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Original Article

Evaluation of the genetic damage to workers in a Greek shipyard

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Abstract: Shipyards are industrial areas where workers are likely exposed to environmental pollutants such as welding fumes, fine organic solvent and dye dust, that render the occupational environment a high risk one. Assessing the risk that workers are exposed to is a high critical factor in improving their working conditions. The present study aims to investigate the potential genetic damage to workers exposed to a harsh environment in a Greek shipyard. It is focused on assessing the percentage of induced micronuclei, as well as on changes in the various cell types of shipyard workers' oral mucosa epithelium by implementing the buccal micronucleus cytome assay. Exposed workers appeared with statistically significant induced micronuclei as compared to office employees. Statistically, significant cell lesions were detected and are related to workers' exposure to environmental conditions. The workers' smoking habit contributed as well to the observed buccal epithelial cell alterations. The observed data signify the high-risk workers are exposed; resulting in the shipyard's management the need to implement measures improving the working environment conditions and to reevaluate the workers' personal protective equipment requirements.

Key words: Occupational exposure, Buccal micronucleus assay, Welding fumes, Organic solvents, Dye dust, Smoking habit, Micronuclei induction

Introduction

Shipyards are industrial areas where a variety of ships that may require repair, maintenance and construction services take place. Shipward facilities employ a large number of skilled and non-skilled workers that despite regulations sometimes do not use the appropriate protective equipment. Some services provided take place in the open air, while other services take place in enclosed areas. Thus, shipbuilding facilities, being a hard-metal industry, should be considered a hazardous occupational environment. Among the building and repairing services provided are welding and spray painting.

Metal welding is expected to generate fumes and fine particulate dust that may present a serious hazard not only to the operator but to all those present in the area. Additionally, welders are exposed to extremely low-frequency magnetic fields (ELF-MF)[1–5] at higher workday mean exposures[6]. The chemical composition of the welding fumes depends upon various factors, such as the type of welding,

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metal coatings that may be present, the material of the electrode and the type of metal being welded. Metal welding fumes contain various metals, including chromium (Cr), manganese (Mn), nickel (Ni), copper (Cu), arsenic (As), cadmium (Cd), lead (Pb), iron (Fe), mercury (Hg), molybdenum (Mo), antimony (Sb) and aluminum (Al)[7–9].

Spray painting, when required, generates fine organic solvent and dye dust. The chemical composition of the various dyes and their organic solvents comprise a serious health threat to those involved in their production and occupational use. Organic solvents have been reported to induce neurobehavioral disorders[10], as well as potential genetic damage[11]. Paint industry workers are on the first line of those to be affected by dyes and their organic solvents[11–13]. The occupationally exposed workers, where paints are extensively used, are on the second line to paint industry workers. Such workers are employed in the painting of buildings, cars and ships employ various painting systems, from brushes to rollers to electrostatic or not spraying. Regardless of the painting system utilized and the protective means used, painting has been found to induce various health problems[10, 14–21].

During welding and painting, the primary targets of the generated fumes and fine particulate dust are the oral and nasal cavities and the lungs. The cells of the oral epithelium, forming the first barrier to the respiratory and digestive tract, are capable of metabolizing harmful chemicals into active products[22]. Thus, alterations observed in buccal and nasal epithelium cells may provide indications of possible side effects from the exposure to various harmful agents. Studies support the suggestion that noxious agents, such as Ni, are affecting the physiological process of the buccal epithelium inducing DNA damage, karyorrhexis, pyknosis and karyolysis[23], an observation that is compatible with the reported effect of cigarette smoke upon buccal epithelial cells[24]. An extended period of electroplating was associated with increased micronuclei (MN) induction in buccal mucosa cells in non-smokers[25], as well as in blood leucocytes[26], where Ni and Cr were measured in the subjects' plasma. According to Grimsrud *et al.*[27], there is a dose-dependent relationship between lung cancer and the water-soluble Ni compounds. Excessive exposure to noxious agents may lead to a risk of cancer, asthma, interstitial lung disease (hard-metal disease), or other adverse effects on the respiratory tract[28–30].

Many bio-monitoring studies investigate the possibility of side effects from the exposure to various harmful agents applied the micronucleus assay in human buccal mucosa cells (Buccal Micronucleus Cytome assay, BMCyt assay)[31–34].

MN are small masses of chromatin or chromosome fragments (DNA fragments) that during mitosis do not arrive at the poles of the spindle in telophase and remain encapsulated as a separate core outside the main cell nucleus[35, 36] leading to potential aneuploidies. Because aneuploidies are the key factors in the development of malignancies[37], MN act as internal dosimeters for the disclosure of specific genotoxic tissue damage in workers who are exposed to carcinogens[38, 39].

