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Evaluation of Respiratory, Genotoxic and Cytotoxic Effects from Occupational Exposure to Typography Activities

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Abstract: This cross-sectional study was structured to allow the evaluation of the respiratory, genotoxic, and cytotoxic effects of occupational exposure to products resulting from the activity of printers in typographies and, to determine the risk of genotoxicity associated with such exposure. This study comprised 69 subjects, 25 individuals occupationally exposed to the products of typographies (study group), and 44 individuals non-exposed to the environment studied (reference group). The frequency of micronucleated cells and other nuclear anomalies (binucleated, karyolitic, pyknotic, and karyorrhectic cells) in the oral epithelia of each subject were analyzed. The frequency of micronucleated cells was significantly higher in the study group when compared to the reference one (12.96 MN/2000 cells vs. 4 MN/2000 cells, respectively). Occupational exposure to products of typography is a risk factor for the occurrence of micronucleated cells in the study group (RR = 3.2; 95% CI, 2.7–3.9; $p < 0.001$). The results of the spirometry test did not reveal significant respiratory effects between the reference and study groups.

Keywords: micronuclei; spirometry; printing office; VOC

Citation: Linhares, D.; Rocha, J.; Rodrigues, A.; Camarinho, R.; Garcia, P. Evaluation of Respiratory, Genotoxic and Cytotoxic Effects from Occupational Exposure to Typography Activities. *Atmosphere* **2023**, *14*, 562. https://doi.org/10.3390/atmos14030562

Academic Editor: Pasquale Avino

Received: 23 January 2023
Revised: 3 March 2023
Accepted: 13 March 2023
Published: 15 March 2023

1. Introduction

Modern civilization spends on average one-third of their life at their work, representing roughly 90,000 h of exposure to the working environment [1]. As it is recognized that yearly more than 1 billion workers are exposed to noxious substances and that, because of such exposures, many of them lose their lives, succumbing to fatal diseases, such as cancers [2], it is important to increase the efforts in certain countries and occupational contexts to ensure the right of all workers to safe and healthy working conditions.

Exposure to different working environments can contribute to the development of different occupational diseases, being the most reported dermatitis, respiratory illnesses, cancer, hearing loss, stress, and mental health disorders. A workplace where most of these occupational diseases have been reported is the typographies, as the workers are continuously exposed to toxic components derived from photocopiers during printing activities [3]. These workers are particularly exposed to the substances that make up ink cartridges [4] and to polycyclic aromatic hydrocarbons (PAHs), styrene, magnetite (Fe_3O_4), and nitropyrene [1]. In addition to the ink cartridge components, when printers are in operation, they release various hazardous compounds, such as toxic gases (ozone, nitrogen dioxide), and volatile organic compounds (VOCs), and produce low-frequency electromagnetic emissions [4].

Some studies have linked exposure to photocopier ink cartridges to many illnesses, such as sick building syndrome and sarcoidosis-like pulmonary diseases [5,6]. Nevertheless, since VOCs emissions are well-known for negatively impacting the quality of indoor air in the workplace [7,8], the association between occupational exposure to VOCs and the effects

on the health of printing workers needs to be further addressed [8]. VOCs have carcinogenic components, such as benzene, with a high potential to damage human health, targeting specific organs (e.g., the liver, spleen) and systems (such as the respiratory system as exposure to VOCs may cause upper and lower respiratory symptoms and contribute to the worsening of asthma [9]. Moreover, it has been scientifically proved that exposure to VOCs induces a significant increase in the frequency of micronuclei in oral epithelial cells [8,10,11] and in lymphocytes [3,12], as well as the appearance of other nuclear anomalies [13]. As the oral mucosa is an important barrier to potential genotoxic agents [8], the buccal micronucleus cytometry assay (BMcyt assay) provides a trustworthy measurement of the genotoxic effects resulting from the malfunction of a person's DNA repair system [14].

