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Evaluation of Genetic Damage to Workers in a Nickel Smelting Industry

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Abstract

Objectives: Occupational exposure to nickel is affecting millions of employees around the world. Potential alterations in the genetic material of workers in the mining and processing of nickel, possibly resulting from exposure to nickel in the production process, were investigated. The study focused on assessing the percentage of induced micronuclei, as well as on changes in the various cell types of oral mucosa epithelium. **Methods:** The buccal micronucleus assay was employed to assess possible induced genetic alterations to production line workers in comparison to office employees of the same nickel mining and pyrometalurgic processing industry. Subjects were also compared with regard to their smoking habit. **Results:** Very low soluble nickel levels were measured in certain workplaces and only in one workplace insoluble nickel was above the acceptable level. Statistically significant micronuclei differences among smokers and non-smokers, in both study groups as well as in total, were observed (p less than 0.01). Production line workers appeared with statistically significant induced micronuclei compared to office employees. Non-smoker production line workers compared to non-smoker office employees revealed statistically significant induced micronuclei. Statistically significant cell lesions were detected between non-smokers and smokers among office employees and production line workers documenting Ni engagement in their induction. **Conclusions:** The observed frequencies of micronuclei and cell lesions in the oral mucosa of workers, in certain positions of the production line, in relation to their smoking habit document the synergistic effect of Ni and cigarette smoking as effectors in their induction.

Keywords

Metallic Nickel, Soluble Nickel, Micronuclei, Occupational Exposure, Buccal

Micronucleus Cytome Assay, Smoking Habit

1. Introduction

Nickel (Ni) is the fifth most common element on Earth and is mainly found as sulfur, oxides and salts minerals. It is an important commercial product due to its special characteristics and is mainly used in the production of stainless steel as ferronickel (FeNi).

Occupational exposure to Ni affects millions of workers worldwide working in industrial mining, refining and production of Ni alloys, welding procedures and chemical catalysts with Ni compounds. The primary way of exposure to Ni is through inhalation of aerosols, dust and smoke containing Ni [1]. Ni is found in cigarettes and its content is estimated at 2.32 - 4.20 mg/kg and 2.20 - 4.91 mg/kg in smoke [2]. The absorption of inhaled Ni particles depends on the particle size and their solubility. The soluble Ni sulfate is absorbed faster than other Ni minerals [3] [4].

Exposure to sulfides, NiS and Ni_3S_2, was associated with lung cancer in several epidemiological studies in Ni mills in Canada [5] and Norway [6]. Studies indicate that metallic Ni is less likely to be carcinogenic to humans [7] [8]. According to Grimsrud *et al.* [9], there is a dose dependent relationship between lung cancer and the water-soluble Ni compounds. Soluble forms of Ni enhance the risk associated with exposure to insoluble forms of Ni [10]. The International Agency for Research on Cancer (IARC) indicated that there is sufficient evidence for the carcinogenicity of Ni sulfate and combinations of Ni sulfides and oxides in professional environments and thus classified soluble forms of Ni in group 1. Furthermore, IARC concluded that there is insufficient evidence that metallic Ni is carcinogenic to humans, and classified metallic Ni and Ni carbonyl as potentially carcinogenic to man in category 2B, based on sufficient evidence of being carcinogenic to animals [11].

The primary targets, for carcinogenesis induced by Ni, are the nasal cavity and the lungs. The cells of the oral epithelium, forming the first barrier to respiratory and digestive tract, are capable of metabolizing carcinogens into active products [12]. Thus, alterations observed in buccal and nasal epithelium cells may provide indications of possible side effects from the exposure to various harmful agents.

Many bio-monitoring studies, in order to investigate the possibility of side effects from the exposure to various harmful agents, used the micronucleus assay in human buccal mucosa cells (Buccal Micronucleus Cytome assay, BMCyt assay) [13]-[26].

Micronuclei 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 [27] [28] leading to potential aneuploidies. Because aneuploidies are key factors

in the development of malignancies [29], micronuclei act as internal dosimeters for the disclosure of specific genotoxic tissue damage in workers exposed to carcinogens [30] [31].