Thus, monitoring workers' health conditions, mainly of those working in enclosed areas, and persuading them to use their personal protective equipment (PPE) and improving the PPE standards are essential measures to reducing the risk to which they are exposed.

The present study aims to investigate the potential genetic damage of workers exposed to a harsh environment in a Greek shipyard employing the buccal micronucleus Cytome assay (BMCyt assay).

Subjects and Methods

Study group

The study was carried out on the workers of a Greek shipyard. Participants were selected based on the length of their working life. Office employees (white-collar workers, WCWs) and production line workers (blue-collar workers BCWs) with less than one year in the shipyard were excluded from the study. Participants' smoking habit was the main parameter scored in the present study, while their alcohol consumption was recorded as being an important socioeconomic parameter. An additional parameter that was recorded, without been taken into consideration, was the incidence of cancer in the participant's family with the important notice that none of the participants reported personal cancer incidence. A full and updated medical record was kept for all participants in the study by the company's occupational physician.

The participants were divided into two groups: a) control group: 26 office employees (WCWs) with no contact with the fumes and dust of the production process and b) exposed group. The exposed group consisted of 38 employees in the production line (BCWs). BCWs, in turn, were subdivided into those that were exposed to welding fumes and fine particulate dust (WFPD) and those exposed to fine organic solvents and dye dust (OSDD). Those exposed to welding fumes were the welders, the piping technicians and the metal technicians. Their average working period was 22.5 years with a maximum of 40 and a minimum of

Table 1. Distribution and demographic characteristics of the participants in the study groups

	Subjects					
	Control (26)[a] (office employees, WCW)					
	Smokers		**Alcohol**		**Cancer**	
	Yes	No	Yes	No	Yes	No
	3	23	2	24	6	20
Age	40.30 ± 13.54*					
	Exposed Workers (38)[a] (BCW)					
	Smokers		**Alcohol**		**Cancer**	
	Yes	No	Yes	No	Yes	No
Weld + Pip + Met (WFDP)	23	7	4	26	7	23
Maint (OSDD)	6	2	0	8	2	6
Total	29	9	4	34	9	29
Age	49.32 ± 8.89*					

*mean ± standard deviation

[a]number of employees

WCW: white-collar workers. BCW: blue-collar workers. Weld: welders. Pip: piping technicians. Met: Metal technicians. Maint: maintenance workers. WFPD: welding fumes and fine particulate dust. OSDD: organic solvent and dye dust. Smokers: smoking employees. Alcohol: heavy alcohol consumption. Cancer: cancer incidence in the family.

5 working years. Those exposed to organic solvents and dye dust were the painters and the maintenance workers. Their average working period was 16.1 years with a maximum of 35 and a minimum of 3 working years. While working, WFPD workers were not exposed to OSDD and *vice versa*, rendering their exposure to hazardous conditions rather homogeneous. Each of these groups could then be subdivided into smokers and non-smokers (Table 1). The 50% of the participants in the present study were smokers and the other 50% non-smokers. Non-smokers comprised the 88.5% of the white-collar and 23.7% of the blue-collar workers. Conversely, 11.5% of the white-collar and 76.3% of the blue-collar workers were smokers. The comparison of smoker versus the non-smoker of WCW is 3/23, while the corresponding analogy of BCW is 29/9. Heavy smokers, those smoking more than 20 cigarettes per day, were the 67.6% of the smoking participants. The number of non-smoker office employees is more than 3.5 times larger than the corresponding blue-collar workers, while the number of smoker white-collar employees is more than 6.5 times smaller than the corresponding blue-collar workers. Thus no decisive statistical conclusions can be drawn, although their analogy to the total number of participants is 32/64. It is important to note that according to published data, the smoke of cigarettes and tobacco contains Ni. Its content is estimated at 2.32–4.20 mg/kg and 2.20–4.91 mg/kg in smoke, while the presence of Ni in smokers' blood was not significantly different from the non-smokers. On the contrary, Ni concentration in smokers' urine was significantly higher than in non-smokers[40].

The average age of the WCWs was approximately 40 years, while of the BCWs 50 years. BCWs were working in their post for an average period of 20 years. The 7.7% of WCWs and 10.5% of the BCWs reported being heavy alcohol drinkers, while 23.1% and 23.7% respectively reported a cancer incidence in their family. Worthy of note is that no cancer incidence was reported or it was ever indicated in the participants' medical records. The 7.3% of BCWs were both heavy smokers and heavy alcohol drinkers, while none of the WCWs reported being both heavy smoker and heavy alcohol drinker.

The administration of the shipyard reported that all production line workers were provided the appropriate personal protective equipment (PPE). Furthermore, all production lines workers declared using their PPE during their working time.

