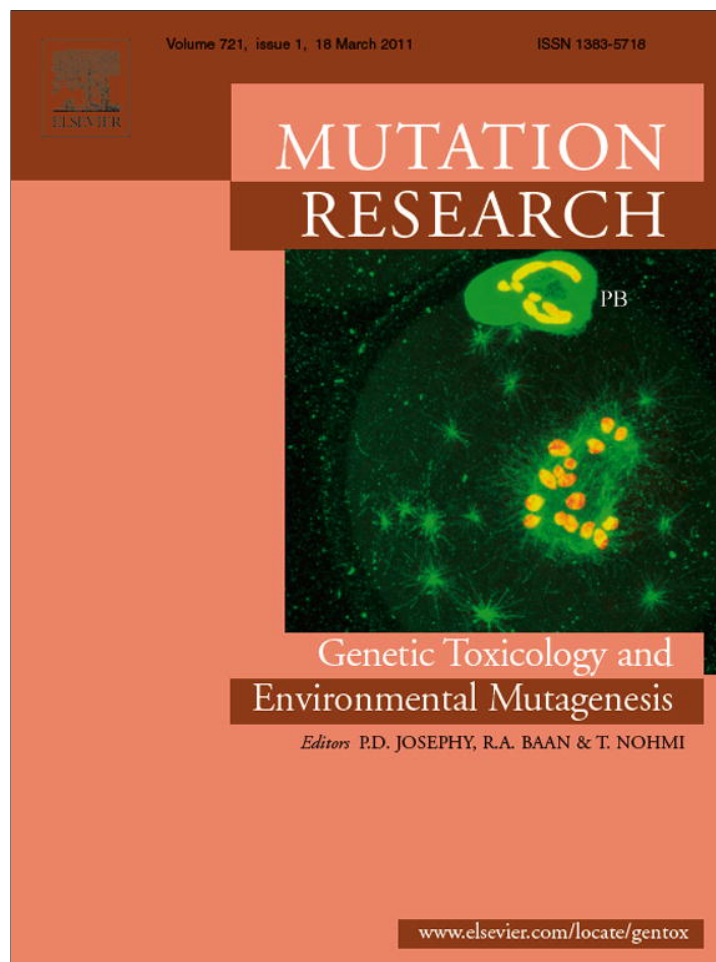


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Genotoxicity biomarkers in occupational exposure to formaldehyde—The case of histopathology laboratories

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ABSTRACT

Formaldehyde, classified by the IARC as carcinogenic in humans and experimental animals, is a chemical agent that is widely used in histopathology laboratories. The exposure to this substance is epidemiologically linked to cancer and to nuclear changes detected by the cytokinesis-block micronucleus test (CBMN). This method is extensively used in molecular epidemiology, since it provides information on several biomarkers of genotoxicity, such as micronuclei (MN), which are biomarkers of chromosomes breakage or loss, nucleoplasmic bridges (NPB), common biomarkers of chromosome rearrangement, poor repair and/or telomere fusion, and nuclear buds (NBUD), biomarkers of elimination of amplified DNA.

The aim of this study is to compare the frequency of genotoxicity biomarkers, provided by the CBMN assay in peripheral lymphocytes and the MN test in buccal cells, between individuals occupationally exposed and non-exposed to formaldehyde and other environmental factors, namely tobacco and alcohol consumption.

The sample comprised two groups: 56 individuals occupationally exposed to formaldehyde (cases) and 85 unexposed individuals (controls), from whom both peripheral blood and exfoliated epithelial cells of the oral mucosa were collected in order to measure the genetic endpoints proposed in this study.

The mean level of TWA_{8h} was 0.16 ± 0.11 ppm (< detection limit until 0.51 ppm) and the mean of ceiling values was 1.14 ± 0.74 ppm (0.18–2.93 ppm). All genotoxicity biomarkers showed significant increases in exposed workers in comparison with controls (Mann–Whitney test, $p < 0.002$) and the analysis of confounding factors showed that there were no differences between genders.

As for age, only the mean MN frequency in lymphocytes was found significantly higher in elderly people among the exposed groups ($p = 0.006$), and there was also evidence of an interaction between age and gender with regards to that biomarker in those exposed.

Smoking habits did not influence the frequency of the biomarkers, whereas alcohol consumption only influenced the MN frequency in lymphocytes in controls ($p = 0.011$), with drinkers showing higher mean values. These results provide evidence of the association between occupational exposure to formaldehyde and the presence of genotoxicity biomarkers.

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1. Introduction

Formaldehyde (FA) is a reactive, flammable and colourless gas with a strong and very characteristic pungent odour. When combined with air, FA can form explosive mixtures. FA occurs as an endogenous metabolic product of N-, O- and S-demethylation reactions in most living systems. It is used mainly in the production of resins and their applications, such as adhesives and binders in wood product, pulp and paper, synthetic vitreous fibre indus-

tries, production of plastics, coatings, textile finishing, and also as an intermediate in the synthesis of other industrial chemical compounds. Common non-occupational sources of exposure to FA include vehicle emissions, particle boards and similar building materials, carpets, paints and varnishes, food and cooking, tobacco smoke and its use as a disinfectant [1–5].

Commercially, FA is manufactured as an aqueous solution called formalin, usually containing 37–40% by weight of dissolved FA [6], which is commonly used in histopathology laboratories as a cytological fixative to preserve the integrity of cellular architecture for diagnosis.