The present study aims to investigate the potential genetic damage of workers occupationally exposed in a large Greek industry of mining, manufacturing and processing Ni. The company uses pyro-metallurgy to extract Ni and the annual production is 18,000 - 20,000 tons of Ni. For this purpose the buccal micronucleus assay was used in order to determine the extent of genetic damage, in terms of micronuclei formation, from exposure to Ni. In addition, potential differences in the degree of oral mucosa cell lesions were estimated in workers exposed to soluble and insoluble Ni. Moreover, since according to several studies smoking causes cellular lesions to oral mucosa [32] [33] [34] [35] and also cigarette smoke contains Ni [2], a further objective of the study was to compare the induced alterations in the genetic material of smoking workers in relation to non-smokers.

2. Materials and Methods

2.1. Study Group

The study was carried out on a group of 59 workers of a large industry of mining, manufacturing and processing Ni, occupationally exposed to different concentrations of Ni (soluble and in-soluble). The participants in the present study were divided into two groups: a) control group: 13 office employees (nine males and four females) with no contact with the dust of the production process (white collar workers, WCW) and b) exposed group: 46 employees in the production procedure of Ni (blue collar workers, BCW, they all were males). Each of these groups was then subdivided into smokers and non-smokers (**Table 1**).

In the present study one shift office employees and exposed workers were included. Newcomer workers were excluded from the study. Furthermore, a number of employees and workers were excluded from the study as they were under medication, at least for a three months period prior to the sampling day, according to the instructions of their occupational physician. A full and updated medical record was kept for all participants in the study. In their records there was no report of heavy alcohol consumption or cancer incident in their family.

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

2.2. Nickel Measurements

The concentration of Ni, in the breathable and inhalable fraction of suspended particles in the air of 10 workplaces in the production process of the company, was measured using a portable personal sampler, (3-stages personal impactor RESPICONTM—Helmut Hund GmbH). The determination of Ni and its inorganic

Table 1. Distribution of the participants in the study groups.

Subjects	Number of Employees			
	Gender			
Control (office employees, WCW)	**Males**	**Females**		**Total**
Smokers	1	2		3
Non-smokers	8	2		10
Total (controls)	9	4		13
Exposed Workers (BCW)[a]	**Years of Exposure**			
	2 - 10	**11 - 20**	**20 - over**	**Total**
Smokers	23	5	4	32
Non-smokers	6	3	5	14
Total (exposed workers)	29	8	9	46

WCW: white collar workers, BCW: blue collar workers, a: all BCWs were males.

compounds in the respirable fraction of suspended particles (RSP) was conducted according to the Standard—MDHS Procedure 42/2 [36] [37] [38] [39] and was expressed in mg/m^3 (Table 2). The Ni Institute (NI) reports different Ni limit values for various countries, however, a limit value of 0.01 mg/m^3 is widely acceptable [40].

According to the participants in the present study the plant was divided into: 1) Offices, where Ni processing by-products are not present in the air, 2) Sander, in the proximity of the mining place but far away of the pyro-metallurgic processing plant, and 3) the main Ni processing plant comprised by the Demineralization, Cranes, Dome, Electrical Furnaces, Granulation, Steelworks, and Forge workplace areas.

2.3. Epithelial Cells Sampling

Buccal cell samples were collected, after the end of work-shifts. Subjects prior to sampling washed thoroughly their mouth with tap water. Exfoliated epithelial cells of buccal mucosa were obtained by means of a manual toothbrush which was rotated ten times, with light pressure, on the middle part of the inner cheek, paying attention not to strike on teeth. The epithelial cells collected from buccal mucosa were transferred in sterile tubes containing phosphate buffered saline (PBS) and properly transported under refrigeration to the laboratory for further processing.

The buccal micronucleus Cytome assay (BMCyt assay) was used to measure biomarkers of DNA damage (micronucleated cell, micronuclei and/or elimination of nuclear material by budding-buds), cytokinetic defects (binucleated cells), proliferative potential (basal and differentiated cell frequency) and/or cell death (condensed chromatin, Karyorrhectic, Pyknotic and Karyolytic cells). BMCyt assay was performed according to standard procedures [20] [41] with minor modifications.

Table 2. Nickel (soluble and/or insoluble) concentration (mg/m^3) in the various workplaces of the plant.

