established tool extensively used in human biomonitoring studies to assess DNA damage at chromosomal level [4]. Micronuclei are extra-nuclear DNA containing bodies formed as a result of chromosomal fragments or whole chromosomes not being incorporated into the daughter nuclei during nuclear division. Since MN represents a measure of both chromosome breakage and chromosome loss, an increased frequency of micronucleated cells can reflect exposure to genotoxic agents with clastogenic or aneugenic modes of action. In human studies, peripheral blood lymphocytes are usually the most frequently used tissue for MN test. However exfoliated epithelial cells (urothelial, buccal or nasal cells) are increasingly popular, as they can be easily collected and in some cases are better models than lymphocytes, since they are target tissues of some cancers.

Formaldehyde (FA) is a building-block for many chemical compounds with a wide range of industrial and medical uses. It is a high-volume production chemical produced worldwide, which many people are exposed to. At room temperature it is a flammable and colorless gas with a strong pungent odor. In 2006, the International Agency for Research on Cancer, IARC, reclassified FA from Group 2A (probably carcinogenic to humans) to Group 1 (carcinogenic to

humans) [5]. More recently, FA carcinogenic status was also revised by the US National Toxicology Programme (NTP). After a rigorous scientific review, FA was reassigned, in the NTP 12th Report on Carcinogens, as *known to be a human carcinogen* [6]. Both reclassifications are based on sufficient evidence of carcinogenicity from studies in humans, supporting studies on mechanisms of carcinogenesis and experimental evidence in animals.

Epidemiological studies demonstrated a causal relationship between occupational exposure to FA and cancer [5, 6]. Consisting findings of increased risks of certain types of rare cancers, namely nasopharyngeal and sinonasal carcinomas were found among workers with higher measures of exposure to FA (exposure level or duration). Studies have also suggested that FA may affect the lymphatic and blood systems and that exposure to FA may cause leukemia, particularly myeloid leukemia, in humans [7–9], yet due to mix results and biological implausibility the evidence for FA leukemogenicity remains controversial and needs further investigation [5, 10]. FA also caused tumors in two rodent species (rats and mice), at several different tissue sites, and by two different routes of exposure (inhalation and ingestion) [6].

The highest level of human exposure to FA occurs in occupational settings, namely in pathology and anatomy laboratories where it is used as a fixative and tissue preservative for more than 100 years. Indoor air analyses have consistently shown that the levels of airborne FA in anatomy laboratories exceed recommended exposure criteria [11, 12]. In these settings, absorption of FA occurs mainly through inhalation, affecting primarily the upper airways.

In the last decade a large number of toxicological studies were published about FA. FA's genotoxicity is confirmed in a variety of experimental systems ranging from bacteria to rodents. Although these positive findings may provide a basis for extrapolation to humans, the cytogenetic assays in humans have been inconsistent with both positive and negative outcomes [5]. Biological evidence of toxicity on distant-site such as peripheral lymphocytes and bone marrow is still insufficient and conflicting [13]. Some authors stated that since inhaled FA is rapidly metabolized it would not be expected to enter the systemic circulation and for that reason genotoxic and carcinogenic effects (leukemia) in animals and humans are limited to local effects, in the area of first contact [14, 15]. The principal aim of the present study was to evaluate MN frequency in both peripheral blood lymphocytes (PBLs) and in exfoliated buccal cells of FA-exposed workers from pathology anatomy laboratories. An association on MN frequency in these two biological tissues, a first contact tissue and a systemic tissue, was also investigated. Air sampling was also performed in order to determine FA-level of exposure of each worker.