Exogenous FA can be absorbed following inhalation, dermal or oral exposure, the extent of absorption being dependent on the route of exposure. The International Agency for Research on Cancer

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(IARC) reclassified FA as a human carcinogen (group 1) in June 2004 based on “sufficient epidemiological evidence that FA causes nasopharyngeal cancer in humans” [3,6]. In their review, IARC also concluded that there was “strong but not sufficient evidence for a causal association between leukaemia and occupational exposure to formaldehyde” [6,7]. Subsequently, on the basis of additional information the IARC concluded in 2009 that there is sufficient evidence that formaldehyde causes nasopharyngeal cancer and leukaemia [8]. However, some studies have also led to mixed results and inconclusive evidence [4,9].

The inhalation of vapours can produce irritation to eyes, nose and the upper respiratory tract. Whilst occupational exposure to high FA concentrations may result in respiratory irritation and asthmatic reactions, it may also aggravate a pre-existing asthma condition. Skin reactions following exposure to FA are very common, because the chemical is both irritating and allergenic [2]. FA induces genotoxic and cytotoxic effects in bacteria and mammalian cells [10] and its carcinogenicity and genotoxicity have been proven, respectively, in epidemiological studies and in experimental studies that used proliferating cultured mammalian cells and human lymphocytes [2,11] to determine DNA–protein cross-links, chromosome aberrations, sister chromatid exchange, and micronuclei [6].

The cytokinesis-block micronucleus cytome assay (CBMN) is a comprehensive system for measuring DNA damage, cytostasis and cytotoxicity. DNA-damage events are scored specifically in once-divided binucleated cells and comprise micronuclei (MN), nucleoplasmic bridges (NPB) and nuclear buds (NBUD). This assay has been applied successfully for bio-monitoring of *in vivo* exposure to genotoxins, *in vitro* genotoxicity testing, and in areas like nutrigenomics and pharmacogenomics [12–14].

MN originate from chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division and are not included in the main daughter nuclei. Thus MN provide a measure of both chromosome breakage and loss and they have been shown to be at least as sensitive an indicator of chromosome damage as the classical metaphase chromosome analysis [13,15–17].

The analysis of NPB was validated as a biomarker of DNA damage in human WIL2-NS cells treated with hydrogen peroxide, superoxide or after co-incubation with activated human neutrophils [18]. NPB should be scored because they provide a measure of chromosome rearrangement, which is otherwise not assessed if only MN are scored [17,19]. This event occurs when centromeres of dicentric chromosomes are pulled to opposite poles of the cell at anaphase. NPB are therefore biomarkers of dicentric chromosomes resulting from telomere end-fusions or DNA mis-repair [19–22].

NBUD are characterized by the same morphology as MN, except that they are linked to the nucleus by a narrow or wide stalk of nucleoplasmic material, depending on the stage of the nuclear budding process. They are classified as biomarkers of the elimination of amplified DNA and/or DNA-repair complexes [19,21,22].

The goal of this study is to compare the frequencies of genotoxicity biomarkers, provided by the CBMN assay in peripheral lymphocytes and the MN test in buccal cells, between workers of histopathology laboratories exposed to formaldehyde and individuals not exposed to formaldehyde, and other environmental factors, i.e. tobacco and alcohol consumption.

2. Methods

2.1. Subjects

The study population consisted of 56 workers occupationally exposed to FA from six hospital-associated histopathology laboratories located in Portugal (Lisbon and Tagus Valley region), and 85 administrative staff without occupational exposure to FA. The characteristics of both groups are described in Table 1.

Ethical approval for this study was obtained from the institutional Ethical Board and the service directors of the participating hospitals, and all subjects gave

Table 1
Characteristics of the sample.

	Control group	Exposed group
Number of subjects	85	56
Gender		
Females	54 (64%)	37 (66%)
Masculine	31 (36%)	19 (34%)
Age (mean ± standard deviation, in years)		
Range	32.42 ± 8.1 20–53	39.45 ± 11.5 20–61
Years of employment (mean ± standard deviation, in years)		
Range	–	14.5 1–33
Tobacco consumption		
Non-smokers	60 (70,6%)	45 (80,4%)
Smokers	25 (29,4%)	11 (19,6%)
Alcohol consumption		
Non-drinkers	19 (22,4%)	19 (33,9%)
Drinkers	66 (77,6%)	37 (66,1%)

informed consent to participate in this study. Every person completed a questionnaire aimed at identifying exclusion criteria like history of cancer, radio or chemotherapy, use of therapeutic drugs, exposure to diagnostic X-rays in the past six months, intake of vitamins or other supplements like folic acid, as well as information related to working practices (such as years of employment and the use of protective measures). In this study, none of the participants were excluded.

2.2. Environmental monitoring of FA exposure

Exposure assessment was based on two techniques of air-monitoring conducted simultaneously. First, environmental samples were obtained by air-sampling with low-flow pumps for 6–8 h, during a typical working day. FA levels were measured by gas chromatography analysis and the time-weighted average (TWA_{8h}) was estimated according to a method described by the National Institute of Occupational Safety and Health (NIOSH 2541) [23].

The second method was aimed at measuring ceiling values of FA using photoionization detection (PID) equipment (11.7-eV lamps) with simultaneous video recording. Instantaneous values for FA concentration were obtained on a per second basis in both methods. A relationship can thus be established between worker activities and ceiling values, and serve to reveal the main exposure sources [24,25].

Measurements and sampling were always performed in the workers' breathing zone, in a room that was equipped with fume hoods.

2.3. Cytokinesis-block micronucleus cytome assay

Evaluation of genotoxic effects was performed by applying the CBMN assay in peripheral blood lymphocytes and exfoliated cells from the buccal mucosa.