Workplace	Insoluble Ni	Soluble Ni
Offices	Not detected	Not detected
Sander	Not measured	Not measured
Demineralization	0.0203	0.0012
Cranes	Not detected	Not detected
Dome	0.0066	0.001
Dome Caretaker	Not detected	Not detected
Electrical Furnaces	0.0048	0.0002
Granulation	Not detected	Not detected
Steel Works	Not detected	0.0012
Forge Caretaker	0.0059	Not detected

The phosphate buffered saline with the epithelial cells collected from buccal mucosa was centrifuged at 2000 rpm for 5 min to sediment buccal cells that next were 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 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 1000 cells and the number of micronuclei in a total of 2000 cells were scored. 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 [20] [41]. Annotated microscope slide preparations were observed with a Leica DMLB (400× magnification) microscope by a researcher who was not aware of any parameter of the samples.

2.4. Statistical Analysis

To overcome the small number of subjects participated in the present study and their distribution in the various workplaces non-parametric analysis (Mann-Whitney and Kruskal-Wallis tests with the use of SPSS17 [SPSS Inc.]) was employed in order to compare the calculated data from the BMCyt assay. The various employee groups were compared according to their smoking habits and their exposure to Ni compounds, soluble or insoluble. In addition to non-parametric statistical analysis, χ^2 and G-test for independence on 2×2 tables were used for additional data comparison with the use of Minitab statistical software (Minitab Inc., Pennsylvania, USA).

3. Results

3.1. Smoking Habit—Exposure

The subjects that participated in the present study were 42.7% non-smokers and 57.3% smokers (Table 1). The 68% of blue collar workers were smokers. The average exposure of BCW to Ni according to their working years, in positions where Ni at any form was detected, it was 10.83 years. 17 out of the 46 BCWs were exposed to metallic Ni in their working place, 15 were exposed to soluble Ni, while 14 were simultaneously exposed to soluble and insoluble Ni.

The difficulties encountered in the study focused on: 1) the heterogeneity of the sample in relation to smoking habits, as most of the production workers are smokers and 2) the exposure time of the individuals to Ni based on measurements made per workplace. Because of the extremely difficult working conditions at certain places of the production process (increased number of accidents, high temperatures, musculoskeletal strain, etc) a shift of workers between workplaces takes regularly place. This shifting renders all BCWs to be homogeneously exposed to either soluble or insoluble Ni during their working years.

3.2. Nickel (Ni) Measurements

With the use of a personal cascade impactor sampler and following the Standard—MDHS Procedure 42/2 for the determination of Ni and its inorganic compounds, insoluble and soluble Ni was measured in the various working places of company's production process. Table 2 depicts the results of these measurements. The main observation is that, in all places that measurements were performed, insoluble Ni concentration was below the international limit of 0.01 mg/m^3, except for the Demineralization area, while soluble Ni was not measured above the international limit at any working place [40]. In the Sander area, located near the mines, there was no Ni measurement performed as it is located in a large distance from the main plant where soluble and insoluble Ni may be suspended in the air.

3.3. Buccal Micronucleus Cytome (BMCyt) Assay Measurements and Analysis

Oral mucosa cell samples, from all participants in the study, were prepared and observed microscopically by an independent researcher. Cells were classified into various types (Basal, Differentiated, Binucleated, Micronucleated, Condensed, Karyorrhectic, Pycnotic, Karyolytic) according to Thomas *et al.* [20]. All subjects were divided into smokers and non-smokers. 1000 cells were observed per slide and scored to identify the various cell types.

Non-parametric analysis between smokers at the various work-places (Table 3) revealed statistically significant differences (Table 4) between office employees (WCWs) and exposed workers (BCWs) in cells with micronuclei. Statistically significant differences were also detected between non-smokers and smokers regardless their work-places. In the WCWs, between non-smokers and

Table 3. The frequencies (‰) of the various cell types in exfoliated epithelial cells of control and exposed subjects[a].