According to records, kept in the administration and storage room of the shipyard, the electrodes used contain

Table 2. Frequencies (‰) and the non-parametric analysis of the various cell types in exfoliated epithelial cells of control and exposed subjects as well as of non-smoker and smokers

Cell types	Subjects														
	WCWs (26)*	WCW (NSm) (23)	WCW (Sm) (3)	BCWs (38)	BCWs (NSm) (9)	BCWs (Sm) (29)	WFDP (30)	OSDD (8)	WCWs/ BCWs (26/38)	WCW NSm/Sm (23/3)	BCW NSm/Sm (9/29)	NSm WCW/BCW (23/9)	Sm WCW/BCW (3/29)	WCW/ WFDP (26/30)	WCW/ OSDD (26/8)
	M ± SE								[a]*p*						
Basal	0.62 ± 0.10	0.61 ± 0.10	0.67 ± 0.33	0.61 ± 0.08	0.67 ± 0.17	0.59 ± 0.09	0.63 ± 0.09	0.63 ± 0.18	1.000	1.000	0.718	1.000	1.000	1.000	1.000
Differentiated	453.50 ± 16.81	454.04 ± 19.04	449.33 ± 5.92	401.21 ± 7.52	396.33 ± 7.35	402.72 ± 9.64	400.93 ± 8.58	402.25 ± 16.66	0.000	0.079	0.911	0.543	0.080	0.000	0.047
Binucleated	1.58 ± 0.31	1.26 ± 0.27	4.00 ± 1.15	3.76 ± 0.25	4.00 ± 0.74	3.69 ± 0.24	3.77 ± 0.30	3.75 ± 0.41	0.000	0.825	0.904	0.012	0.823	0.000	0.001
Micronucleated	2.81 ± 0.47	2.30 ± 0.41	6.67 ± 1.33	6.76 ± 0.29	5.89 ± 0.35	7.03 ± 0.35	6.83 ± 0.35	6.63 ± 0.46	0.000	0.901	0.068	0.009	0.898	0.000	0.000
Condensed	3.04 ± 0.42	3.26 ± 0.45	1.33 ± 0.67	3.37 ± 0.26	3.44 ± 0.69	3.34 ± 0.28	3.30 ± 0.26	3.63 ± 0.84	0.293	0.018	0.921	0.141	0.019	0.321	0.535
Karyorrhectic	39.50 ± 3.05	37.13 ± 2.91	57.67 ± 10.52	26.08 ± 1.03	25.56 ± 1.28	26.24 ± 1.30	26.00 ± 1.23	26.38 ± 1.75	0.000	0.004	0.850	0.078	0.003	0.001	0.013
Pyknotic	82.69 ± 7.33	76.13 ± 7.04	133.00 ± 14.42	86.58 ± 3.59	98.78 ± 8.40	82.79 ± 3.72	85.23 ± 4.00	91.63 ± 8.37	0.287	0.002	0.131	0.012	0.003	0.342	0.413
Karyolytic	422.73 ± 18.98	432.57 ± 20.59	347.33 ± 6.67	472.39 ± 9.16	466.67 ± 12.19	474.17 ± 11.47	474.17 ± 10.06	465.75 ± 22.94	0.002	0.000	0.874	0.034	0.001	0.002	0.121

*number of employees.

[a]*p*: 2 tailed Monte Carlo *p* Value.

M ± SE: Mean ± standard error.

WCWs: white-collar workers (office employees), BCWs: blue-collar workers (exposed employees). WFPD: welding fumes and fine particulate dust, OSDD: organic solvent and dye dust, NSm: Non-Smokers, Sm: Smokers.

iron, titanium dioxide, nickel, silicon, calcium carbonate, magnesium and copper. As a consequence, during welding works they prevail in the generated fumes.

All participants in the present study signed a consent document declaring that they are aware of the study and its purposes. The study was approved by the Ethical Committee of the National School of Public Health, Greece.

Epithelial cells sampling

Buccal cell samples from the participants were collected, after the end of work-shifts. Before sampling, participants thoroughly washed their mouth with tap water. Buccal mucosa cells were obtained by utilizing a manual toothbrush which was rotated with light pressure on the middle part of both inner cheeks, paying attention not to strike on teeth. Epithelial cells were transferred in sterile tubes which contained phosphate-buffered saline (PBS) and were transported under refrigeration to the laboratory for further processing.

The buccal micronucleus cytome assay (BMCyt assay) was used to a) measure biomarkers of DNA damage (micronucleated cells and micronuclei), b) cytokinetic defects (binucleated cells), c) proliferative potential (basal and differentiated cell frequency) and/or d) cell death (condensed chromatin, karyorrhectic, pyknotic and karyolytic cells). The BMCyt assay was performed according to standard procedures[31, 33] with minor modifications as previously reported[23].