Whole blood and exfoliated cells (buccal mucosa cells) were collected from every subject between 10 a.m. and 12 p.m. and processed for testing. All samples were coded and analyzed under blind conditions. The criteria for scoring the nuclear abnormalities in lymphocytes and MN in the buccal cells were described by, respectively, Fenech et al. [17] and Tolbert et al. [26].

2.3.1. Peripheral blood lymphocytes

Heparinized blood samples were obtained by venipuncture from all subjects and freshly collected blood was directly used for the micronucleus test. Lymphocytes were isolated by use of a Ficoll-Paque gradient and placed in RPMI1640 culture medium with L-glutamine and phenol red added, with 10% inactivated fetal calf serum, 50 µg/ml streptomycin + 50 U/ml penicillin, and 10 µg/ml phytohaemagglutinin. Duplicate cultures from each subject were incubated at 37 °C in a humidified 5% CO₂ incubator for 44 h, and cytochalasin-b (6 µg/mL) was added to the cultures in order to prevent cytokinesis. After 28 h of incubation, cells were spun onto microscope slides by use of a cytocentrifuge. Smears were air-dried and double-stained with May–Grünwald–Giemsa and mounted with Entellan®. One thousand cells were scored from each individual by two independent observers on a total of two slides. Each observer visualized 500 cells/individual.

2.3.2. Buccal mucosa cells

Cells from the buccal mucosa were sampled by endobrushing. Exfoliated cells were smeared onto the slides and fixed with Mercofix®. The standard protocol used was Feulgen staining without counterstain. Two thousand cells were scored from each individual by two independent observers on a total of two slides. Each observer visualized 1000 cells/individual. Only cells containing intact nuclei that were neither clumped nor overlapping were included in the analysis.

Table 2
FA ceiling values (ppm) by tasks in the macroscopy room.

Tasks	Ceiling values (ppm)
Macroscopic specimen's exam	2.93
Disposal of specimen and used solutions	0.95
Jar filling	2.51
Specimen wash	2.28
Biopsy exam	1.91

2.4. Statistical analysis

The deviation of variables from the normal distribution was evaluated by the Shapiro–Wilk goodness-of-fit test. The association between each of the genotoxicity biomarkers and occupational exposure to FA was evaluated by binary logistic regression. The biomarkers were dichotomized (absent/present) and considered as the dependent variable in regression models where exposure was an independent variable. Odds ratios were computed to evaluate the risk of the biomarkers' presence and their significance was assessed. The non-parametric Kruskal–Wallis and Mann–Whitney *U*-tests were also used to evaluate interactions involving confounding factors. All statistical analyses were performed with the SPSS package for Windows, version 15.0.

3. Results

3.1. FA exposure levels

FA exposure was determined with the two methods described—the NIOSH 2541 method [23] for average concentrations (TWA_{8h}) and the PID method for ceiling concentrations. For the first exposure metric, the FA mean exposure level of the

56 individuals studied was 0.16 ppm (0.04–0.51 ppm), a value that lies below the OSHA reference value of 0.75 ppm. The mean ceiling concentration found in the laboratories was 1.14 ppm (0.18–2.93 ppm), a value well above the reference of the American Conference of Governmental Industrial Hygienists (ACGIH) for ceiling concentrations (0.3 ppm). As for the different tasks developed in histopathology laboratories, the highest FA concentration was identified during macroscopic examination of specimens. This task involves careful observation and grossing of the specimen preserved in FA, which involves direct and prolonged contact of the observer with FA vapours (Table 2).

3.2. Genotoxicity biomarkers

For all genotoxicity biomarkers under study, the workers exposed to FA had significantly higher mean values than the controls (Table 3).

In peripheral blood lymphocytes, significant differences (Mann–Whitney test, *p* < 0.001) were observed between subjects exposed and those not exposed to FA, namely in mean MN (respectively, 3.96 ± 0.525 vs 0.81 ± 0.172), NPB (3.04 ± 0.523 vs 0.18 ± 0.056), and NBUD (0.98 ± 0.273 vs 0.07 ± 0.028). In buccal mucosa cells, the mean MN frequency was also significantly higher (*p* = 0.002) in exposed subjects (0.96 ± 0.277) than in controls (0.16 ± 0.058).

The odds ratios indicate an increased risk for the presence of biomarkers in those exposed to FA, compared to non-exposed (Table 3); all odds ratios were significant (*p* < 0.001).

Table 3
Descriptive statistics of MN in lymphocytes and buccal cells, NPB and NBUD in the studied population (mean ± mean standard error, range), *p*-value of the Mann–Whitney test and results of binary logistic regression concerning the association between exposure and genotoxicity biomarkers, as evaluated by the odds ratio (OR).

	MN in lymphocytes Mean ± S.E. (range)	NPB Mean ± S.E. (range)	NBUD Mean ± S.E. (range)	MN in buccal cells Mean ± S.E. (range)
Controls	0.81 ± 0.172 (0–7)	0.18 ± 0.056 (0–3)	0.07 ± 0.028 (0–1)	0.16 ± 0.058 (0–2)
Exposed	3.96 ± 0.525 (0–14)	3.04 ± 0.523 (0–15)	0.98 ± 0.273 (0–13)	0.96 ± 0.277 (0–9)
<i>p</i> -value*	<0.001	<0.001	<0.001	0.002
OR	9.665	11.97	9.631	3.990
CI 95%	3.81–24.52	4.59–31.20	3.12–29.70	1.38–11.58
<i>p</i> -value**	<0.001	<0.001	<0.001	0.011

* Mann–Whitney test.
** Binary logistic regression.

Table 4
Descriptive statistics in the exposed group of MN in lymphocytes and buccal cells, NPB, and NBUD (mean ± mean standard error, range) by years of exposure to formaldehyde.

