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Exposure of thermoelectric power-plant workers to volatile organic compounds from fuel oil: Genotoxic and cytotoxic effects in buccal epithelial cells

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ABSTRACT

Thermoelectric power-plant workers are constantly exposed to high levels of potentially genotoxic gaseous substances, such as volatile organic compounds (VOCs) from the combustion of fuel oil or the processing of naphtha. The aim of the present study was to estimate the association between such occupational exposure and the frequency of micronucleated cells and cells with other nuclear anomalies.

Buccal epithelial cells were collected from a total of 44 power-plant workers (exposed group) and 47 administrative workers (non-exposed group), and examined for the frequency of micronucleated cells (MNC) and of cells with other nuclear anomalies (ONA: pyknosis, karyolysis, and karyorrhexis) by means of the micronucleus assay. The frequencies of MNC and ONA per 1000 cells in the exposed group (1.8‰ and 82.4‰, respectively) were significantly higher than in the non-exposed group (0.2‰ and 58.3‰, respectively). The exposed group had a twelve-fold increase in risk for formation of MNC compared with non-exposed individuals (RR = 12.1; 95% CI, 5.0–29.2; $P < 0.001$). The confounding factors analyzed (age, smoking status, alcohol consumption, and mouthwash use) did not show any significant association with the frequency of MNC or ONA.

The findings of this study show that workers from power plants exposed to VOCs have a significantly elevated risk for DNA damage. Therefore, bio-monitoring of DNA damage is recommended for this group of workers.

1. Introduction

In thermoelectric power plants, fuel oil and naphtha are combusted to produce heat, which is then used to create steam that turns a turbine-generator, leading to the production of electricity [1]. During this process workers are exposed to toxic volatile organic compounds (VOCs). These include several chemicals, some of which with potential to cause mild to moderate health effects, such as conjunctival and airway irritation and inflammation, or more adverse health outcomes, such as cancer [2–4]. Benzene and formaldehyde are examples of VOCs classified as human carcinogens [5]. Epidemiological studies have shown an increased risk for lung and gastric cancers in subjects exposed at the workplace to high concentrations of diesel exhaust [6–8].

Bio-monitoring the effect of exposure to genotoxic substances in the workplace is essential for the development of strategies to improve occupational health and safety conditions [9]. Human bio-monitoring may be done with various cytogenetic tests that evaluate genotoxic effects by detecting DNA damage, such as sister chromatid exchange and micronuclei [10,11]. Micronuclei in exfoliated buccal epithelial cells emerge during mitosis of the basal layers of the epithelium as extra-chromosomal DNA particles, when chromosome fragments or whole chromosomes lag behind and fail to be included in the main nuclei of the daughter cells, as a result of a clastogenic or aneugenic events [11,12]. Micronuclei in epithelial cells reflect genotoxic events occurring in the dividing basal cell layer of the putative target organ 1–3 weeks earlier [13,14], which allows for cytogenetic surveillance of groups at high risk of developing organ-specific cancer (*e.g.*, upper aerodigestive tract) or oral premalignant lesions [15–17]. Chromosomal damage in epithelial cells also leads to nuclear anomalies other than micronuclei, such as karyorrhexis, karyolysis, pyknosis, condensed chromatin, 'broken eggs' and bi-nucleated cells [11,18]. Karyorrhexis, karyolysis and pyknosis are associated with both cytotoxicity (necrosis and keratinization) and genotoxicity (apoptosis) accompanying the early stages of apoptosis [12,14], and thus are also considered effective

Abbreviations: VOCs, volatile organic compounds; MNC, micronucleated cells; ONA, cells with other nuclear anomalies.

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Table 1
Age and main lifestyle habits of the groups exposed (power-plant workers) and non-exposed (administrative workers) to VOCs [data are reported as the number of subjects (% between brackets)]; VOCs concentration (mean ± SD) from both working areas (thermoelectric power-plant facilities and offices of administrative services).