2 Methods

2.1 Study population

The general characteristics of the studied population are summarized in Table 1. In total, 80 women were involved in the study, 38 working for at least one year

in pathology and anatomy laboratories located in Portugal, and 42 non-exposed control females, working in administrative offices in the same area and without occupational exposure history to formaldehyde (FA). Both groups were similar in age and smoking habits. Health conditions, medical history, medication, diagnostic tests (X-rays, etc) and important individual information namely age and smoking habits was elicited by means of questionnaires. Subjects that stopped smoking for more than two years were considered non-smokers. Workers also gave information related to working practices such as use of protective measures, years of employment, specific symptoms related to FA-exposure and chronic respiratory diseases and others. Ethical approval for this study was obtained from the Ethical Board of the National Institute of Health. All subjects were fully informed about the procedures and aims of this study and each subject prior to the study signed an informed consent form.

Table 1: Characteristics of the study population.

	Control Group (*N*=42)	**Exposed Group** (*N*=38)	*P*-value
Age (years) [a]	38.90 ± 11.99 (*20–61*)	39.68 ± 8.49 (*26–56*)	*0.74*[b]
Years of employment [a]	—	11.82 ± 7.10 (*1–32*)	
Smoking status			
Non-smokers	34 (81%)	30 (79%)	
Smokers	8 (19%)	8 (21%)	
Packs per year [a]	13.14 ± 9.76 (*2.85–32.25*)	9.07 ± 6.32 (*0.25–18.00*)	*0.34*[b]

[a]Mean ± SD (*range*).
[b]Student's *t*-test.

2.2 Environmental monitoring

Air sampling was performed in the workers breathing zone for representative working periods; analysis of the samples allowed the calculation of the 8h-TWA (time weighed average) level of exposure to FA for each subject. Air sampling and FA analysis were performed according to the NIOSH method no. 3500 [16].

2.3 Biologic samples collection

For lymphocytes culture peripheral blood samples were collected by venipuncture from each donor. After rinsing the mouth with tap water, buccal cells were collected from each donor. This was performed inside of both cheeks with different cytobrush to sample left and right areas of the mouth, to eliminate any unknown bias that may be caused by sampling one cheek only. All samples were collected between 10 and 11 am, coded, and analyzed under blind conditions.

2.4 Lymphocyte cytokinesis-block micronucleus assay

Aliquots of 0.5 mL of whole blood were used to establish duplicate lymphocyte cultures for cytokinesis-blocked micronucleus (MN) test, as described by Teixeira *et al.* [17]. Microscopic analyses were performed on a Nikon Eclipse E400 light microscope. To determine the total number of MN in binucleated cells, a total of 1000 binucleated cells with well-preserved cytoplasm (500 per replicate) were scored for each subject. MN were scored blindly by the same reader and identified according to the criteria defined by Fenech [18].

2.5 Buccal micronucleus cytome assay

Buccal micronucleus cytome assay was performed as described by Thomas *et al.* [19] with minor modifications.Briefly, for every subject, an individual sample from each cheek was collected and suspended in a 10 mL tube with buccal cell buffer (0.01M Tris-HCl, 0.1M EDTA, 0.02 NaCl, pH7). Cells were then spun for 10 min at 1500 rpm. Supernatant was removed and replaced with fresh buffer and washed twice more. After the last wash, cells were resuspended in small volume of buffer and placed in clean labeled slides (3 slides for each cheek). After air-drying, slides were fixed with cold ethanol: acetic acid (3:1, v/v) solution for 20 min. Air-dried slides were then treated in 5M HCl for 30 min and washed in running tap water for 3 min. Slides were then stained with Schiff's reagent at room temperature, in the dark, for at least 60 min. Next, slides were washed in running tap water for 5 min and 1 min in deionised water and left to dry in the dark for 10 min. Slides were then stained for 5 sec in 1% Fast Green solution and washed in ethanol 3 times, 2min each. After that, slides were allowed to air dry, then covered with coverslips and mounted with Entellan®. Slides were scored blindly by the same person using a Nikon Eclipse E400 light microscope. For each subject a total of 1000 cells were scored for basal cells, differentiated cells, binucleated cells and cell death parameters such as condensed chromatin, karyorrhectic, pyknotic and karyolitic cells. A total of 2000 differentiated cells were scored for micronuclei, nuclear buds and nucleoplasmic bridges. Cells containing micronuclei were confirmed under fluorescence to eliminate false positives. The scoring criteria were based in Tolbert *et al.* [20] and Thomas *et al.* [19].