Years of exposure	<i>N</i>	MN in lymphocytes Mean ± S.E. (range)	NPB Mean ± S.E. (range)	NBUD Mean ± S.E. (range)	MN in buccal cells Mean ± S.E. (range)
<5	8	2.75 ± 0.940 (0–8)	5.13 ± 1.381 (0–10)	1.38 ± 0.498 (0–3)	0.63 ± 0.625 (0–5)
6–10	19	3.05 ± 0.775 (0–12)	2.42 ± 0.668 (0–9)	1.53 ± 0.731 (0–13)	0.63 ± 0.326 (0–6)
11–20	12	5.50 ± 1.317 (0–14)	3.33 ± 1.443 (0–14)	0.33 ± 0.188 (0–2)	0.83 ± 0.458 (0–5)
>21	15	5.00 ± 1.151 (0–13)	2.33 ± 1.036 (0–15)	0.73 ± 0.248 (0–2)	1.20 ± 0.8 (0–9)

Table 5
Descriptive statistics of MN in lymphocytes and buccal cells, NPB, and NBUD means by gender and exposition (mean ± mean standard error, range).

Groups	Exposed		Controls	
	Females ^a <i>N</i> = 37	Males ^a <i>N</i> = 19	Females ^a <i>N</i> = 54	Males ^a <i>N</i> = 31
MN in lymphocytes Mean ± S.E. (range)	4.43 ± 0.676 (0–14)	3.47 ± 0.883 (0–13)	0.87 ± 0.229 (0–7)	0.71 ± 0.255 (0–6)
NPB Mean ± S.E. (range)	3.03 ± 0.699 (0–15)	2.95 ± 0.818 (0–14)	0.22 ± 0.078 (0–3)	0.10 ± 0.071 (0–2)
NBUD Mean ± S.E. (range)	1.34 ± 0.418 (0–13)	0.42 ± 0.158 (0–2)	0.11 ± 0.043 (0–1)	0.00
MN in buccal cells Mean ± S.E. (range)	1.14 ± 0.353 (0–8)	0.74 ± 0.495 (0–9)	0.11 ± 0.057 (0–2)	0.26 ± 0.122 (0–2)

^a Gender.

Table 6
Age effects on descriptive statistics of MN in lymphocytes and buccal cells, NPB and NBUD means in the studied population (mean \pm mean standard error, range).

Groups	Age	N	MN in lymphocytes Mean \pm S.E. (range)	NPB Mean \pm S.E. (range)	NBUD Mean \pm S.E. (range)	MN in buccal cells Mean \pm S.E. (range)
Exposed	20–30	18	2.19 \pm 0.526 (0–8)	3.56 \pm 0.926 (0–10)	1.63 \pm 0.816 (0–13)	0.75 \pm 0.470 (0–6)
	31–40	11	3.00 \pm 0.775 (0–8)	1.20 \pm 0.467 (0–4)	0.50 \pm 0.224 (880–2)	0.40 \pm 0.221 (0–2)
	>41	27	5.54 \pm 0.876 (0–14)	3.00 \pm 0.879 (0–15)	0.69 \pm 0.234 (0–5)	1.46 \pm 0.503 (0–9)
Controls	20–30	36	0.47 \pm 0.157 (0–3)	0.14 \pm 0.071 (0–2)	0.08 \pm 0.047 (0–1)	0.19 \pm 0.96 (0–2)
	31–40	35	1.14 \pm 0.326 (0–7)	0.20 \pm 0.099 (0–3)	0.06 \pm 0.040 (0–1)	0.14 \pm 0.83 (0–2)
	>41	14	0.86 \pm 0.501 (0–6)	0.21 \pm 0.155 (0–2)	0.07 \pm 0.71 (0–1)	0.14 \pm 0.143 (0–2)

Regarding the impact of the duration of exposure to FA, the mean values of MN in lymphocytes and in buccal cells tended to increase with years of exposure (Table 4) but the association was not statistically significant.

Gender and age are considered the most important demographic variables affecting the MN index. However, Table 5 shows that the mean of all the genotoxicity biomarkers did not differ between men and women within the exposed and the controls ($p > 0.05$).

In order to examine the effect of age, exposed and non-exposed individuals were stratified by age groups: 20–30, 31–40, and ≥ 41 years old (Table 6). There was no consistent trend regarding the variation of biomarkers with age, the only exception being the MN frequency in lymphocytes in the exposed group (Kruskal–Wallis, $p = 0.006$), where the higher means were found in the older group. According to the Mann–Whitney test, there is a statistically significant difference between the younger and the older group (20–30 and >41 years old, $p = 0.02$), however the comparison between 20–30 and 31–40 groups ($p = 0.262$) and 31–40 and >41 groups ($p = 0.065$) did not reach statistical significance.

The interaction between age and gender in determining the frequencies of genotoxicity biomarkers was investigated and found to be significant only for MN in lymphocytes in exposed subjects (Kruskal–Wallis, $p = 0.04$). In general the MN tended to be more frequent in the >41 years old category in both genders; however women had the higher means (Table 7).

Regarding smoking habits, a non-parametric analysis rejected the null hypothesis that biomarkers are the same for the four categories (control smokers and non-smokers, exposed smokers and non-smokers) (Kruskal–Wallis, $p < 0.001$). However, the analysis of the interactions between FA exposure and tobacco smoke between exposed and controls (Mann–Whitney test) showed that FA exposure, rather than tobacco, has a preponderant effect upon the determination of biomarker frequencies. In the control group, non-smokers had slightly higher MN means in buccal cells in comparison with smokers; although the result did not reach statistical significance (Mann–Whitney, $p > 0.05$).