Cell types	Subjects			
	WCW ns	WCW s	BCW ns	BCW s
Basal	0.60 ± 0.16	0.50 ± 0.19	0.86 ± 0.10	0.89 ± 0.06
Differentiated	418.00 ± 36.26	439.38 ± 26.20	375.29 ± 33.70	310.89 ± 19.89
Binucleated	2.40 ± 0.34	3.24 ± 0.49	2.57 ± 0.31	2.96 ± 0.22
Micronucleated	4.30 ± 0.30	6.50 ± 0.46	6.21 ± 0.33	8.63 ± 0.49
Condensed	1.60 ± 0.37	1.63 ± 0.26	2.50 ± 0.36	2.63 ± 0.22
Karyorrhectic	39.90 ± 4.90	66.88 ± 6.54	53.07 ± 3.57	57.15 ± 3.02
Pycnotic	101.90 ± 10.13	131.00 ± 5.79	110.79 ± 6.89	119.41 ± 5.89
Karyolytic	431.30 ± 44.19	350.88 ± 25.83	449.50 ± 35.86	498.56 ± 21.65

a: Mean ± standard error, WCW: white collar workers, BCW: blue collar workers, ns: non-smokers, s: smokers.

Table 4. Non parametric analysis of WCWs versus BCWs as well as non-smokers versus smokers.

Cell Types		Subjects						
		WCWs		BCWs		WCWs versus BCWs		
		NS/S		NS/S		WCWs	BCWs	
		Mean ± s.e.[a]	*p	Mean ± s.e.[a]	*p	Mean ± s.e.[a]	Mean ± s.e.[a]	*p
Basal	NS	0.60± 0.16	>0.05	0.86 ± 0.10	>0.05	0.60 ± 0.16	0.86 ± 0.10	>0.05
	S	0.50± 0.19		0.89 ± 0.06		0.50 ± 0.19	0.89 ± 0.06	<0.05
Differentiated	NS	418.00 ± 36.26	>0.05	375.29 ± 33.70	>0.05	418.00 ± 36.26	375.29 ± 33.70	>0.05
	S	439.38 ± 26.20		310.89 ± 19.89		439.38 ± 26.20	310.89 ± 19.89	<0.05
Binucleated	NS	2.40 ± 0.34	>0.05	2.57 ± 0.31	>0.05	2.40 ± 0.34	2.57 ± 0.31	>0.05
	S	3.24 ± 0.49		2.96 ± 0.22		3.24 ± 0.49	2.96 ± 0.22	> 0.05
Micronucleated	NS	4.30 ± 0.30	<0.05	6.21 ± 0.33	<0.05	4.30 ± 0.30	6.21 ± 0.33	<0.05
	S	6.50 ± 0.46		8.63 ± 0.49		6.50 ± 0.46	8.63 ± 0.49	<0.05
Condensed	NS	1.60 ± 0.37	> 0.05	2.50 ± 0.36	>0.05	1.60 ± 0.37	2.50 ± 0.36	>0.05
	S	1.63 ± 0.26		2.63 ± 0.22		1.63 ± 0.26	2.63 ± 0.22	< 0.05
Karyorrhectic	NS	39.90 ± 4.90	<0.05	53.07 ± 3.57	>0.05	39.90 ± 4.90	53.07 ± 3.57	>0.05
	S	66.88 ± 6.54		57.15 ± 3.02		66.88 ± 6.54	57.15 ± 3.02	>0.05
Pycnotic	NS	101.90 ± 10.13	<0.05	110.79 ± 6.89	>0.05	101.90 ± 10.13	110.79 ± 6.89	>0.05
	S	131.00 ± 5.79		119.41 ± 5.89		131.00 ± 5.79	119.41 ± 5.89	>0.05
Karyolytic	NS	431.30 ± 44.19	>0.05	449.50 ± 35.86	>0.05	431.30 ± 44.19	449.50 ± 35.86	>0.05
	S	350.88 ± 25.83		498.56 ± 21.65		350.88 ± 25.83	498.56 ± 21.65	<0.05

a: Mean ± standard error, *p: Two tailed Monte Carlo p Value, WCW: white collar workers, BCW: blue collar workers, NS: Non-smokers. S: Smokers.

smokers, statistically significant differences were measured in pycnotic cells. In the meantime between smokers in WCWs and BCWs statistically significant differences were observed in differentiated, condensed and karyolytic cells.