	Power-plant workers	Administrative workers
Subjects (n)	44	47
Mean age[a]	36.2 ± 9.6	42.1 ± 7.6
Age		
≤40 years[b]	19 (43.2%)	13 (27.7%)
>40 years[b]	25 (56.8%)	34 (72.3%)
Smoking status		
Regular smoker	9 (20.5%)	10 (21.3%)
Non-smoker	35 (79.5%)	37 (78.7%)
Alcohol consumption		
Yes	29 (65.9%)	28 (60%)
No	15 (34.1%)	19 (40%)
Use of mouthwash elixir		
Yes	11 (25%)	15 (32%)
No	33 (75%)	32 (68%)
VOCs concentration, mg/m^3 (mean ± SD)	3.64 ± 7.36	0.08 ± 0.05

[a] Age is expressed in years and reported as the group mean ± standard deviation.
[b] Cut-off defined according the mean value (*i.e.*, 39.2 years) of the observed age distribution in the whole population.

biomarkers for populations exposed to mutagenic and carcinogenic agents [19]. According to Holland et al. [11], the observation of both micronuclei and these other nuclear anomalies provides a more comprehensive assessment of chromosomal damage than is the case from micronuclei only, particularly when cytotoxic effects are also considered. The micronucleus assay with exfoliated cells is emerging as a preferential method to measure this type of damage [11]; it is considered a sensitive and non-invasive method for monitoring DNA damage in human populations [15,20]. Recently, such approaches have been frequently used in bio-monitoring studies of occupational exposure to carcinogenic substances [*e.g.*, 21–25], but to our knowledge no study has been done for thermoelectric power-plant workers exposed to VOCs derived from fuel oil. Thus, the main objective of this study is to assess the association between such occupational exposure to VOCs and the frequency of genotoxic and cytotoxic events in the human buccal epithelium.

2. Materials and methods

2.1. Study participants

Forty-four men were recruited from the large thermoelectric power plant on S. Miguel Island (Azores, Portugal). These were workers handling fuel oil and naphtha, truck drivers and maintenance men (engaged in various tasks within the thermoelectric power-plant facilities). A group of 47 administrative workers from the offices of administrative services of the same company were also recruited. Their offices were located in a building about 10 km away from the power plant. Power-plant workers were inhabitants from the same localities as the administrative workers, to minimize the influence of other environmental factors on DNA damage. All individuals gave written informed consent to participate in this study. A standard questionnaire was used to interview each person about their age, nature of occupation, smoking habits (smoking of cigarettes and/or the use of smokeless tobacco), alcohol consumption, frequent use of mouthwash, exposure to X-rays during the previous week, and general health status.

2.2. Assessment of exposure to VOCs

Air concentrations of total VOCs inside the power-plant facilities and the offices of administrative services were measured by photo-ionization with the detector PhoCheck 5000 Plus (IonScience, Cambridge, UK). This equipment is a highly sensitive photo-ionization detector with a measurement range of 0.01–10,000 ppm. Since inside the power-plant facilities the main source of VOCs is residual fuel oil (EINECS No.: 270-675-6, CAS No.: 68476-33-5), the major hazardous components are polycyclic aromatic hydrocarbons and hydrogen sulphide.

Two 5-min measurements (one in the morning, between 8 and 10 am, and the other in the afternoon, between 1 and 3 pm) were done in each room within the thermoelectric power-plant facilities and offices of administrative services. The average concentrations from both working areas are summarized in Table 1.

2.3. Analysis of micronuclei and other nuclear anomalies in buccal epithelial cells

Exfoliated buccal epithelial cells were obtained from the inside of both cheeks by gently scrubbing the mucosa with a sterile cytobrush. The cells were smeared onto pre-cleaned glass slides. After air-drying, cells were fixed in methanol for 20 min at 0 °C, and the Feulgen method, modified from Tolbert et al. [12,14], was applied for staining. Feulgen-stained slides were evaluated under a light microscope with 1000× magnification, using immersion oil. For each individual, 1000 epithelial cells were analyzed for the frequency of cells with one or more micronuclei (MNC) and other nuclear anomalies (ONA). The following other nuclear anomalies were considered: karyolysis (nucleus depleted of DNA, in which a Feulgen-negative ghost-like image of the nucleus remains), pyknosis (the nucleus has one-third to two-thirds the size of a nucleus in normal differentiated cells), and karyorrhexis (nuclear disintegration associated with loss of nuclear membrane integrity). The scoring of cells with MNC or ONA was done according to the criteria set by Thomas et al. [18].