In brief, the phosphate-buffered saline with the epithelial cells collected from buccal mucosa was centrifuged at 2,000 rpm for 5 min in order to sediment buccal cells that were then twice washed with saline and once more with Carnoy's fixative (methanol and glacial acetic acid 3:1) under the same centrifugation conditions.

Cell suspensions were dropped onto thoroughly alcohol-cleaned slides and allowed to air dry at room temperature. The slides were then stained with 7% Giemsa solution for 5 min, rinsed in distilled water, and air-dried. For each individual, the frequency of the various buccal cell types per 1,000 cells and the number of micronuclei in a total of 2,000 cells were recorded. Duplicate microscope slides were prepared and analyzed per subject. The study focused on observing changes in the various cell types of the oral mucosa epithelium, as well as on assessing the percentage of induced micronuclei, as biomarkers. Thus, oral epithelium samples were processed according to the buccal micronucleus assay protocol[31, 33]. Annotated microscope slide preparations were observed with a Leica DMLB (400X magnification) microscope by an independent researcher, unaware of any parameter of the samples.

Statistical analysis

To overcome the small number of participants in this study, non-parametric analysis (Mann-Whitney and Kruskal-Wallis tests with the use of SPSS17 [SPSS Inc.]) was employed to compare the calculated data from the BMCyt assay. The various employee groups were compared according to their exposure to electroplating fumes and particulate dust. In addition to non-parametric statistical analysis, χ^2 and G-test for independence on 2x2 tables were used for additional data comparisons with the use of Minitab statistical software (Minitab Inc., Pennsylvania, USA).

Results

Buccal Micronucleus Cytome (BMCyt) Assay Measurements and Analysis

Oral mucosa cell samples were prepared as previously reported[23] and observed microscopically by an independent researcher. Cells were classified into various types (Basal, Differentiated, Binucleated, Micronucleated, Condensed, Karyorrhectic, Pyknotic and Karyolytic) according to Thomas *et al*.[33]. 1,000 cells were observed per slide and recorded to identify the various cell types.

The mean values of the various cell types and the non-parametric analysis of the calculated data between the various workers' groups are presented in Table 2.

The initial analysis revealed statistically significant differences in almost all cell types between WCWs and BCWs which indicates that the exposed workers are influenced by the environmental conditions of their working area. Comparing the data between office employees (WCWs) and exposed workers (BCWs) we observe statistically significant differences in differentiated, binucleated and micronucleated cells as well as in karyorrhectic and karyolytic cells.

Further analyzing the calculated data for the exposed workers according to their exposure, we observed that those who were exposed to welding fumes and fine particulate dust (WFPD) revealing statistically significant differences towards the WCWs in differentiated, binucleated, micronucleated, karyorrhectic and karyolytic cells. Additionally, those who were exposed to fine organic solvent and dye dust (OSDD) revealed statistically significant differences towards the WCWs in differentiated, binucleated, micronucleated and karyorrhectic cells. The observed data indicate that the participants involved in welding are exposed to more hazardous working conditions than the ones involved in painting and maintenance.

Table 3. Non-parametric analysis of non-smoking WCWs versus BCWs Heavy smokers

Cell types	Subjects						
	WCW NSm (23)*	BCWs HSm (22)	WFPD HSm (17)	OSDD HSm (5)	WCW NSm / BCW HSm (23/22)	WCW NSm / WFPD HSm (23/17)	WCW NSm / OSDD HSm (23/5)
	M ± SE				[a]*p*		
Basal	0.61 ± 0.10	0.59 ± 0.11	0.65 ± 0.12	0.40 ± 0.24	1.000	1.000	0.627
Differentiated	454.04 ± 19.04	401.95 ± 10.70	405.76 ± 12.15	389.00 ± 24.15	0.005	0.017	0.075
Binucleated	1.26 ± 0.27	3.64 ± 0.25	3.53 ± 0.27	4.00 ± 0.63	0.000	0.000	0.001
Micronucleated	2.30 ± 0.41	6.95 ± 0.33	6.88 ± 0.40	7.20 ± 0.58	0.000	0.000	0.000
Condensed	3.26 ± 0.45	3.27 ± 0.35	2.94 ± 0.26	4.40 ± 1.25	0.861	0.858	0.392
Karyorrhectic	37.13 ± 2.91	27.18 ± 1.64	27.65 ± 2.00	25.60 ± 2.69	0.009	0.027	0.074
Pyknotic	76.13 ± 7.04	80.91 ± 3.73	81.53 ± 4.50	78.80 ± 6.76	0.198	0.216	0.545
Karyolytic	432.57 ± 20.59	476.09 ± 12.65	471.71 ± 13.94	491.00 ± 31.37	0.012	0.029	0.091

M ± SE: Mean ± standard error.
*number of employees.
[a]*p*: 2 tailed Monte Carlo *p* Value.
WCW NSm: non-smoking white-collar workers (office employees), BCWs: blue-collar workers (exposed employees), HSm: heavy smokers, WFPD: welding fumes and fine particulate dust, OSDD: organic solvent and dye dust.