Nuclear anomalies, such as the micronuclei, that emerge during mitosis, are associated with cytotoxic and genotoxic effects and are correlated with the processes of initiation and/or tumoral promotion [15]. Damage to cells, including epithelial cells, also leads to nuclear anomalies other than micronuclei, such as binucleates, pyknosis, karyorrhexis, and karyolysis [16]. Another valuable tool for evaluating the health status of the respiratory system, due to exposure to air contaminants, is the pulmonary function testing. In simple screening spirometry, the most used parameters are [17]: (i) FVC (forced vital capacity in liters)—The maximal volume of air exhaled using maximal effort following maximal inspiration; (ii) FEV1 (forced expiratory volume in one second in liters), the volume of air exhaled during the first second, and (iii) FEV1/FVC Ratio (Index of Tiffenau), the ratio is used to detect airways obstruction. The interpretive strategy of the spirogram involves establishing a pattern of abnormality (obstructive, restrictive, or mixed) and grading the severity of the abnormality.

Considering that in typographies the workers are continuously exposed to toxic components, especially VOCs, derived from photocopiers and, that there is little information regarding the genotoxic and cytotoxic potential of these compounds in this working environment, the present study aims to evaluate whether the toxic components produced during printing activity: (i) affect the lung function of typography workers; and (ii) increase the risk of DNA damage (genotoxic and cytotoxic effects) in the oral epithelial cells of these workers. For that, spirometry tests were carried out and the frequency of micronucleated cells and other nuclear anomalies in the oral epithelia was determined.

2. Materials and Methods

2.1. Study Participants

The study group was formed by 25 individuals working in local typographies. There were only considered eligible to take part of the study workers that were in the selected typographies for over six months and that worked regularly with photocopiers to ensure their exposure to carbon black, resin, polycyclic aromatic hydrocarbons (PAHs), styrene, magnetite (Fe_3O_4), nitropyrene, toxic gases such as ozone, nitrogen dioxide and volatile organic compounds (VOCs). All individuals carrying typographic activities were occupationally exposed to photocopiers, toners, paints, and polishes on a routine daily basis for at least 8 h.

The selected working places had a mean area of 44 m^2 (ranging from 12 m^2 to 60 m^2) and all presented windows and ventilation systems (e.g., air conditioning).

The reference group was composed of 44 individuals randomly selected from the local population inhabiting Ponta Delgada city (Azores-Portugal), where the studied typographies are also located. Individuals from this group had no prior or present work that could be related to the exposure to photocopier hazardous substances. Both groups were matched considering age and gender.

Individuals were only considered appropriate to participate in the study if they had no exposure to X-rays in the week prior to the collection of buccal epithelial cells.

All individuals signed a written informed consent to participate in this study. A questionnaire, prepared by the authors, was applied to compile data regarding their professional occupations, the occurrence of respiratory diseases, and lifestyle habits, such

as smoking, alcohol intake, and use of mouthwash (summarized in Table 1). Occupational history included the types of toners used in their respective photocopier machines, the total number of working hours/day, workdays/week, and years of service in the photocopier industry.

Table 1. Description of the demographic characteristics, lifestyle habits, and health status of the studied population groups (mean ± standard error—for continuous variables or % for categorical variables).

	Study Group (n = 25)	Reference Group (n = 44)	*p*-Value [a]
Age [b]	42.5 ± 1.5	41.9 ± 1.3	0.297
Gender			
Male	19 (76)	32 (72.7)	
Female	6 (24)	12 (27.3)	0.766
Tobacco consumption			
Yes	4 (16)	16 (36.4)	
No	21 (84)	28 (63.6)	0.073
Alcohol consumption			
Yes	9 (36)	22 (50)	
No	16 (64)	22 (50)	0.261
Use of mouthwash			
Yes	16 (64)	25 (56.8)	
No	9 (36)	19 (43.2)	0.559
Asthma			
Yes	2 (8)	8 (18.2)	
No	3 (92)	36 (81.8)	0.248
Bronchitis			
Yes	3 (12)	3 (6.8)	
No	22 (88)	41 (93.2)	0.463
Rhinitis			
Yes	3 (12)	6 (13.6)	
No	22 (88)	38 (86.4)	0.846
DNA damage			
MNC	12.9 ± 0.7	4 ± 0.4	<0.001
ONA	219.8 ± 14.5	51.5 ± 4.6	<0.001

[a] *p*-value comparing reference and study groups using the Mann-Whitney for continuous variables and the χ^2 for categorical variables. [b] Age is reported as the group mean ± standard deviation and is expressed in years.