As for alcohol consumption, because uptake reported in enquires may differ considerably from real consumption, all con-

sumers were gathered into a single entity, in contrast with non-consumers. Nevertheless, no one acknowledged having “heavy drinking habits” in the questionnaires.

Overall, biomarkers in controls exhibited higher mean frequencies among alcohol consumers than among non-consumers. Among those exposed, however, mean frequencies were slightly lower among drinkers, suggesting that exposure was the major predominant factor in determining the high biomarker frequencies of those who are exposed. Differences between drinkers and non-drinkers were not statistically significant, except with respect to the MN frequency in lymphocytes in controls (Mann–Whitney, $p = 0.011$), where drinkers have higher means. The interaction between alcohol consumption and smoking habits was statistically significant (Kruskal–Wallis, $p = 0.043$), as subjects that do not smoke and do not drink tend to have lower frequencies of MN in buccal cells than those who drink and smoke, with a gradient of frequencies in between.

4. Discussion

Long-term exposures to FA, such as those to which some workers are subjected for occupational reasons, are suspected to be associated with genotoxic effects, which can be evaluated by analysis of biomarkers [1,3,5,6]. In this study the results suggest that workers in histopathology laboratories are exposed to FA levels that exceed recommended exposure limits. In particular, macroscopic examination of specimens is the task that involves higher exposure, because it requires a greater proximity to anatomical preparations impregnated with FA, as supported by the studies of Goyer et al. [27] and Orsière et al. [28].

A statistically significant association was found between FA exposure and biomarkers of genotoxicity, namely micronuclei (MN) in lymphocytes, nucleoplasmic bridges, nuclear buds and MN in buccal cells. Chromosome damage and effects on lymphocytes arise because FA escapes from sites of direct contact, such as the mouth, causing nuclear alterations in the lymphocytes of those exposed [6,10,28,29]. Our results thus corroborate previous reports [30] that lymphocytes can be damaged by long-term FA exposure.

Table 7
Descriptive statistics of MN in lymphocytes and buccal cells, NPB and NBUD means by exposition, gender and age (mean \pm mean standard error, range).

Groups		N	MN lymphocytes Mean \pm S.E.	NPB Mean \pm S.E.	NBUD Mean \pm S.E.	MN buccal cells Mean \pm S.E.
Exposed	Females 20–30	12	2.42 \pm 0.67	4.17 \pm 1.22	2.00 \pm 1.07	1.00 \pm 0.62
	Females 31–40	7	2.71 \pm 0.68	0.86 \pm 0.46	0.57 \pm 0.3	0.29 \pm 0.18
	Females >41	18	6.00 \pm 1.12	3.22 \pm 1.09	1.06 \pm 0.39	1.44 \pm 0.55
	Males 20–30	6	1.00 \pm 0.52	3.50 \pm 1.02	0.50 \pm 0.34	0.00 \pm 0.0
	Males 31–40	4	3.00 \pm 1.78	1.50 \pm 0.96	0.25 \pm 0.25	0.50 \pm 0.50
	Males >41	9	5.33 \pm 1.45	3.22 \pm 1.56	0.44 \pm 0.24	1.33 \pm 1.01
Controls	Females 20–30	23	0.43 \pm 0.20	0.13 \pm 0.70	0.13 \pm 0.7	0.13 \pm 0.10
	Females 31–40	22	1.32 \pm 0.44	0.27 \pm 0.15	0.09 \pm 0.06	0.14 \pm 0.10
	Females >41	9	0.89 \pm 0.68	0.33 \pm 0.24	0.11 \pm 0.11	0.00 \pm 0.00
	Males 20–30	13	0.54 \pm 0.27	0.15 \pm 0.15	0.00 \pm 0.00	0.31 \pm 0.21
	Males 31–40	13	0.85 \pm 0.48	0.08 \pm 0.08	0.00 \pm 0.00	0.15 \pm 0.15
	Males >41	5	0.80 \pm 0.80	0.00 \pm 0.00	0.00 \pm 0.00	0.40 \pm 0.40

Moreover, the changes in peripheral lymphocytes indicate that the cytogenetic effects triggered by FA can reach tissues far away from the site of initial contact [31]. Long-term exposures to high concentrations of FA indeed appear to have a potential for inducing DNA damage; these effects were well demonstrated in experimental studies with animals, in which local genotoxic effects following FA exposure were observed, i.e. DNA–protein cross-links and chromosome damage [3].

In humans, FA exposure is associated with an increase in the frequency of MN in buccal epithelium cells [32–34], as corroborated by the results presented here.

Suruda et al. claim that although changes in oral and nasal epithelial cells and peripheral blood cells do not indicate a direct mechanism leading to carcinogenesis, they present evidence that DNA alteration took place [31]. It thus appears reasonable to conclude that FA is a cancer risk factor for those who are occupationally exposed in histopathology laboratories [3].

In epidemiological studies, it is important to evaluate the role played by common confounding factors, such as gender, age, smoking and alcohol consumption, in the association between disease and exposure [17,35]. Concerning gender, studies realized by Fenech et al. and Wojda et al. reported that biomarker frequencies were higher in females than in males by a factor of 1.2–1.6, depending on the age group [17,36]. Except for MN in the buccal cells of controls, the results presented here point to females having higher frequencies than males in all genotoxicity biomarkers, although the differences usually lacked statistical significance. Such a trend is concordant with previous studies that reported higher MN frequency in lymphocytes in females and a slightly higher MN frequency in buccal cells in males [37], which can be explained by preferential aneuploidic events involving the X-chromosome. A possible explanation is the micro-nucleation of the X-chromosome, which has been shown to occur in lymphocytes in females, both *in vitro* and *in vivo*, and which can be accounted for by the presence of two X chromosomes. This finding may explain the preferential micro-nucleation of the inactive X [38–40].