Table 4. Non-parametric analysis of measured micronuclei based on the exposure of shipyard's employees with regard to their smoking habit as well

Subjects	M ± SE	[a]*p*
WCWs (26)*	5.42 ± 0.70	0.000
BCWs (38)	13.61 ± 0.65	
WCWs (26)	5.42 ± 0.97	0.000
WFPD (30)	13.90 ± 0.79	
WCWs (26)	5.42 ± 0.70	0.000
OSDD (8)	12.50 ± 0.80	
NSm WCWs (23)	4.39 ± 0.84	0.011
Sm WCWs (3)	13.33 ± 2.67	
NSm BCWs (9)	12.00 ± 0.73	0.201
Sm BCWs (29)	14.10 ± 0.80	
NSm WCWs (23)	4.39 ± 0.84	0.000
NSm BCWs (9)	12.00 ± 0.73	
Sm WCW (3)	13.33 ± 2.67	0.897
Sm BCW (29)	14.10 ± 0.80	
NSm WCWs (23)	4.39 ± 0.84	0.000
HSm BCWs (22)	13.91 ± 0.80	
NSm WCWs (23)	4.39 ± 0.84	0.000
HSm WFPD (17)	14.18 ± 0.97	
NSm WCWs (23)	4.39 ± 0.84	0.000
HSm OSDD (5)	13.00 ± 1.26	

M ± SE: Mean ± standard error.

*number of employees.

[a]*p*: Two-tailed Monte Carlo *p* Value.

WCWs: white-collar workers, BCWs: blue-collar workers, WFPD: welding fumes and fine particulate dust, OSDD: organic solvent and dye dust, NSm: non-smokers, Sm: smokers, HSm: heavy smokers.

Taking into account office employees' smoking habit (Table 2), a pronounced increase of micronucleated, karyorrhectic and pyknotic cells and a corresponding decrease of condensed and karyolytic cells it was observed. Furthermore, the decrease of differentiated and pyknotic cells appeared not to be so pronounced comparing the data of no-smoking versus the smoking participants. In the meantime, taking into account BCWs smoking habits, we observed that it does not contribute to any additional effect compared to the ones observed and induced by their working environment. Further analyzing the observed data between the smoking and the non-smoking participants statistically significant differences were observed in binucleated, micronucleated, pyknotic and karyolytic cells between non-smokers WCWs and BCWs, while statistically significant differences were observed in condensed, karyorrhectic, pyknotic and karyolytic cells between smoking and non-smoking WCWs and BCWs.

Analyzing the data of BCWs by comparing the heavy smokers (the ones smoking more than 20 cigarettes per day) versus the non-smoking BCWs or all the BCWs no differences were detected (Tables 2 and 3). Additionally, comparing WCWs, no differences were detected if WCW were segregated according to their smoking habit. However, comparing the BCW heavy smokers to the WCWs, pronounced differences were observed in differentiated, binucleated, micronucleated, karyorrhectic and karyolytic cells (Table 3). Segregating the BCWs according to their working environment (i.e. the WFPD and the OSDD ones) pronounced differences were observed in differentiated, binucleated, micronucleated, karyorrhectic and karyolytic cells in the WFPD exposed ones, while the OSDD ones revealed

differences only in binucleated and micronucleated cells. This observation indicates that the differences in the BCW heavy smokers came mainly from the WFPD ones.

It should be taken into consideration that the number of the smoking WCWs is very small (3 smoking over 23 non-smoking ones) and accordingly the non-smoking BCWs are not proportional to the smoking ones (9 non-smoking over 29 smoking ones). Thus, it is rather not safe to reach decisive conclusions.

The number of the participants that were heavy drinkers is very small (Table 1), 2 out of 26 of the WCW and 4 out of 38 of the BCW. The statistical analysis performed, taking into consideration participant's drinking habit, did not reveal any differences in their buccal cells. Thus, this observation cannot be taken into consideration.