2.6 Statistical analysis

All analyses were conducted using the SPSS for Windows statistical package 16.0. The statistical differences between means in the characteristics of the study population were assessed by means of students' *t*-test. All results obtained in the study were assessed for normal distribution using Kolmogorov-Smirnov test and graphic evaluation (histograms, Q-Q plots). Since both dependent variables departed from normal distribution non-parametric tests were applied to data. The effect of exposure on the level of genotoxicity was preliminarily tested through Mann-Whitney *U*-test. Multivariant analysis with Negative Binomial model (with log link) was applied to evaluate the contribution of exposure and potential

confounding factors to the response variables considered. Correlation between variables was analyzed by Spearman's correlation test. The level of significance considered was 0.05.

3 Results and discussion

To evaluate current exposure to formaldehyde (FA) in pathology and anatomy laboratories air samples were collected in the worker's breathing zone. The mean level of worker's exposure to FA was 0.35 ± 0.03 ppm (range 0.18–0.69 ppm). Current Portuguese occupational exposure limit is 0.30 ppm (ceiling level), meaning the maximum safe FA concentration that should never be exceeded during any length of time in a worker's breathing zone**.** The American Conference of Governmental Industrial Hygienists (ACGIH) also set a ceiling exposure limit of 0.30 ppm [21]. Our results show that in the laboratories analysed the pathology anatomy workers are exposed to air concentrations of FA that exceed national guidelines and ACGIH recommended exposure criteria. Several reports on indoor FA levels have consistently shown that the airborne concentrations in anatomy laboratories approach or exceed recommended guidelines [11, 12]. Implementation of security and hygiene measures, such as periodic air sampling, efficient air extraction, temperature control, as well as good practice campaigns, may be crucial to decrease FA exposure in this workplace.

The results of MN frequency in peripheral blood lymphocytes (PBLs) and buccal cells are shown in Table 2. Compared to unexposed controls MN frequency was significantly increased in FA-exposed females in both PBLs and exfoliated buccal cells. MN frequency in PBLs was found to be 2.1-times higher in the exposed workers compared to control subjects, whereas in buccal cells a 5.9-fold increase was observed.

Table 2: Results of biomarkers of genotoxicity in studied groups (mean ± SE and range).

	Control Group	**Exposed Group**
PBLs MN (‰)	1.71 ± 0.25 (*0–6*)	3.51 ± 0.35* (*0–8*)
Buccal MN (‰)	0.12 ± 0.06 (*0–2*)	0.71 ± 0.12 * (*0–4*)

S.E., mean standard error.
*P< 0.05.

Our results are in agreement with data obtained in other studies that reported an increased frequency of MN in PBLs and/or epithelial cells among mortuary students and, more recently, in hospital staff exposed to FA. In 1993, Suruda *et al.* [22] reported a significant increase in post-exposure MN frequency in lymphocytes (26%) and buccal mucosa cells (12-fold) among mortician students exposed to FA (TWA=0.33ppm), whereas in nasal epithelial cells the increase

(22%) was not significant. Also, a significant dose-response relationship was found between buccal micronuclei increase and cumulative exposure to FA, but only in male subjects. Ying *et al.* [23] also assessed MN frequency in lymphocytes, oral and nasal mucosa cells of 25 anatomy students exposed to FA over an 8-week period. A higher frequency of MN was found in nasal and oral exfoliated cells, but no significant increase in lymphocytes was observed. In contrast, a significant elevated frequency in micronucleated lymphocytes was found among 59 pathology and anatomy laboratory workers in a study conducted by Orsière *et al.* [24]. Burgaz *et al.* [25, 26] also evaluated MN induction on pathology and anatomy workers exposed to FA but only in buccal and nasal epithelium; an increased frequency of MN in both epithelial cells was found in exposed subjects compared to controls. More recently in a group of Portuguese histopathology laboratory workers (Ladeira *et al.* [27]) reported elevated MN frequencies in PBLs and buccal epithelial cells, confirming previous reports by Costa *et al.* [13, 28] and Viegas *et al.* [29].