Ageing in humans appears to be associated with genomic instability. Cytogenetically, ageing is associated with a number of gross cellular changes, including altered size and morphology, genomic instability and changes in expression and proliferation [41,42]. It has been shown that a higher MN frequency is directly associated with decreased efficiency of DNA repair and increased genomic instability [28,43]. The data show a significant increase of the MN frequency in lymphocytes in the exposed group. This can be explained in the light of genomic instability, understood as an increased number of mutations and/or chromosomal aberrations, which cytogenetically translate into a higher frequency of changes in chromosome number and/or structure and in the formation of micronuclei [41]. The involvement of micro-nucleation in age-related chromosome loss has been supported by several studies showing that the rate of MN formation increases with age, especially in women [38].

This study provides evidence that age and gender interact to determine the frequency of MN in the lymphocytes of exposed subjects. The higher incidence of MN in both genders is more manifest in older age groups and the effect of gender becomes more pronounced as age increases. Several reports link this observation to an elevated loss of X chromosomes [44].

Tobacco smoke has been epidemiologically associated with a higher risk for cancer development, especially in the oral cavity, larynx, and lungs, as these are places of direct contact with the carcinogenic tobacco compounds. In this study, smoking habits did not influence the frequency of the genotoxicity biomarkers; moreover, the frequencies of MN in buccal cells were unexpectedly higher in exposed non-smokers than in exposed smokers, although the difference was not statistically significant. In most reports, the results

about the effect of tobacco upon the frequency of MN in human lymphocytes were negative, as in many instances smokers had lower MN frequencies than non-smokers [45]. In the current study, the analysis of the interaction between FA exposure and smoking habits indicates that exposure is preponderant in determining the frequency of biomarkers. Nevertheless, the effect of smoking upon these biomarkers remains controversial. Some studies [46,47] reported an increased frequency of MN in lymphocytes, NPB, and NBUD as a consequence of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Still in this study no associations were observed between tobacco and nuclear abnormalities.

Alcohol consumption did not appear to influence the frequency of genotoxicity biomarkers in this study, except that of MN in lymphocytes in controls (Mann–Whitney, $p=0.011$), with drinkers having higher means. Alcohol is definitely a recognized genotoxic agent, being cited as able to potentiate the development of carcinogenic lesions [48]. In our study, drinkers in the control group had higher mean frequencies of all biomarkers than non-drinkers, but the differences were only significant for MN in lymphocytes. Stich and Rosin [49] studied alcoholic individuals and reported the absence of significant differences concerning MN frequencies in buccal cells. This is important to corroborate our result, because of the lack of “heavy drinkers” in our study. The same study [49] concluded that neither alcohol nor smoking, alone, increased MN frequency in buccal cells, but a combination of both resulted in a significant elevation in micronucleated cells in the buccal mucosa. However, the synergism between alcohol consumption and tobacco has not been observed to act upon all biomarkers and, in several studies of lifestyle factors, it was difficult to differentiate the effect of alcohol from that of smoking [37].

The CBMN assay is a simple, practical, low-cost screening technique that can be used for clinical prevention and management of workers subject to occupational carcinogenic risks, namely through exposure to a genotoxic agent such as formaldehyde. The results obtained in this study provide unequivocal evidence of an association between occupational exposure to formaldehyde in histopathology laboratory workers and the presence of nuclear changes.

Given these results, preventive actions must prioritize safety conditions for those who perform macroscopic examination of specimens. In general, reduction of exposure to FA in this occupational setting may be achieved through adequate local exhaust ventilation and by keeping the biological specimen containers closed during the macroscopic examination.

Conflict of interest

None.

References

- [1] S. Viegas, J. Prista, Cancro Nasofaríngeo e Exposição a Formaldeído: avaliação da história profissional em 63 casos registados, Soc. Port. Med. Trabalho 6 (2007) 13–22.
- [2] M. Pala, D. Ugolini, M. Ceppi, F. Rizzo, L. Maiorana, C. Bolognesi, T. Schilirò, G. Gilli, P. Bigatti, R. Bono, D. Vecchio, Occupational exposure to formaldehyde and biological monitoring of Research Institute workers, Cancer Detect. Prev. 32 (2008) 121–126.
- [3] IARC, 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.
- [4] S.J. Franks, A mathematical model for the absorption and metabolism of formaldehyde vapour by humans, Toxicol. Appl. Pharmacol. 206 (2005) 309–320.
- [5] C. Conaway, J. Whysner, L. Verna, G. Williams, Formaldehyde mechanistic data and risk assessment: endogenous protection from DNA adduct formation, Pharmacol. Ther. 71 (1996) 29–55.