2.4. Statistical analysis

The Mann–Whitney *U* test was used to compare the frequencies of MNC and of ONA between power-plant workers and administrative workers (groups of high *vs* low exposure to VOCs, respectively). To estimate the association between occupational exposure to VOCs and frequency of MNC, relative risks (RRs) and 95% confidence intervals (95% CIs) were calculated by use of negative binomial regression models [26,27], adjusting for age (≤40 *vs* >40 years), smoking status (non-smoker *vs* regular smoker), alcohol consumption (yes *vs* no), and mouthwash use (yes *vs* no). The same approach was applied for the ONA. All statistical analyses were performed with SPSS 15.0 for Windows [28], and the level of statistical significance was set at $P \leq 0.05$.

3. Results

The two groups were comparable in terms of age, smoking status, alcohol drinking, and mouthwash use (Table 1). Among regular smokers, none used smokeless tobacco. None of the participants had an X-ray taken in the week prior to the collection of exfoliated buccal epithelial cells. The average concentration of VOCs in the power-plant workplace was 3.64 mg/m^3, while the concentration in the offices of the administrative services was 0.08 mg/m^3 (Table 1).

3.1. MNC and ONA frequencies

The frequency (mean ± S.E.) of MNC per 1000 cells in the exposed group was significantly higher than in the non-exposed group (1.8 ± 1.6‰ *vs* 0.2 ± 0.4‰, respectively; $P < 0.001$; Fig. 1A). Similarly, the frequency (mean ± S.E.) of ONA per 1000 cells in the exposed group was also significantly higher than in the non-exposed group (82.4 ± 39.0‰ *vs* 58.3 ± 16.4‰, respectively; $P < 0.001$; Fig. 1B).

3.2. Association between exposure to VOCs and MNC or ONA frequencies

Exposure to VOCs was a significant predictor of the MNC frequency, both in the univariate and multivariate analysis. After adjustment for age, smoking status, alcohol consumption and mouthwash use, a higher MNC frequency was found associated with exposure to VOCs (RR = 12.1; 95% CI, 5.0–29.2; $P < 0.001$) (Table 2).

Although the frequency of ONA was significantly higher in the exposed group (Fig. 1B), no significant association was found between this variable and the exposure to VOCs (Table 3).

4. Discussion

Several cytogenetic endpoints have been extensively used for bio-monitoring of human exposure to carcinogenic substances [26,29], with the increase in MNC being considered as predictive of an elevated cancer risk [15–17,30]. According to Holland et al. [11], buccal epithelial cells represent a recognized target site for early