Increased frequencies of this biomarker were also found among FA-exposed workers from industrial units. In fact, Ballarin *et al.* [30] were the first to describe an increase in micronucleated nasal cells collected from 15 non-smoking workers from a plywood factory. TWA levels of exposure to FA were 0.08 ppm in the saw mill and shearing-press departments and 0.32 ppm in the warehouse area, there was also a concurrent exposure to wood dust (0.19 to 0.6 ppm). Later on, in a population of 151 plywood factory workers exposed to FA, Yu *et al.* [31] also reported a significantly higher frequency of MN but in PBLs, compared to a control group.

Overall, the majority of the studies show a link between the exposure to this chemical and the increase of this cytogenetic endpoint in lymphocytes, oral and nasal epithelium, confirming that MN is a sensitive indicator for the mutagenic action of FA.

Micronuclei formation may result from aneugenic or clastogenic actions. On additional slides from Suruda *et al.* [22] study, Titenko-Holland *et al.* [32] detected a greater increase in centromere-negative micronuclei content in buccal and nasal tissues from FA-exposed subjects suggesting chromosome breakage as the primary mechanism of FA micronucleus formation. In contrast, Orsière *et al.* [24], in their study found higher significant frequencies of centromere-positive micronuclei (monocentric) in the FA-exposed workers. However, recent studies [28, 33] indicate a clastogenic effect of FA, confirming earlier results from Titenko-Holland *et al*. [32] study.

In the present work we found a significant positive association (r=0.449, P<0.001) between MN frequencies in PBLs and exfoliated buccal cells. The concomitant increase of MN formation in buccal cells, a local FA-target tissue, and in PBLs indicates not only that inhaled FA is able to induce cytogenetic alterations in circulating systemic lymphocytes (distal tissue) but also that the damage between this two tissues may be associated, giving relevance to Zhang *et al.* [9, 34] hypothesis of FA capability to induce directly or indirectly genotoxic damage in distal cells. Although the biological mechanisms associated with FA-induced cancer are not completely understood, it is important to acknowledge

that chemicals can act through multiple toxicity pathways and modes and/or mechanisms of action to induce cancer or other health outcomes [35].

Potential carcinogenic modes of actions for FA include DNA reactivity, gene mutation, chromosomal breakage, aneuploidy, enzyme-mediated DNA damage/repair, cell signaling other than nuclear-receptor mediated, immune response modulation, inflammation, and cytotoxicity [6].

Among the exposed group, was also found a near significant positive association (r=0.324, P=0.051) between MN frequency in PBLs and FA exposure levels, which reinforces the relevance of using this cytogenetic damage biomarker to assess FA genotoxic effects in occupational exposed populations.

The evaluation of the effects of age and smoking habits in addition to exposure was performed using a multivariate model. Table 3 summarizes the results obtained.

Table 3: Influence of exposure, smoking habits and age on MN frequency in PBLs and buccal cells.

Model	β	SE	95% Wald CI	Wald χ^2	P-value
1. PBLs MN (‰)					
Exposed vs. non-exposed	0.72	0.27	0.19 to 1.25	7.20	**<0.05**
Smokers vs. non-smokers	0.03	0.33	-0.62 to 0.67	0.01	0.93
Age (years)	0.01	0.01	-0.01 to 0.04	1.26	0.26
2. Buccal MN (‰)					
Exposed vs. non-exposed	1.92	0.56	0.81 to 3.02	11.54	**<0.05**
Smokers vs. non-smokers	0.85	0.46	-0.06 to 1.75	3.36	0.07
Age (years)	0.04	0.03	-0.01 to 0.09	2.25	0.13

In the present study no significant influence of age, smoking habits or years of employment was observed. A significant positive correlation was found between age and MN frequency in buccal cells (r=0.257, P<0.05). The increase of MN frequency in buccal cells with age is a documented fact [36] confirmed in a recent pooled analysis by Bonassi *et al.* [37] with more than 5000 subjects, being this increase significant from age forty.