- [6] L. Zhang, C. Steinmaus, D. Eastmond, X. Xin, M. Smith, Formaldehyde exposure and leukemia: a new meta-analysis and potential mechanisms, *Mut. Res.* 681 (2009) 150–168.
- [7] L. Zhang, X. Tang, N. Rothman, Occupational exposure to formaldehyde, hematotoxicity, and leukemia-specific chromosome changes in cultured myeloid progenitor cells, *Cancer Epidemiol. Biomarkers Prevention* 19 (2010) 80–88.
- [8] R. Baan, Y. Grosse, K. Straif, B. Secretan, F. El Ghissassi, V. Bouvard, L. Benbrahim-Tallaa, N. Guha, C. Freeman, L. Galichet, V. Coglianò, On behalf of the WHO International Agency for Research on Cancer Monograph Working Group, A review of human carcinogens. Part F. Chemical agents and related occupations, *The Lancet Oncol.* 10 (2009) 1143–1144.
- [9] G. Speit, H.-P. Gelbke, D. Pallapies, P. Morfeld, Occupational exposure to formaldehyde, hematotoxicity and leukemia-specific chromosome changes in cultured myeloid progenitor cells—letter, *Cancer Epidemiol. Biomarkers Prev.* 19 (7) (2010) 1882–1884.
- [10] X. Ye, W. Yan, H. Xie, M. Zhao, C. Ying, Cytogenetic analysis of nasal mucosa cells and lymphocytes from high-level long-term formaldehyde exposed workers and low-level short-term exposed waiters, *Mut. Res.* 588 (2005) 22–27.
- [11] G. Speit, P. Schütz, J. Högel, O. Schmid, Characterization of the genotoxic potential of formaldehyde in V79 cells, *Mutagenesis* 22 (6) (2007) 387–394.
- [12] M. Fenech, Cytokinesis-block micronucleus cytome assay, *Nat. Protoc.* 5 (2007) 1084–1104.
- [13] R. Mateuca, N. Lombaert, P. Aka, I. Decordier, M. Kirsch-Volders, Chromosomal changes: induction, detection methods and applicability in human biomonitoring, *Biochimie* 88 (2006) 1515–1531.
- [14] L. Serrano-García, R. Montero-Montoya, Micronuclei and chromatid buds are results of related genotoxic events, *Environ. Mol. Mutagen.* 38 (2001) 38–45.
- [15] M. Fenech, Chromosomal biomarkers of genomic instability relevant to cancer, *Drug Discov. Today* 22 (2002) 1128–1137.
- [16] G. Iarmarcovai, S. Bonassi, A. Botta, R.A. Baan, T. Orsière, Genetic polymorphisms and micronucleus formation: a review of the literature, *Mut. Res.* 658 (2008) 215–233.
- [17] M. Fenech, N. Holland, W. Chang, E. Zeiger, S. Bonassi, The Human MicroNucleus Project—an international collaborative study on the use of the micronucleus technique for measuring DNA damage in humans, *Mut. Res.* 428 (1999) 271–283.
- [18] K. Umegaki, M. Fenech, Cytokinesis-block micronucleus assay in WIL2-NS cells: a sensitive system to detect chromosomal damage induced by reactive oxygen species and activated human neutrophils, *Mutagenesis* 15 (3) (2000) 261–269.
- [19] M. Fenech, Cytokinesis-block micronucleus assay evolves into a “cytome” assay of chromosomal instability, mitotic dysfunction and cell death, *Mut. Res.* 600 (2006) 58–66.
- [20] M. Fenech, The Genome Health Clinic and Genome Health Nutrigenomics concepts: diagnosis and nutritional treatment of genome and epigenome damage on an individual basis, *Mutagenesis* 20 (2005) 225–269.
- [21] P. Thomas, K. Umegaki, M. Fenech, Nucleoplasmic bridges are a sensitive measure of chromosome rearrangement in the cytokinesis-block micronucleus assay, *Mutagenesis* 18 (2003) 187–194.
- [22] M. Fenech, J. Crott, Micronuclei, nucleoplasmic bridges and nuclear buds induced in folic acid deficient human lymphocytes—evidence for breakage-fusion-bridge cycles in the cytokinesis-block micronucleus assay, *Mut. Res.* 504 (2002) 131–136.
- [23] NIOSH – Manual of analytical methods, 4th ed., DHHS (NIOSH) Publication 94–113, 1994 August.
- [24] J.D. McGlothlin, et al., Occupational exposure assessment and control using video exposure monitoring in the pharmaceutical industry, in: International Scientific Conference (IOHA 2005), 6, 19–23 September 2005, Pilanesberg National Park North West Province, South Africa. Pilanesberg, IOHA—International Occupational Hygiene. MVS—Mine Ventilation of South Africa, 2005.
- [25] S. Viegas, C. Ladeira, C. Nunes, J. Malta-Vacas, M. Gomes, M. Brito, P. Mendonça, J. Prista, Genotoxic effects in occupational exposure to formaldehyde: a study in anatomy and pathology laboratories and formaldehyde-resins production, *J. Occup. Med. Toxicol.* 5 (25) (2010) 1–8.
- [26] P. Tolbert, C. Shy, J. Allen, Micronuclei and other nuclear anomalies in buccal smears: a field test in snuff users, *Am. J. Epidemiol.* 8 (1991) 840–850.
- [27] N. Goyer, C. Beaudry, D. Bégin, M. Bouchard, S. Buissonet, G. Carrier, O. Gely, M. Gérin, L. Lavoué, P. Lefebvre, N. Noisel, G. Perraut, B. Roberge, Impacts d'un abaissement de la valeur d'exposition admissible au formaldéhyde: industries de fabrication de formaldéhyde et de résines à base de formaldéhyde, Institut de Recherche Robert-Sauvé en Santé et Sécurité du Travail, Montréal, 2004.