2.2. Buccal Micronucleus Cytome Assay: DNA Damage

The buccal micronucleus cytometry assay (BMcyt assay) was used to determine DNA damage and cell death of buccal epithelial tissue [18]. This minimally invasive technique [19] is often used in biomonitoring studies regarding exposure to carcinogenic substances either under occupational or environmental contexts [11,20,21].

The samples of exfoliated buccal epithelial cells were obtained from inside both cheeks using a sterile cytobrush to scrap the mucosa surface. Then, the sampled cells were spread on pre-cleaned glass slides and then transferred into sterile tubes filled with alcohol.

The glass slides containing the sampled cells were dried up in the air and then methanol fixed. After fixation, cells were stained using the Feulgen method (adapted from Tolbert et al. [15,22]). The Feulgen-stained slides were evaluated under a light microscope (LEICA DM 1000, Leica Microsystems®, Wetzlar, Germany) with a 400-fold-magnification; for each participant, 2000 epithelial cells were analyzed and scored for the frequency of cells with one or more micronuclei, and other nuclear anomalies. In this study, we considered the following other nuclear anomalies: karyorrhexis (destructive fragmentation of the nucleus whereby its chromatin is distributed irregularly throughout the cytoplasm with the loss of nuclear membrane integrity), karyolysis (complete dissolution of the chromatin with nuclei completely depleted of DNA and therefore appear as Feulgen-negative ghost-like), pyknosis (shrinkage of the cell nucleus, intensively stained nucleus) and binucleation (two nuclei with similar size, morphology and with the equivalent intensity in coloring) [16,23] (representative photomicrographs are given in Supplementary Material Figure S1). The scor-

ing of micronucleated cells and cells with other nuclear anomalies was conducted following the criteria defined by Thomas et al. [16] and Bolognesi et al. [18].

2.3. Spirometry Tests: Respiratory Effects

The forced vital capacity (FVC) and the forced expiratory volume in one second (FEV1) values were obtained by spirometry in all participants. For the maneuver, the procedure was explained and demonstrated to each participant. Participants were instructed that they should sit in an up position wearing a nose clip and that they should completely fill the lungs, place the disposable mouthpiece in the mouth and close the lips tightly around it exhaling the tube until no more air could be expelled. The equipment used to perform these tests was the EasyOne automated portable spirometer (ndd, Zürich, Switzerland) that is equipped with software that checks for unacceptable maneuvers and compares the measured values with reference tables which, and meets ATS/ERS spirometry standards [24]. The standardized operating procedures were employed and controlled by the technician that maneuvered the equipment. Participants performed three attempts to provide technically acceptable maneuvers, the spirogram was examined for acceptability and repeatability criteria as recommended by the ATS [25] and the guidelines of the European Respiratory Society [24]. We did not apply post-bronchodilator tests.

Results from the spirometry were classified into one of three spirometric categories: normal pulmonary function, a restrictive defect, or an obstructive defect. Individuals with normal FEV1/FVC and with FVC <80% predicted were considered to have restrictive defects. According to the third United States National Health and Nutrition Examination Survey (NHANES III) for adult Caucasians, an FEV1/FVC < 70% was used as a fixed cut-point for obstruction (COPD). COPD was further classified in the following ranks given by the spirometer output: mild (FEV1 $\geq$ 80%), moderate (FEV1 50–79%), and severe (FEV1 30–49%), following the GOLD guidelines [26,27].

2.4. Statistical Analysis

The frequency of micronucleated cells and of cells with other nuclear anomalies, the FVC and FEV1 results were compared between individuals working in typographies and individuals without exposure to a typographic environment (study and reference groups, respectively) using the non-parametric Mann-Whitney *U* test; Chi-Square test was used to compare both groups regarding the consumption of tobacco (yes vs. no), alcohol, the use of mouthwash (yes vs. no) and the general health status of the participants (asthma, bronchitis, rhinitis; all yes vs. no). The *t*-student test was applied to compare both groups regarding age and respiratory patterns.

