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Assessment of Workers' Contamination Caused by Air Pollution Exposure in Industry Using Biomonitors

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Abstract. The worker's health service is guided to look for risks. There is not an evaluation of the onset of the disease linked to a long-term exposure to low levels of toxic agents. Besides, there are few records of the level of metal concentration in the environmental air in industry, as well as few records of the level of contamination of factory workers. To assess the level of pollution in workplace, galvanising industry was chosen as the object of this study. The worker exposure and contamination levels were assessed by means of airborne particulate matter collected in air filters and biomonitors. The analysis of the air filter, hair and toenail samples were carried out by Instrumental Neutron Activation Analysis, and urine samples by Graphite Furnace Atomic Absorption Spectrophotometry. The statistical multivariate analysis and ANOVA methods were applied to elemental concentration. Copper and iron elements were highlighted as the main contributors on the differentiation of the classes. The results point out the effectiveness of biomonitors in occupational studies.

Key words: biomonitor, copper, iron, neutron activation analysis, workplace exposure.

1. Introduction

In general, there are diverse sources of pollution in the industrial workplace but there are few records of the level of metal concentration in the environmental air in industry, as well as few records of the level of contamination of factory workers (International Atomic Energy Agency, 2000; Maitre *et al.*, 2003; Menezes *et al.*, 2000, 2002, 2004) Menezes, 2002. Statistical surveys about occupational diseases usually refer to accidents and damages and not to the occupational diseases developed through long exposures to hazardous working conditions (Almeida, 1998; Oliveira, 2001; Guth *et al.*, 1996; Schaller, 1990). Many processes take time to produce the sinister effects, and because of that, contamination is difficult to be identified. Not only the effects may appear in a long-term but also the onset of occupational diseases is similar to other chronic diseases. There is no careful investigation to identify occupational causes and the worker's health service is guided to look for risks. There is not an evaluation of the onset of the disease linked to a long-term exposure to low levels of toxic agents. All these points make an occupational diagnosis a very complex task (Almeida, 1998; Oliveira, 2001; Guth *et al.*, 1996; Schaller, 1990).

Several industries have some characteristic pollutants that are the worker's health service awareness main concern. Chromium is one of these risky elements related to galvanising industry (Silva, 1998), because it is among the ones responsible for the great majority of injuries caused to galvanising workers in a short time (Lin *et al.*, 1994; Silva, 1998). The toxicity of Cr is well known (Lin *et al.*, 1994): The oxidising agent chromic acid and the dichromate salts cause several injuries and, in some instances, septum perforation.

Aiming at assessing the level of other elements besides chromium, a study (International Atomic Energy Agency, 2000; Menezes *et al.*, 2000, 2002, 2004; Menezes 2002) was carried out in galvanising factories in Belo Horizonte, capital of the Brazilian State of Minas Gerais. Because the galvanisation process is responsible for the majority of patients who look for medical assistance due to metal contamination, the galvanising industry was chosen to be studied. This assessment also had as its objective to alert the Worker's Health Awareness Program of the Secretaria Municipal de Saúde (Municipal Department of Health) of Belo Horizonte, Minas Gerais, for the need of a study about long-term exposure to pollutants in the workplace associated with the worker's contamination. Up to now this is not the health programme task.

It is important to mention that the studywas approved by the Ethics Committee of the Federal University of Minas Gerais. During the study, the assessment of exposure levels to metals revealed that 92.3% of the elements from air filters were also determined in hair and toenail samples (Ag, Al, Au, Cl, Cr, Cu, Fe, Mn, Na, Sb). The present paper is related only to copper and iron, elements classified as essential to human being (Mertz, 1981).

2. Experimental Procedures

According to the statistical planning (International Atomic Energy Agency, 2000; Menezes *et al.*, 2000, 2002, 2004; Menezes, 2002), three workplaces (G1, G2 and G3) were studied. They were chosen at random downtown, Belo Horizonte city, and work with the same procedures called 'decorative chromium', a kind of galvanisation whose main industrial process is to plate several metals over small items like taps, trays and other decorative items. In order to assess the air pollution impact on workers' contamination, two evaluations were applied: the characterisation of work environment through airborne particulate matter (Kucèra *et al.*, 1996; Linch, 1981) and workers' biomonitoring (Cheng *et al.*, 1995; Ryabukhin, 1978; Saiki *et al.*, 1998) using hair, toenail and urine from volunteer workers. The total number of volunteers was 26. Another group was also studied, the Comparative Group, made up of 22 subjects not exposed to the same workplace environment. No other aspect such as diet and personal habit was controlled.

2.1. SAMPLING PROCEDURE AND PREPARATION OF AIRBORNE PARTICULATE MATTER

The level of elemental exposure in the indoor environment of the plants was evaluated carrying out the stationary air sampling. The samples collected came from places that would reflect, as much as possible, the indoor environment—near the polishing activities, as close to the chrome and copper baths as possible, in the office and in the items reception desk.

The sampling of airborne particulate matter (APM) was conducted by using both 0.8 μm pore size to get the breathable fraction (corresponding to APM having equivalent aerodynamic diameter less than 5 μm) and by using 5.0 μm pore size to get inhalable fraction. Both filters were housed in polystyrene cassettes. Each two samplers, each one with one type of filter, collected the APM simultaneously at the same place for 8 h—one-day working hours at 4 $L.\,min^{-1}$. After the sampling the cassettes were carefully opened, the air filters were folded and inserted into their respective polyethylene vial for irradiation. The samples were collected only once according to the strategy chosen (International Atomic Energy Agency, 2000; Menezes, 2002).