Micronuclei Measurements

Micronuclei were evaluated in a total of 2,000 cells per subject according to Thomas *et al.*[33] Comparisons were made between non-exposed and exposed groups as well as to those exposed to either welding and fine particulate dust (WFPD) or fine organic solvent and dye dust (OSDD) (Table 4). Significant induction of micronuclei was observed between office employees and exposed workers regardless of their working post. However, comparing the smoking WCWs versus the smoking BCWs or the BCWs according to their working environment, no induction was observed indicating that their smoking habit possessed no additional effect. In the meantime, the participant's drinking habit did not reveal any induction, possibly due to the small number of those being heavy drinkers.

Discussion

In recent years, there is serious concern over the threat of the occupational environment on human health. Thus investigating its effect on human health will contribute to improving both occupational environment conditions and worker's health status. By occupational environment we mean the area into which one spends most of his working time. Works take place either indoor or outdoor and therefore the conditions differ. However, there are indoor working places where conditions are better controlled by the appropriate ventilation systems and/or PPE supplied by officials and used by workers. Following generally accepted guidelines contribute to eliminate the risks for which workers are exposed. It should be taken into consideration that there are cases where despite the appropriate measures are kept, the risks to which the workers are exposed can't be properly controlled. Furthermore, there are cases where either PPE are not used or the standards of the appropriate PPE are not applied. Such cases apply for those involved in picking rags[41], car technicians[42, 43], construction workers[44], painters[14–21] or road construction workers[45] and markers[46]. However, there are occupational environments where the working conditions are better controlled, but the works performed produce particular fumes that may threaten workers' health independently of the use or not use of PPE.

Among socioeconomic parameters indicative of a poor life-style, contributing to the possible increase in the risk the workers are exposed to, are smoking, alcohol drinking, poverty and the incidence of cancer. Smoking has been related with MN induction and buccal cell lesions[47–49], while alcohol drinking has not been associated with increased MN frequencies[50] although there are reports indicating a minor association[51, 52]. However an association was observed when additional parameters were considered together with alcohol drinking[53, 54]. Thus the consideration of confounding factors has to be taken seriously when one attempts to study the involvement of the working environment on workers' health.

Our intention, in the present study, was to investigate possible side effects of the occupational environment of a Greek shipyard, on its worker's health. Therefore, we employed the BMCyt assay which is a quick and minimally invasive method able to identify DNA damage, cytotoxic and genotoxic effects, as well as the regenerative capacity of oral mucosa epithelial cells[51, 55, 56].

The BMCyt assay detects the effects of exposure to inhaled or ingested genotoxic agents that can induce alterations to the physiological process of the buccal epithelium. Among such alterations are the karyorrhexis, pyknosis and karyolysis as well as the induction of MN. It is worth mentioning that a strong correlation between MN frequency in buccal exfoliated cells and human peripheral blood lymphocytes has been previously pointed out[51, 57]. As a consequence, the use of the BMCyt assay provides useful information regarding the influence of inhaled and ingested harmful agents on human health status in occupational environments.

During ship construction, maintenance and repair there is an increased production of fumes and suspended particles that possess a serious threat to human health. The fumes derive mainly from the welding process while the suspended particles from either the welding process and/or the maintenance of ships. Additionally, workers involved in the welding process are exposed to extremely low-frequency electromagnetic fields (ELF-MF).

In recent years, there is increased serious concern over the threat of the occupational environment on human health. Thus investigating its effect on human health will contribute to improving both occupational environment conditions and worker's health status and possibly of reduced insurance costs. It is well documented that both welding and solvent fumes and suspended particles, as well as ELF-MF in various occupational environments, possess a serious threat to human health[1–6, 9–21, 58–64]. In the meantime, results that indicate no threat to human health were also reported[65–67].

As mentioned before, our intention was to investigate possible side effects of the occupational environment of a Greek shipyard, on its worker's health, by employing the BMCyt assay which is quick and minimally invasive method able to identify DNA damage, cytotoxic and genotoxic effects, as well as the regenerative capacity of oral mucosa epithelial cells[51, 55, 56].

The BMCyt assay detects the effects of exposure to inhaled or ingested genotoxic agents that can induce alterations to the physiological process of the buccal epithelium. Among such alterations are karyorrhexis, pyknosis and karyolysis as well as the induction of MN. It is worth mentioning that a strong correlation between MN frequency in buccal exfoliated cells and human peripheral blood lymphocytes has been pointed out[51, 57]. As a consequence, the use of the BMCyt assay provides useful information regarding the influence of inhaled and ingested harmful agents on human health status in occupational environments.

In this study, the shipyard's employees were divided into the office employees (WCWs) that are not subjected to the environmental conditions existing in the area of the production line and to the exposed workers (BCWs) that were sub-divided into those exposed to welding fumes and fine particulate dust (WFPD) and those exposed to fine organic solvent and dye dust (OSDD).