In conclusion, results found in the present study substantiate an association between MN formation and occupational exposure to FA, confirming this endpoint as a sensitive indicator for evaluating FA genotoxic effect in occupational exposed populations. Moreover, a significant positive correlation between MN frequency in PBLs and MN frequency in buccal cell was found. Such results along with the recent implications of human carcinogenicity, point out for the need of close monitoring of FA exposures. Development of training programs, medical surveillance programs, valuable data for program evaluation and effective purchase and implementation of control measures may be crucial to decrease the risk associated to FA occupational exposure.

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References

[1] Clapp, R.W., Jacobs, M.M. and Loechler, E.L., Environmental and occupational causes of cancer: new evidence 2005–2007. *Rev Environ Health*, **23(1)**, pp. 1–37, 2008.
[2] Cebulska-Wasilewska, A., Srám, R. J. and Au, W.W., Human Monitoring for Genetic Effects (NATO ASI SERIES), *IOS Press*: Amsterdam, volume 351, 2003.
[3] Bonassi, S., Znaor, A., Ceppi, M., Lando, C., Chang, W.P., Holland, N., Kirsch-Volders, M. and *et al*., An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. *Carcinogenesis*, **28(3)**, pp. 625–31, 2007.
[4] Fenech, M., Holland, N., Chang, W. P., Zeiger, E. and Bonassi, S., The Human MicroNucleus Project – An international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans. *Mutat. Res.*, 428, pp. 271–28, 1999.
[5] International Agency for Research on Cancer, IARC Monographs on the Evaluation of Carcinogenic Risks to Human, Volume 88: Formaldehyde, 2-Butoxyethanol and 1-*tert*-Butoxy-2-propanol. *World Health Organization*: Lyon, 2006.
[6] National Toxicology Program, Final Report on Carcinogens Background Document for Formaldehyde. *Rep Carcinog Backgr Doc*. 10-5981: i-512, 2010.
[7] Bosetti, C., McLaughlin, J.K., Tarone, R.E., Pira, E., and La Vecchia C., Formaldehyde and cancer risk: a quantitative review of cohort studies through 2006. *Ann Oncol*, **19(1)**, pp. 29–43, 2008.
[8] Beane-Freeman L.E., Blair, A., Lubin, J.H., Stewart, P.A., Hayes, R.B., Hoover, R.N. and Hauptmann, M., Mortality from lymphohematopoietic malignancies among workers in formaldehyde industries: the National Cancer Institute Cohort. *J Natl Cancer Inst*, **101(10)**, pp. 751–761, 2009.
[9] Zhang, L., Steinmaus, C., Eastmond, D.A., Xin, X.K. and Smith, M.T. Formaldehyde exposure and leukemia: a new meta-analysis and potential mechanisms. *Mutat Res*, **681(2–3)**, pp. 150–168, 2009.
[10] Speit, G., Zeller, J., Schmid, O., Elhajouji, A., Ma-Hock. L and Neuss S. Inhalation of formaldehyde does not induce systemic genotoxic effects in rats. *Mutat Res*, **677(1–2)**, pp. 76–85, 2009.
[11] Akbar-Khanzadeh, F. and Pulido, E.V., Using Respirators and Goggles to Control Exposure to Air Pollutants in an Anatomy Laboratory. *Am. J. Ind. Med.*, **43**, pp. 326–331, 2003.
[12] Ohmichi, K., Komiyama, M., Matsuno, Y., Takanashi, Y., Miyamoto, H., Kadota, T., Maekawa, M., Toyama, Y., Tatsugi, Y., Kohno, T., Ohmichi,