- [28] T. Orsière, I. Sari-Minodier, G. Iarmarcovai, A. Botta, 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, *Mut. Res.* 605 (2006) 30–41.
- [29] J.-L. He, H.-Y. Jin, Detection of cytogenetic effects in peripheral lymphocytes of students exposed to formaldehyde with cytokinesis-blocked micronucleus assay, *Biomed. Environm. Sci.* 11 (1998) 87–92.
- [30] X. Ye, W. Yan, M. Zhao, C. Ying, Cytogenetic analysis of nasal mucosa cells and lymphocytes from high-level long-term formaldehyde exposed workers and low-level short-term exposed waiters, *Mut. Res.* 588 (2005) 22–27.
- [31] A. Suruda, P. Schulte, M. Boeniger, R. Hayes, G. Livingston, K. Steenland, P. Stewart, R. Herrick, D. Douthit, M. Fingerhut, Cytogenetic effects of formaldehyde exposure in students of mortuary science, *Cancer Epidemiol. Biomarkers Prev.* 2 (1993) 453–460.
- [32] G. Speit, O. Schmid, M. Fröhler-Keller, I. Lang, G. Triebig, Assessment of local genotoxic effects of formaldehyde in humans measured by the micronucleus test with exfoliated buccal mucosa cell, *Mut. Res.* 627 (2007) 129–131.
- [33] G. Speit, O. Schmid, Local genotoxic effects of formaldehyde in humans measured by the micronucleus test with exfoliated epithelial cells, *Mut. Res.* 613 (2006) 1–9.
- [34] S. Burgaz, O. Erdem, G. Çakmak, A. Karakaya, 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* 2 (2002) 151–161.
- [35] S. Bonassi, M. Fenech, C. Lando, Y.-P. Lin, M. Ceppi, W. Chang, N. Holland, M. Kirsch-Volders, E. Zeiger, S. Ban, R. Barale, M. Bigatti, C. Bolognesi, C. Jia, M. Di Giorgio, L. Ferguson, A. Fucic, O. Lima, P. Hrelia, A. Krishnaja, T.-K. Lee, L. Migliore, L. Mikhalevich, E. Mirkova, P. Mosesso, W.-U. Müller, Y. Odagiri, M. Scarfi, E. Szabova, I. Vorobtsova, A. Vral, A. Zijno, Human MicroNucleus Project: international database comparison for results with cytokinesis-block micronucleus assay in human lymphocytes. I. Effect of laboratory protocol, scoring criteria, and host factor on the frequency of micronuclei, *Environ. Mol. Mutagen.* 37 (2001) 31–45.
- [36] A. Wojda, E. Zietkiewicz, M. Witt, Effects of age and gender on micronucleus and chromosome nondisjunction frequencies in centenarians and younger subjects, *Mutagen. Adv. Access* (2007) 1–6.
- [37] N. Holland, C. Bolognesi, M. Kirsch-Volders, S. Bonassi, E. Zeiger, S. Knasmüller, M. Fenech, 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. Rev. Mutat. Res.* (2008), doi:10.1016/j.mrrev.2008.03.007.
- [38] J. Catalán, K. Autio, E. Kuosma, H. Norppa, Age-dependent inclusion of sex chromosomes in lymphocyte micronuclei in man, *Am. J. Hum. Genet.* 63 (1998) 1464–1472.
- [39] J. Catalán, G. Falck, H. Norppa, The X chromosome frequently lags behind in female lymphocyte anaphase, *Am. J. Hum. Genet.* 66 (2000) 687–691.
- [40] J. Catalán, J. Surrallés, G. Falck, K. Autio, H. Norppa, Segregation of sex chromosomes in human lymphocytes, *Mutagenesis* 3 (2000) 251–255.
- [41] E. Zietkiewicz, A. Wojda, M. Witt, Cytogenetic perspective of ageing and longevity in men and women, *J. Appl. Genet.* 50 (2009) 261–273.
- [42] C. Bolognesi, C. Lando, A. Forni, E. Landini, R. Scarpatò, L. Migliori, S. Bonassi, Chromosomal damage and ageing: effect of micronuclei frequency in peripheral blood lymphocytes, *Age Ageing* 28 (1999) 393–397.
- [43] M. Kirsch-Volders, R. Mateuca, M. Roelants, A. Tremp, E. Zeiger, S. Bonassi, N. Holland, W. Chang, P. Aka, M. DeBoeck, L. Goddenis, V. Haufroid, H. Ishikawa, B. Laffon, R. Marcos, L. Migliore, H. Norppa, J. Teixeira, A. Zijno, M. Fenech, The effects of GSTM1 and GSTT1 polymorphisms on micronucleus frequencies in human lymphocytes in vivo, *Cancer Epidemiol. Biomarkers Prev.* 15 (5) (2006) 1038–1042.
- [44] J.M. Battershill, K. Bumett, S. Bull, Factors affecting the incidence of genotoxicity biomarkers in peripheral blood lymphocytes: impact on design of biomonitoring studies, *Mutagenesis* 6 (2008) 237–423.
- [45] S. Bonassi, M. Neri, C. Lando, M. Ceppi, Y. Lin, W. Chang, N. Holland, M. Kirsch-Volders, E. Zeiger, M. Fenech, Effect of smoking habit on the frequency of micronuclei in human lymphocytes: results from the Human MicroNucleus project, *Mut. Res.* 543 (2003) 155–166.
- [46] R. El-Zein, M. Schabath, C. Etzel, M. Lopez, J. Franklin, M. Spitz, Cytokinesis-blocked micronucleus assay as a novel biomarker for lung cancer risk, *Cancer Res.* 66 (2006) 6449–6456.
- [47] R. El-Zein, M. Fenech, M. Lopez, M. Spitz, C. Etzel, Cytokinesis-blocked cytome assay biomarkers identify lung cancer cases amongst smokers, *Cancer Epidemiol. Biomarkers Prev.* 17 (2008) 1111–1119.
- [48] A. Ramirez, P. Saldanha, Micronucleus investigation of alcoholic patients with oral carcinoma, *Genet. Mol. Res.* 1 (2002) 246–260.
- [49] H. Stich, M. Rosin, Quantitating the synergistic effect of smoking and alcohol consumption with the micronucleus test on human buccal mucosa cells, *Int. J. Cancer* 31 (1983) 305–308.