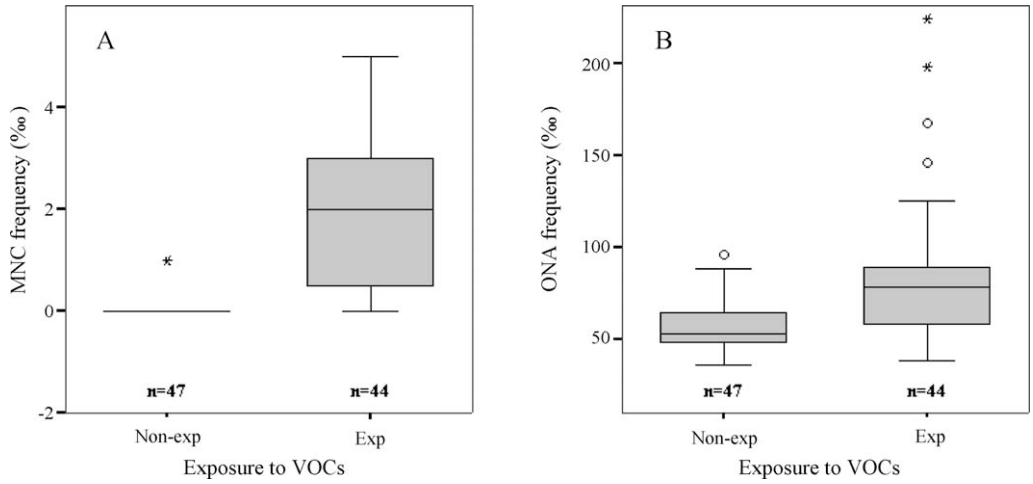

Fig. 1. Box-plots diagrams showing frequency distribution of cells with micronuclei (A) and of cells with other nuclear anomalies (B) per 1000 cells in the groups exposed (power-plant workers) and non-exposed (administrative workers) to VOCs; Line within the box, median; thin horizontal lines represent minimum and maximum values; extreme values (*) and outliers (◯).

Table 2
Association between exposure to VOCs and characteristics of study participants as well as frequency of micronucleated cells in the buccal epithelium.

	N (%)	Micronucleated cells[a]		Univariate analysis		Multivariate analysis[c]	
		Mean	Maximum	RR[b] (95% CI)	*P*-value	RR[b] (95% CI)	*P*-value
Age							
≤40	40 (44)	1.18	5	1.0			
>40	51 (56)	0.80	5	0.7 (0.4–1.2)	0.207		
Smoking status							
Non-smoker	72 (79)	1.00	5	1.0			
Regular smoker	19 (21)	0.84	5	0.8 (0.4–1.8)	0.649		
Alcohol consumption							
No	33 (36)	1.03	5	1.0			
Yes	58 (64)	0.93	5	0.9 (0.5–1.7)	0.743		
Mouthwash use							
No	65 (71)	0.95	5	1.0			
Yes	26 (29)	1.00	4	1.1 (0.6–2.0)	0.886		
Exposure to VOCs							
No	47 (52)	0.17	1	1.0		1.0	
Yes	44 (48)	1.82	5	10.7 (4.6–24.6)	<0.001	12.1 (5.0–29.2)	<0.001

[a] Micronucleated cells per 1000 cells.
[b] RR, relative risk; 95% CI, 95% confidence interval.
[c] Adjusted for age, smoking status, alcohol consumption, and mouthwash use.

Table 3
Association between exposure to VOCs and characteristics of study participants as well as the frequency of cells with other nuclear anomalies (karyorrhexis, karyolysis and pyknosis) of the buccal epithelium.

	N (%)	Cells with other nuclear anomalies[a]		Univariate analysis		Multivariate analysis[c]	
		Mean	Maximum	RR[b] (95% CI)	*P*-value	RR[b] (95% CI)	*P*-value
Age							
≤40	40 (44)	80.70	224	1.00			
>40	51 (56)	61.47	110	0.8 (0.50–1.16)	0.201		
Smoking status							
Non-smoker	72 (79)	72.00	224	1.0			
Regular smoker	19 (21)	62.05	88	0.9 (0.5–1.4)	0.567		
Alcohol consumption							
No	33 (36)	72.18	224	1.0			
Yes	58 (64)	68.64	167	1.0 (0.6–1.5)	0.819		
Mouthwash use							
No	65 (71)	74.02	224	1.0			
Yes	26 (29)	59.69	92	0.8 (0.5–1.3)	0.358		
Exposure to VOCs							
No	47 (52)	58.28	96	1.0		1.0	
Yes	44 (48)	82.36	224	1.4 (0.9–2.1)	0.102	1.3 (0.9–2.1)	0.202

[a] Micronucleated cells per 1000 cells.
[b] RR, relative risk; 95% CI, 95% confidence interval.
[c] Adjusted for age, smoking status, alcohol consumption, and mouthwash use.

genotoxic events induced by carcinogenic substances that enter the body *via* inhalation or ingestion.

Our results show an association between occupational exposure to VOCs and the frequency of MNC, with a twelve-fold increase in the risk among the group of power-plant workers. The frequency of MNC in the administrative workers was lower than 1 per 1000 cells, which is within the normal range for the human oral epithelium (0.5–2.5 MNC/1000) as reported by Holland et al. [11] and Ceppi et al. [26]. The higher frequency of MNC observed among the group exposed to VOCs is also in line with other studies, especially those focusing on exposure to diesel fuel and vehicle exhaust [21,22,31–33]. On the other hand, exposure to VOCs was not related to the frequency of ONA (karyorrhexis, karyolysis, and pyknosis).