M. and Mori, C., Formaldehyde exposure in a gross anatomy laboratory – personal exposure level is higher than indoor concentration. *Environ. Sci. Pollut. Res. Int*., **13**, pp. 120–4, 2006.
[13] Costa, S., Coelho, P., Costa, C., Silva, S., Mayan, O., Santos, L.S, Gaspar, J. and Teixeira, J.P., Genotoxic damage in pathology anatomy laboratory workers exposed to formaldehyde. *Toxicology*, **252(1–3)**, pp. 40–48, 2008.
[14] Heck, H. and Casanova, M., The implausibility of leukemia induction by formaldehyde: a critical review of the biological evidence on distant-site toxicity. *Regul Toxicol Pharmacol* **40(2)**, pp. 92–106, 2004.
[15] Pyatt, D., Natelson, E. and Golden, R. Is inhalation exposure to formaldehyde a biologically plausible cause of lymphohematopoietic malignancies? *Regul Toxicol Pharmacol* **51(1)**, pp. 119–133, 2008.
[16] National Institute for Occupational Safety and Health, Formaldehyde: Method 3500 (Issue 2), in: NIOSH (Eds.), NIOSH Manual of Analytical Method. U.S. Department of Health and Human Services: Cincinnati, Ohio, pp. 2–5, 1994.
[17] Teixeira, J., Gaspar, J., Silva, S., Torres, J., Silva, S., Azevedo, M., Neves, P., Laffon, B., Mendez, J., Goncalves, C., Mayan, O., Farmer, P. and Rueff, J., Occupational exposure to styrene: modulation of cytogenetic damage and levels of urinary metabolites of styrene by polymorphisms in genes CYP2E1, EPHX1, GSTM1, GSTT1 and GSTP1. *Toxicology* **195**, pp. 231–42, 2004.
[18] Fenech, M., Cytokinesis-block micronucleus cytome assay. *Nat. Protoc*. **2**, pp. 1084–1104, 2007.
[19] Thomas, P., Holland, N., Bolognesi, C., Kirsch-Volders, M., Bonassi, S., Zeiger, E., Knasmueller, S. and Fenech M., Buccal micronucleus cytome assay. *Nat Protoc.* **4(6)**, pp. 825–37, 2009.
[20] Tolbert, P.E., Shy, C.M. and Allen, J.W., Micronuclei and other nuclear anomalies in buccal smears: methods development. *Mutat. Res.* **271**, pp. 69–77, 1992.
[21] American Conference of Governmental Industrial Hygienists TLVs and BEIs, Based on the Documentation of the Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. *American Conference of Governmental Industrial Hygienists*: Cincinnati, 2008.
[22] Suruda, A., Schulte, P., Boeniger, M., Hayes, R.B., Livingston, G.K. and Steenland, K., Cytogenetic effects of formaldehyde exposure in students of mortuary science. *Cancer Epidemiol. Biomarkers Prev.* **2**, pp. 453–460, 1993.
[23] Ying, C.J., Yan, W.S., Zhao, M.Y., Ye, X.L., Xie, H., Yin, S.Y. and Zhu, X.S., Micronuclei in nasal mucosa, oral mucosa and lymphocytes in students exposed to formaldehyde vapor in anatomy class. *Biomed. Environ. Sci*.**10**, pp. 451–5, 1997.
[24] Orsière, T., Sari-Minodier, I., Iarmarcovai, G. and Botta, A. Genotoxic risk assessment of pathology and anatomy laboratory workers exposed to