3.4. Micronuclei Measurements

Micronuclei were evaluated in a total of 2000 cells per subject according to Thomas *et al.* [20]. Comparisons were made between smokers and non-smokers (Table 5). Additional non-parametric analysis among WCW and BCW in several working areas revealed statistically significant differences in the case of Steelworks and secondarily in the case of Demineralization. Comparing non-smokers, Office employees and those working in the production line, a strong correlation of micronuclei induction and Ni exposure is observed. The correlation is statistically more significant when in the comparison smokers are included. There is a strong correlation between smoking and increased micronuclei frequency between smokers and non-smokers in all workplaces. However, comparing total smokers per workplace not a very strong correlation is observed. In the meantime, there is a very strong correlation when comparing non-smokers.

4. Discussion

Humans are exposed to harmful agents either consciously or unconsciously. Conscious exposure occurs either occupationally or in lifestyle and can be controlled, while unconscious exposure is very dangerous. One of their primary targets is the oral epithelium. Alterations observed in buccal and nasal epithelium cells may provide indications of possible side effects from conscious or unconscious exposure to air or water soluble harmful agents. Oral epithelium cells, forming the first barrier to respiratory and digestive tract absorbing inhaled noxious agents, are capable of metabolizing carcinogens into active by-products [12]. To investigate the possibility of side effects from the exposure to such agents various bio-motoring assays have been developed. Among others, the BMCyt assay [13]-[26] act as internal dosimeter for the disclosure of specific genotoxic tissue damage in workers exposed to carcinogens [30] [31] [42] [43]. In addition, the BMCyt assay is a quick and minimally invasive method able to identify DNA damage as well as the regenerative capacity of oral mucosa epithelial cells in humans [34] [43].

Table 5. Non parametric analysis of measured micronuclei.

Subjects	Mean ± s.e.[a]		*p
WCWs	Non-smokers	8.60 ± 0.60	<0.05
	Smokers	13.00 ± 0.93	
BCWs	Non-smokers	12.64 ± 0.64	<0.05
	Smokers	17.96 ± 1.10	
WCWs / BCWs	Non-smokers	8.60 ± 0.60	<0.05
		12.64 ± 0.64	
	Smokers	13.00 ± 0.93	<0.05
		17.96 ± 1.10	

a: Mean ± standard error, *p: Two tailed Monte Carlo p Value, WCWs: white collar workers, BCWs: blue collar workers.

The intention in the present study was to investigate possible side effects to Ni exposed workers in an important Greek Ni mining, manufacturing and processing industry. Thus, the level of Ni in the air of the previously referred Ni industry was monitored in its various workplaces. Ni measurements did not reveal increased concentrations of either soluble or insoluble Ni. Soluble Ni was detected in concentrations lower than the widely acceptable 0.01 mg/m^3 level [40] only in the Demineralization, Dome, Electric Furnaces and Steel Works work-places. Insoluble Ni was measured below the acceptable level in the Dome, Electrical Furnaces and Forge Caretaker work-places and only in the Demineralization area it was found higher than the widely acceptable 0.01 mg/m^3 level [40]. In all other work places insoluble or soluble Ni was not detected. Thus, the reported Ni measurements data indicate that the above referred industry should be considered as a rather safe working place in case that personally protective outfit is applied throughout the plant.

According to published data, cigarettes and tobacco and in consequence their smoke 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 non-smoker ones. On the contrary, Ni concentration in smokers' urine was significantly higher than in non-smokers [2].

The subjects of the present study were grouped in relation to their work-place, on the basis of Ni measurements and their smoking habit. Analyzing the collected samples a statistically significant increase of the number of micronuclei is observed in smoker office employees in relation to non-smoker ones indicating the contribution of smoking habit to DNA induced damage. This observation is compatible with previously reported data [44] [45] [46] [47]. In the meantime buccal epithelium appears to be affected by smoking. A statistically significant increase in micronucleated, karyorrhectic and pycnotic cells was observed in smoker office employees compared to non-smoker ones indicating a process to eliminate possible DNA damaged cells by inducing cell death. Naderi and Pasha [48] reported a compatible observation comparing cigarette and water-pipe smokers to no-smokers suggesting the cytotoxicity of cigarette smoking. Cigarette smoke cytotoxicity on alveolar and nasal epithelial cells was previously reported [49] [50].