A comparison between white- and blue-collar workers revealed statistically significant differences in almost all buccal cell types indicating the induction of cytotoxic damage possibly due to BCWs exposure to harmful environmental conditions. This observation is compatible with previously published data regarding welders[68, 69], tannery[70] and paint industry workers[71]. Higher DNA damage to welders was reported compared to controls, when tested after a one-week work, applying the cytogenetic endpoints of the comet and CBMN assays to peripheral blood lymphocytes[72, 73]. Furthermore, applying the BMCyt assay, an increase of binucleated and condensed chromatin cells when compared to unexposed subjects was reported in a Mexican welders[74] study, while increased cytotoxicity in a Brazilian welders study[69] was reported. In the meantime, increased rates of chromosome aberrations (CA) and sister chromatid exchanges (SCE) in blood lymphocytes of Mexican public building painters[15] were reported. Additionally, statistically significant DNA damage measured by the comet assay in blood lymphocytes of Brazilian paint industry workers exposed to low toluene levels[20] were observed. Testa *et al.*[42] reported higher frequencies of CA and SCE in blood lymphocytes of Italian automobile painters, while Kianmehr *et al.*[75], in Iranian construction painters study, observed increased DNA damage in blood lymphocytes, measured by the comet assay, compared to non-painter controls and Cassini *et al.*[76] reported increased DNA damage, measured by the comet assay, in blood lymphocytes of Brazilian painters. Increased frequencies of binucleated, karyorrhectic and karyolytic cells in buccal epithelial cells of Indian painters[77], but only of micronucleated cells in a Brazilian car painters and technicians study compared to office employees[43] were reported. In the meantime, Lee *et al.*[60] in a Korean shipyard painters' study, assessing several biomarkers potentially related to polycyclic aromatic hydrocarbons (PAH) exposure, reported elevated DNA-adduct levels to painters compared to on-site controls.

Shipyard's blue-collar workers being specialized in different works are exposed to different environmental hazards. Thus, it is important to study the possible side effects of the environmental conditions prevailing in the various work areas.

Analyzing our data according to BCWs specialized work and comparing them to the WCWs we observe that the ones involved in welding works, i.e. the ones mainly exposed to WFPD, appear with decreased differentiated and karyorrhectic cells and increased binucleated, micronucleated and karyolytic cells. However, the BCW that are involved in maintenance works appear with decreased differentiated and karyorrhectic cells and increased binucleated and micronucleated cells. These results indicate the possibility of triggering cytotoxic as well as genotoxic events that have to be taken into consideration.

Studies have demonstrated that smoking is associated with cellular lesions in oral mucosa[24, 47–49, 56, 78–83]. Furthermore, cigarette smoke contains between 2.20 and 4.91 mg Ni per tobacco kilogram[40]. Ni, being one of the components present in welding fumes[8, 9], has been reported to induce cell lesions and micronuclei induction in Nickel industry workers[23]. As a consequence, a further objective of the study was to compare the possible induced alterations

in the genetic material of smoking workers in relation to non-smoker ones.

Thus, by further analyzing the produced data, comparing exposed and non-exposed employees according to their smoking habit, although there is no good proportion in their numbers, it is speculated that there might be an induction of DNA damage (micronucleated cells and micronuclei) as well as of cell death (pyknotic cells) in smoker office employees (WCWs) compared to the non-smoker ones. This observation is compatible with previously reported data[23, 51, 55, 56, 78, 79, 84, 85]. There are no differences to the studied biomarkers between non-smoker and smoker blue-collar workers (BCWs) indicating that smoking had no additional effect on them. This observation is compatible with earlier reported observations[25, 86]. Additionally, in a Turkish welders' study[87], implementing the comet assay, significantly higher DNA damage in blood lymphocytes and implementing the BMCyt assay significantly higher DNA damage in buccal epithelial cells was reported, however, no correlation of the observed results with the smoking habit of the participants was found. In the meantime, in an Indian welders study[88], implementing the comet assay in blood lymphocytes, statistically higher DNA damage compared to controls was observed, while the smoking habit of the exposed subjects was not associated with the observed DNA damage. Sellappa *et al.*[89] showed a significant increase in micronucleated cells compared to controls and a larger mean comet tail length in buccal epithelial cells of welders and Danadevi *et al.*[86] reported statistically increased DNA damage in lymphocytes and an increase in micronucleated cells in buccal epithelial cells compared to the controls.

Our data, based on the workers smoking habits, revealed a statistically significant increase of binucleated, micronucleated, pyknotic and karyolytic cells, when non-smokers BCWs were compared with the corresponding WCWs, while differentiated and karyorrhectic cells appeared not significantly decreased in non-smoker BCWs compared to the WCWs. Furthermore, a statistically significant decrease of karyorrhectic and pyknotic cells and an increase of condensed and karyolytic cells was observed when smokers BCWs were compared to the WCWs.