The association between the exposure to a working environment with photocopiers and the frequency of micronucleated cells, the relative risk (RR), and 95% confidence intervals (95% CIs) were estimated using a generalized linear model (Poisson Regression Model), adjusted for the use of mouthwash (yes vs. no), consumption of tobacco (yes vs. no), and consumption of alcohol (yes vs. no).

The IBM SPSS Statistics 24.0 for Windows was used to perform all statistical analyses; the level of statistical significance was set at $p \leq 0.05$.

3. Results

The demographic characteristics, lifestyle habits, and health status of the studied population groups are given in Table 1. Both groups were similar in terms of age, consumption of tobacco (smoking status), alcohol drinking, and the use of mouthwash elixirs (Table 1). Moreover, both groups are comparable in the diagnosis of respiratory health issues such as asthma, bronchitis, or rhinitis (Table 1).

3.1. DNA Damage

Results evidence significant differences between the study and reference groups for DNA damage. The frequency (mean ± SE) of micronucleated cells per 2000 buccal epithelial cells in the exposed group differed significantly from the values observed in the reference one, being higher in the former (12.9 ± 0.70 vs. 4 ±0.37, respectively); the frequency (mean ± SE) of cells with other nuclear anomalies per 2000 buccal epithelial cells was also significantly higher in the exposed group than in the reference one (219.8 ± 14.55 vs. 51.5 ± 4.62, respectively) (Table 1 and Figure 1).

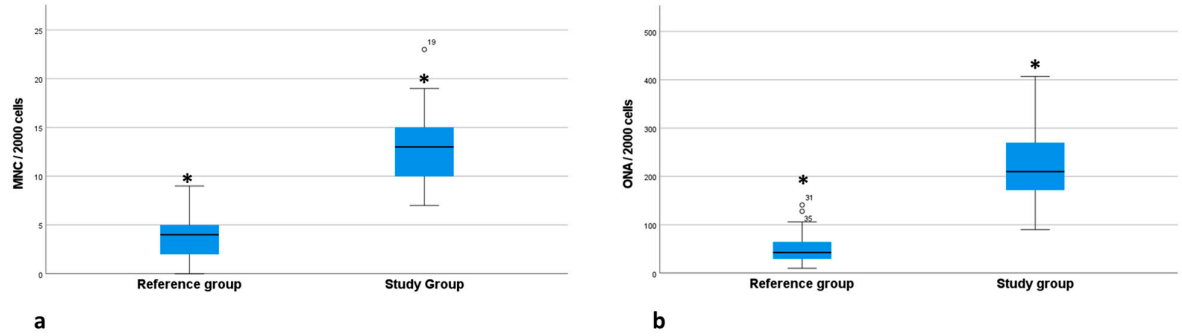

Figure 1. Box plots diagrams displaying the distribution of the frequency of cells with micronuclei (MNC) (**a**) and of cells with other nuclear anomalies (ONA) (**b**) per 2000 buccal epithelial cells in the study and reference groups. The line within the box corresponds to the median; thin vertical lines represent the minimum and maximum values; outliers (°). Significant differences between groups (Mann–Whitney U Test, $p \leq 0.05$) are indicated by the asterisk over the bars.

Exposure to a working environment with photocopiers was revealed to be a significant predictor of the frequency of micronucleated cells in the multivariate analysis. After adjustment for use of mouthwash, tobacco consumption, and alcohol consumption, a higher risk for an increased frequency of micronucleated cells was found associated with exposure to working in an environment with photocopiers (RR = 3.2; 95% CI, 2.7–3.9; $p < 0.001$) (Table 2). The analyzed confounding factors did not show any significant association with the frequency of micronucleated cells (Table 2).

Table 2. Adjusted association between characteristics of study participants, exposure to a working environment with photocopiers, and the frequency of micronucleated cells.

Poisson Regression (GLZ)		**Number of Obs: 69 Prob > χ^2 < 0.001**		
		N (%)	**RR (95% CI) [a]**	***p*-Value**
Mouthwash use				
	Yes	41 (59.4)	1.07 (0.9–1.3)	0.483
	No	28 (40.6)	1	
Tobacco consumption				
	Yes	20 (29)	1.02 (0.8–1.3)	0.846
	No	49 (71)	1	
Alcohol consumption				
	Yes	31 (44.9)	1.07 (0.9–1.3)	0.5
	No	38 (55.1)	1	
Exposure to photocopiers				
	Study group	25 (36.2)	3.2 (2.7–3.9)	<0.001
	Reference group	44 (63.8)	1	

[a] RR, relative risk, 95% CI, 95% confidence interval.