Data of production line workers revealed a statistically increase in the total micronuclei number of smokers compared to non-smokers. Taking into consideration that in certain workplaces of the pyrometallurgic production line soluble and insoluble Ni concentration was detected, it is rather obvious that Ni induced DNA damage to buccal epithelial cells. The statistical analysis of the various buccal epithelial cells, undergoing cell death, among non-smoker and smoker BCW revealed only an increase in micronucleated cells. This observation enhances the suggestion that Ni should be responsible for DNA damage during the maturation of the basal cell layer of this epithelium.

Comparing the observed data between non-smoker office employees (WCW) and production line workers (BCW) a statistically increase of micronucleated

cells and total micronuclei was observed, further enhancing the suggestion that Ni induces DNA damage in buccal epithelial cells. In the meantime a comparison among the smoker ones, WCW compared to BCW, in addition to the significant increase in micronucleated cells and total micronuclei a significant increase in cells undergoing the cell death process is observed.

Our data support the suggestion that Ni is affecting the physiological process of the buccal epithelium inducing both DNA damage and karyorrhexis, pycnosis and karyolysis, an observation being compatible with the reported effect of cigarette smoke upon buccal epithelial cells [48]. Taking into consideration that cigarette smoke contains between 2.20 and 4.91 mg Ni per tobacco kilogram [2], the reported effect should be accounted, among other effectors, to the presence of Ni [14] [16] [32] [34] [35] [48] [51] [52].

Taking into consideration that: 1) micronuclei act as internal dosimeters for the disclosure of specific genotoxic tissue damage in workers exposed to carcinogens [30] [31] [53] [54], 2) IARC has classified soluble Ni in group 1 carcinogenic substances [11] and 3) exposure to soluble forms of Ni has been associated with higher mortality and a high risk for lung cancer [5] [6] [7] [8] [9] [10], it is rather intriguing not to consider the high risk the smoker plant workers, particularly those working to the workplaces where either insoluble or soluble Ni was detected, are exposed, as smoking may play an important role in causing cell damage and triggering early genotoxic events. An elongated period of electroplating was associated with increased micronuclei induction in buccal mucosa cells in non-smokers [23] [54], as well as in blood leucocytes [55] where Ni and Cr were measured in subjects' plasma and not in their workplaces. It is well documented that micronuclei are a product of the early stages of the carcinogenic process in humans [32] [51]-[59].

The analysis of our data supports the micronuclei induction due to Ni inhalation and smoking habit which they possibly work synergistically. It must be noted that significant induction of micronuclei frequencies in human peripheral blood lymphocytes has been observed in heavy smokers occupationally exposed to various noxious agents [33], indicating the relationship and the synergistic effect of smoking and noxious agents in inducing genotoxic damages.

The non-parametric analysis indicates that the soluble form of Ni induces cellular alterations that trigger early genotoxic effects. Smoking appears to act synergistically on the possible side effects of occupational exposure to soluble forms of Ni, as statistically significant induction of micronuclei and/or other cellular lesions were observed in workers in the Demineralization, Electrical Furnaces and Steelworks areas of the plant where soluble forms of Ni were detected.

5. Conclusions

The working environment of this particular Greek nickel processing industry, with regard to nickel concentration in the breathable and inhalable fraction of

suspended particles in the air in the various positions of its production line, appears to be adequate for those participating in its activities taking into consideration that production line workers use their personal protection outfits. Taking into consideration our findings and the potential risks to the health of workers exposed to nickel, the company's management could take the following measures: 1) Air quality measurements every six months to determine the concentration of soluble and insoluble Ni forms 2) To reconsider the personal protective equipment characteristics that are in use at the various sites of its production line 3) The company's occupational physician requires examining the workers of the suspicious areas, for soluble forms of Ni, at least once every three months.

Analyzing the BMCyt data, buccal cell lesions and micronuclei frequencies were observed as a result of workers exposure to Ni. In addition, smoking habit induced the micronuclei frequency to office employees and a synergistic with nickel activity on the induction of the observed lesions is attested.

The above reported data confirm the IARC classification of soluble and metallic nickel forms with regard to their carcinogenic potential [11].

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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