Important observations were revealed when comparing the heavy smoking BCWs to the office employees. The statistical analysis of our data, derived from the office employees, did not reveal differences when all office employees were used for the comparisons performed, without including the non-smokers. The main observations for the heavy smokers involved in welding works are the reduction of differentiated and karyorrhectic cells, and the induction of binucleated, micronucleated and karyolytic cells. In the meantime, for the heavy smokers involved in the maintenance works, the analysis revealed the induction of the binucleated and micronucleated cells.

Another important observation was the pronounced induction of MN in almost all combinations analyzed. The association of welding fumes[15, 68, 72, 73, 77, 86, 87, 90] and organic solvents[4, 12, 21, 42, 43, 91] with genotoxic events have been already reported. However, some reports did not associate the induction of MN with exposure to either welding fumes[69, 73] or organic solvents[20, 76]. Our data is consistent with previously reported observations regarding the association of smoking with cytotoxic and genotoxic effects. The only comparisons that revealed no further induction of the observed micronuclei were the one of the smoking WCWs versus the corresponding BCWs and the one of the non-smoking and the smoking BCWs. In the meantime, Dominici *et al.*[4] reported that welders exposed to ELF-MF showed significantly increased micronuclei frequencies in their lymphocytes compared to non-exposed subjects and that their smoking habit had no important additional effect on micronuclei frequencies. The analysis of our data is consistent with their observation of increased micronuclei frequencies as a result of workers exposure to ELF-MF. Regarding the association of smoking on the induction of genotoxic events, we could speculate that the main contributor could be the Ni content present in both cigarette smoke[40] and welding fumes[8, 9]. The association of smoking with the induction of micronuclei was also reported in various studies of subjects exposed to either welding fumes and fine particulate dust[68, 72, 73, 89, 90] or organic solvents and dye dust[21, 42]. Incidentally, there are reports that in welders there is no correlation between smoking and increased micronuclei numbers[87] or DNA damage[88], as well as in painters exposed to various hazardous substances present in paints, thinners and hardeners[12, 43]. Furthermore, there are reports that do not associate smoking and MN induction in buccal cells of road markers[46] or silica exposed individuals[92]. Meanwhile, Sram *et al.*[91] in a systemic review study reported that smoking is not affecting significantly NM in lymphocytes measured by the CBMN assay.

The non-parametric analysis of our data indicates that the welding and organic solvent fumes and fine particulate dust generated during the various works in a shipyard induce cellular alterations that trigger cytotoxic and early genotoxic effects. Assessing the quantitative risk is the key event to implement control measures in the working areas and to upgrade the personal protective equipment that is the prerequisite for reducing the risk the working force is ex-

posed to. Siemiatycki *et al.*[93] referring to occupational carcinogens stressed that different carcinogens produce different levels of risk, and for a given carcinogen there may be vast differences in the risks incurred by different people exposed under different circumstances, thus there may be threshold effects or interactions with other factors, environmental or genetic ones, that produce no risk for some exposed workers and high risk for others raising the issue of quantitative risk assessment.

The present data reveal that the exposed workers when compared to the office employees showed: a) An increase in binucleated and micronucleated cells as well as in total micronuclei regardless they were exposed to WFPD or to OSDD. b) A decrease in differentiated and karyorrhectic cells regardless they were exposed to WFPD or to OSDD. c) An increase in pyknotic cells regardless they were exposed to WFPD or to OSDD. d) An increase in karyolitic cells in the WFPD exposed ones. Regarding the association of lifestyle parameters, such as smoking habit and alcohol consumption only speculations can be drawn. The proportion of smoking and non-smoking participants is equal, but regarding their analogy between WCWs and BCWs is unequal. The number of those that reported to have been heavy drinkers is very small and no analysis results could be drawn. As a consequence, the main observation is that smoking did not further contribute to the observed buccal cell lesions to the exposed workers in relation to the effects observed to the office employees. In the meantime, heavy smoking appears to be associated with the increase of binucleated and micronucleated cells as well as to the total number of micronuclei of the exposed workers.

Thus our observations further stresses that shipyard workers are risk exposed which suggests that shipyard management should implement measures to improve occupational environment conditions and reconsider workers' personal protective equipment characteristics. Furthermore, our study in association with reviews[9, 21, 91, 93–96] and other previously published studies support the classification by IARC of welding fumes and UV radiation[97] as "carcinogenic to humans" as well as painting[98] as an occupation that increases certain cancers risk.

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