formaldehyde by use of personal air sampling and analysis of DNA damage in peripheral lymphocytes. *Mutat. Res.* **605 (1–2)**, pp. 30–41, 2006.
[25] Burgaz, S., Cakmak, G., Erdem, O., Yilmaz, M. and Karakaya, A.E., Micronuclei frequencies in exfoliated nasal mucosa cells from pathology and anatomy laboratory workers exposed to formaldehyde. *Neoplasma* **48 (2)**, pp. 144–147, 2001.
[26] Burgaz, S., Erdem, O., Cakmak, G., Erdem, N., Karakaya, A., and Karakaya, A.E., Cytogenetic analysis of buccal cells from shoe-workers and pathology and anatomy laboratory workers exposed to n-hexane, toluene, methyl ethyl ketone and formaldehyde. *Biomarkers* **7(2)**, pp. 151–61, 2002.
[27] Ladeira, C., Viegas, S., Carolino, E., Prista, J., Gomes, M.C. and Brito, M., Genotoxicity biomarkers in occupational exposure to formaldehyde – the case of histopathology laboratories.*Mutat Res.*,**721(1)**, pp. 15–20, 2011.
[28] Costa, S., Pina, C., Coelho, P., Costa, C., Silva, S., Porto, B., Laffon, B. and Teixeira, J.P., Occupational exposure to formaldehyde: genotoxic risk evaluation by comet assay and micronucleus test using human peripheral lymphocytes. *J Toxicol Environ Health A*. **74(15–16)**, pp. 1040–51, 2011.
[29] Viegas, S., Ladeira, C., Nunes, C., Malta-Vacas, J., Gomes, M., Brito, M., Mendonca, P., Prista, J., Genotoxic effects in occupational exposure to formaldehyde: A study in anatomy and pathology laboratories and formaldehyde-resins production. *J Occup Med Toxicol*. **5(25)**, pp. 1–8, 2010.
[30] Ballarin C, Sarto F, Giacomelli L, Bartolucci GB, Clonfero E., Micronucleated cells in nasal mucosa of formaldehyde-exposed workers. *Mutat Res* **280(1)**, pp. 1–7, 1992.
[31] Yu, L.Q., Jiang, S.F., Leng, S.G., He, F.S., and Zheng, Y.X., Early genetic effects on workers occupationally exposed to formaldehyde. *Chinese Journal of Preventive Medicine* **39**, pp. 392–5, 2005. [abstract].
[32] Titenko-Holland, N., Levine, A.J., Smith, M.T., Quintana, P.J., Boeniger, M., Hayes, R., Suruda, A. and Schulte, P., Quantification of epithelial cell micronuclei by fluorescence in situ hybridization (FISH) in mortuary science students exposed to formaldehyde. *Mutat. Res.* **371**, pp. 237–48, 1996.
[33] Speit, G., Kuhner, S., Linsenmeyer, R., and Schutz, P., Does formaldehyde induce aneuploidy? *Mutagenesis* **26**, pp. 805–811, 2011.
[34] Zhang, L., Beane-Freeman, L., Nakamura, J., Hecht, S., Vandenburg, J., Smith, M., and Sonawane, B., Formaldehyde and leukemia: Epidemiology, potential mechanisms, and implications for risk assessment. *Environ Mol Mutagen.*, **51(3)**, pp. 181–91, 2009.
[35] Guyton, K.Z., Kyle, A.D., Aubrecht, J., Cogliano, V.J., Eastmond, D.A., Jackson , M., Keshava, N., Sandy, M.S., Sonawane, B., Zhang, L., Waters, M.D., and Smith, M.T. Improving prediction of chemical carcinogenicity by considering multiple mechanisms and applying toxicogenomic approaches. *Mutat Res*, **681(2–3)**, pp. 230–240, 2009.

[36] Heuser, V.D., Erdtmann, B., Kvitko, K., Rohr, P., and da Silva, J., Evaluation of genetic damage in Brazilian footwear-workers: biomarkers of exposure, effect, and susceptibility. *Toxicology*, **232(3)**, pp. 235–47, 2007.
[37] Bonassi, S., Coskun, E., Ceppi, M., Lando, C., Bolognesi, C., Burgaz, S., Holland, N., *et al*. 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(3)**, pp. 88–97, 2011.