3.2. Respiratory Effects

Results evidence the lack of significant differences within the mean of FVC and FEV1 in the studied groups (p = 0.812 and p = 0.201, respectively) (Table 3). As for respiratory patterns, the relationships are slightly different within the groups; the reference group has significantly more cases of possible restriction patterns and moderate obstruction (Table 3).

Table 3. Description of the spirometry tests performed in the studied populations (mean ± standard error—for continuous variables or % for categorical variables).

	Reference Group	Study Group	*p*-Value [a]
FVC	94.2 ± 3.17	97.4 ± 2.76	0.812
FEV_1	83.4 ± 3.70	92.8 ± 3.15	0.201
Respiratory patterns			
Normal	32 (67%)	24 (96%)	<0.001
Restriction	9 (19%)	1 (4%)	0.038
Mild obstruction	1 (2%)	0	0.322
Moderate obstruction	5 (10%)	0	0.024
Severe obstruction	1 (2%)	0	0.322

[a] Mann-Whitney for continuous variables and *t*-test for categorical variables (*p*-value set at 0.05).

4. Discussion

Biological monitoring of exposure to chemical substances in the workplace is essential for evaluating occupational risks to human health. The BMcyt assay is considered a reliable and preferential method to measure humans chromosomal damage [28], being the frequency of micronucleus is extensively used as a predictor of genotoxicity and indicator of pre-carcinogenesis [29–32].

Our results reveal a significant association between working in an environment with photocopiers and the frequency of cells with micronuclei. The individuals occupationally exposed to the substances emitted by photocopiers have a threefold increased risk for the frequency of micronucleated cells when compared to the reference group. The frequency of micronucleated cells in the exposed group (about 13 micronucleated cells per 2000 cells, on average) was greater than the normal range for the human oral epithelia (0.3–1.7 MNC/1000; [33]). The higher frequency of cells with micronuclei observed in the group working in the environment with photocopiers is in line with similar studies that also observed an increase in DNA damage justified by exposure to the constituents of toners and their byproducts [1,34]. More recently, Ekapermana [35], concluded that exposure to toners substantially increases the frequency of micronucleated buccal epithelial cells., Khisroon et al. [36] also found a significant increase in DNA damage in the blood cells of persons working in typographies and that such increase was positively correlated with the duration of occupational exposure. The increased genotoxicity observed in the cells of the typography workers observed in the former studies and ours can be related to occupational exposure to high levels of particulate matter and volatile organic compounds emitted by photocopiers during operation [37]. Our results also revealed a significant increase in the frequency of ONA in the works carrying printing activities (about 220 cells with other nuclear anomalies per 2000 cells) when compared to the reference group (about 52 cells with other nuclear anomalies per 2000 cells). The considered other nuclear anomalies are biomarkers of genotoxicity and cytotoxicity: karyorrhexis and pyknosis are associated with both cytotoxicity (necrosis and keratinization) and genotoxicity (apoptosis), accompanying the early stages of apoptosis [18,25], while karyolysis and binucleation are only associated to cytotoxic events of the buccal epithelial cells [25,38]. Thus, besides the genotoxic effects, our results also revealed increased cytotoxic effects in the buccal epithelial cells of the workers carrying typography activities given their significantly higher frequency of cells with other nuclear anomalies.