Among the confounding lifestyle and host factors, smoking status, alcohol consumption, use of mouthwash, sex, age, and gender have been associated with DNA damage, although many studies are contradictory or inconclusive with respect to establishing statistically significant effects [11].

Our results show no significant association between smoking status and the frequency of MNC or ONA. Tobacco smoke is known to contain numerous genotoxic chemicals [34,35], but conflicting results regarding the genotoxic effects of smoking have been reported. For instance, Çelik et al. [32] and Burgaz et al. [36] found a significant increase in the frequency of MNC in smokers compared with non-smokers, whereas Bolognesi et al. [37] and Martino-Roth et al. [38] reported a small decrease in the frequency of MNC in smokers, although this was not significant. Similar to our observations, several authors also found that the occurrence of MNC was not significantly associated with smoking status in individuals occupationally exposed to fuel derivatives [21,22,31,33] or other potentially carcinogenic substances, such as pesticides [23,24]. Regarding ONA, and fully in line with our observations, Martins et al. [22] found that tobacco smoking did not significantly interfere with the cytotoxicity induced by petroleum derivates in buccal mucosa cells or in the lateral border epithelium of the tongue in petrol-station attendants. On the other hand, Çelik et al. [32] report that tobacco use increased significantly the risk of having a high frequency of bi-nucleated cells, but the individuals in their study were heavy smokers (20–25 cigarettes/day), contrary to those in the present study (7.5–16 cigarettes/day).

Alcohol is also described as a genotoxic substance [39], but in this study, as well as in those of others [21,23,31,32,40] its consumption was not significantly associated with the increase in frequency of MNC or ONA in the buccal epithelium.

Some studies have shown an increase in the frequency of MNC or ONA with age [33,41–44] and with the use of mouthwash [45]. According to Barnett and King [46] the influence of age on genotoxic and cytotoxic endpoints possibly reflects the increase in spontaneous chromosome instability with ageing, associated with an accumulation of DNA damage due to a progressive impairment of overall DNA-repair capacity. Our results show no significant association between age and frequency of MNC or ONA, which can be explained by the short age range of the participants in our study. Opposite to a suggested effect of chlorhexidine on the frequency of MNC in rat oral-mucosa cells [47], we found no relation between the use of mouthwash and the frequency of MNC or ONA.

Although a large sample is always preferable to a small sample, due to the substantial difference in exposure to VOCs between the two groups of individuals in the present study, the present sample size was sufficient to detect a significant genotoxic effect of VOCs on buccal exfoliated cells. Nevertheless, replication of this finding in another and larger population is advisable. Regarding the chosen cell population to monitor for genotoxic damage, Ceppi et al. [27] showed that there is a high correlation between the extent of micronucleus formation in buccal exfoliated cells and in cultured peripheral blood lymphocytes. The latter cell type has been extensively used – in a somewhat more invasive method – to evaluate the presence of chromosomal damage in humans [30,37,39,41–43]. In cases of chronic exposure, the differences in kinetics of replication and half-life between buccal cells and lymphocytes, which may affect the occurrence of micronuclei, become irrelevant, since chronic exposure leads to a steady-state elevated expression of micronuclei regardless of division rate, if the period of exposure exceeds the time required for one nuclear division [27].

In summary, our results suggest that there is a significant association between occupational exposure to VOCs and the occurrence of MNC in buccal epithelial cells. Even though the majority of the power-plant workers declared to use equipment for personal and collective protection, it is important to highlight the significantly higher risk for DNA damage observed in this group, suggesting that preventive measures against exposure to VOCs in the workplace need to be reviewed and perhaps reinforced. Although the present findings require confirmation in larger studies, bio-monitoring for DNA damage (particularly micronuclei) is recommended for power-plant workers, in order to achieve a safer occupational health, especially knowing that some VOCs are carcinogenic.

Conflict of interests

The authors declare that there are no conflicts of interests.

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