Lifestyle variables, such as the use of mouthwash, tobacco consumption, and alcohol consumption, were not significantly associated with the frequency of micronucleated

cells. Even though mouthwash contains products that cause oxidative damage and consequently increase the extent of DNA damage in the buccal cells [39], there are several studies, similar to ours, that do not observe an association between the use of mouthwash and the development of micronuclei [11,39–41]. Tobacco is also known for containing several genotoxic chemicals [42] that could contribute to the increase of the frequency of MN but an association was only found for heavy smokers (i.e., >40 cigarettes/day) [33]; since in our study the mean rates for tobacco consumption (cigarettes/day) were 18.2 ± 6.2 (reference group) and 17.7 ± 4.8 (study group) no significant association was likely to be observed between the participant's smoking habits and the frequency of MN. Alcohol is also considered to be a genotoxic substance, being cited as an effective agent in causing DNA damage. Nevertheless, no association was observed in our study. These results are in line with those obtained by Anlar et al. [43] and Villarini et al. [44] that, similar to our study, had volunteers considered as moderate consumers of alcohol; this indicates that the consumption of a low amount of alcohol may not influence micronuclei formation. To consider the possible existence of respiratory illnesses, such as asthma, bronchitis, and rhinitis, pulmonary function was assessed by spirometry. The spirometry testing revealed no significant differences within the FVC and FEV1 of the studied groups. Although it would be expected that occupational exposure to the air contaminants present in typographies could affect the respiratory system, the study of Karimi et al. [45] revealed that lung function in photocopier workers was not significantly affected by the working environment. However, in our study, significant differences were observed in the restrictive pattern and moderate obstructive cases, with a higher frequency in the reference group. The differences observed may be related to tobacco consumption, as the reference group has more smokers, and smoking habits have been long related to interstitial lung disease and, specifically, pulmonary fibrosis which can contribute to a restrictive pattern when performing a spirometry test [46,47] and, COPD, a progressive lung disease, which includes chronic bronchitis and emphysema, that is associated to smoking habits and revealed by an obstructive pattern [48]. Some limitations of this study should be acknowledged. The small number of typographic workers (limited by dimension and working capacity of the studied typographies) and the lack of the use of a bronchodilator, and consequently, the lack of post-bronchodilator tests that enhance the accuracy of the results from the spirometry tests.

5. Conclusions

This study is the first to examine the genotoxic and cytotoxic potential of typographic environments in the Azores and one of the few worldwide. The obtained results evidence an association between exposure to contaminants from the typographic operation and the frequency of micronucleated cells and cells with other nuclear anomalies, evidencing a higher risk for DNA damage in these workers (particularly of increased genotoxicity, revealed by the higher frequency of cells with micronuclei). Since the increased frequency of micronucleated cells is recognized as a biomarker of pre-carcinogenesis, workers from typographies should be regularly biomonitored for DNA damage.

The spirometry showed that smoking habits have a higher contribution to the development of restrictive and obstructive respiratory patterns than occupational exposure to the typographic environment. This raises awareness of the risk of this habit, especially in working environments, where subjects are often exposed to other cyto-genotoxic contaminants that combined with smoking habits might strongly increase the risk of the development of respiratory illnesses.

Even though biomonitoring studies might be difficult to carry out because studies with humans imply many confounding factors, we highlight that our results reinforce the usefulness of BMCyt assay an important tool for the surveillance of workers potentially exposed to cyto-genotoxic contaminants.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/atmos14030562/s1, Figure S1: Representative photomicrographs of the epithelial cells stained with Feulgen (buccal micronucleus cytome assay): [a] differentiated cell, [b] differentiated cell with micronucleus (arrow), [c] karyolytic cell (arrow), [d] pyknotic cell, [e] karyorrhectic cell, [f] binucleated cell. Magnification of 400X.

Author Contributions: D.L. analyzed and interpreted the data regarding the obtained results in the spirometry tests and in the BMCyt assay. J.R. executed the collection of data and counted the nuclear anomalies. R.C. was responsible for staining and preparing the samples of the BMCyt assay. P.G. and A.R. were major contributors in writing the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by Azores DRCT Plurianual Funding (M1.1.A/Func.UI&D/010/2021-2024) and by the Fundo Regional da Ciência (Regional Government of the Azores) (Diana Linhares post-doc fellowship-M3.1.a/F/004/2018).

Institutional Review Board Statement: This paper studies the effects of the exposure of graphic workers to the interior atmosphere of these factories. However, when the study was done, our university did not yet have an Ethics Committee and therefore we do not have the opinion of an Ethics Committee. However, all participants signed an informed consent, in accordance with the European law.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the participant(s) to publish this paper.

Conflicts of Interest: The authors declare no conflict of interest.

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