2.2. SAMPLING AND BIOMONITOR PREPARATION PROCEDURES

To begin with, physicians explained to the workers the aims of the project and how it would be performed. Afterwards the volunteer workers donated their hair, toenail clippings and also urine samples. A professional hairdresser collected scalp hair samples according to International Atomic Energy Agency (IAEA) protocol (Ryabukhin, 1978).The urine was collected on the last work day of the week and at the end of the shift because of the metal elimination rate (World Health Organisation, 1996). Instructions were followed in order to avoid external contamination. The urine samples were collected in polyethylene flasks and an aliquot of 5 mL was taken to determine the creatinine concentration.

Scalp hair samples were washed following the IAEA procedure (Ryabukhin, 1978) and dried at 40 °C. Toenail clipping samples were washed using the procedure according to the literature (Kucèra, 1996) and air dried. Both biomaterials were weighed in the irradiation container.

The urine samples, after being frozen, were lyophilised (this procedure aimed at concentrating the elements and reaching better detection limits) and solubilized with HNO_3 65% suprapur and diluted with water corresponding to 0.01% of acid (Silva, 1998).

The same preparation procedures were applied to the samples donated by non-exposed subjects. Only one sampling was carried out.

2.3. ANALYTICAL TECHNIQUES

In order to determine the elemental concentration in hair, toenail and air filter matrixes, a mix of k_0 instrumental neutron activation plus monostandard methods (De Corte, 1987; International Atomic Energy Agency, 2000; Menezes *et al.*, 2000, 2002, 2004; Menezes, 2002) was applied. It consisted of schemes of irradiation and gamma spectrometry. The irradiation was performed in the research nuclear reactor TRIGA MARK I IPR-R1 located at the CDTN/CNEN (Nuclear Technology Development Centre), Belo Horizonte, at 100 kW, under thermal neutron flux 6.6×10^{11} neutrons cm^{-2} s^{-1}.

The samples were irradiated simultaneously accompanied by standard comparators of Au and Na, and Human Hair Reference Certified Material, GBW 09101 (Shangai Institute of Nuclear Research, 1988). There is no reference certified material for toenail. Copper was analysed through its radionuclide ^{66}Cu—short half-life, 5.1 min—and iron through ^{59}Fe—long half-life, 44.496 days (De Corte, 1987). The gamma spectroscopy was performed in a HPGe detector 15% of efficiency, resolution of 1.85 keV for the 1332 keV peak of ^{60}Co.

The Neutron Activation was also applied to urine, however, the high interference of other radionuclides made the application of gamma spectrometry unfeasible. Then, urine was analysed by Graphite Furnace Atomic Absorption Spectrophotometry. The work conditions were according to the producer's instructions and literature (Silva, 1998; Perkin Elmer, 1984). The samples were analysed accompanied by Certified Reference Material SRM 2670 from the National Institute of Standards and Technology (National Institute of Standards and Technology, 1994).

3. Statistical Analysis

Data obtained were processed by Principal Component Analysis (PCA) (Georgescu *et al.*, 1998; Johnson and Wichern, 1992). This statistical method takes into account the correlation of several variables analysed simultaneously and reduces a data set to other linear combinations of the original measurements without significant loss of information. It means that the original variables can be condensed into a few new variables, PCs. Apart from the biomonitor data, the additional information used was about the workers such as the galvanising they were working for and the concentration results in air filters from each factory.

PCA was applied using the SYSTAT® program (SYSTAT® 7.0 for Windows®, 1997) and the additional information was coded in classes—matrix, group, activity (officer, item cleaners, trainee, plater, polisher and owner), galvanising- and sub-classes-type of matrix (air, nail, air filter), group (workers and non-exposed) and galvanising (G1, G2, G3). The PCs were determined for the workers using only hair results, only toenail results, only urine results; results for each two monitors together, and all three results together. The same arrangement was made analysing the groups.

The analysis considering air filter results was made using the same arrangement: only air filter results; air filter and hair results; air filter and toenail results; air filter plus two biomonitors and the three matrixes together. Afterwards the same procedure plus the additional information about the workers was used.

After determining the principal components, the method ANOVA was applied using the same SYSTAT® program. The objective was to verify whether there were similarities between the classes—matrix, activity, group and factory—and one PC.

4. Results and Discussions

Table I points out the Fe and Cu elemental concentrations determined in each fraction of airborne particulate matter (APM) for each galvanising studied. The table also shows the established Threshold Limit Values (TLV), i.e., the airborne concentrations of substances and conditions under which it is believed that nearly all workers may be repeatedly exposed to day after day without any adverse health effects, according to the definition of the American Conference of Governmental Industrial Hygienists (ACGIH) (American Conference of Governmental Industrial Hygienists, 2000).

The results suggest that at the time when the airborne particulate matter was collected, the areas presented real risk for the workers concerning Cu and Fe because these elements presented higher concentrations than their foreseen TLV.

However, for better evaluation of the risks it should be considered (American Conference of Governmental Industrial Hygienists, 2000) that the breathable fraction presents an increase in the toxic effects. The ACGIH suggests calculating the TLV for Mixtures. It means to calculate the sum of the ratios between the elemental concentration determined in the breathable fraction and the TLV foreseen for this element concerning this breathable fraction. The sum should be $\leq$ unit value meaning that the cumulative effects will be in a security range of concentration. If the sum is >1, it means that the security limits were exceeded (American Conference of Governmental Industrial Hygienists, 2000). The TLV for Mixtures were calculated for each element, Cu and Fe. Table II shows results and points out that 100% of the presently obtained values are >1 in the Polishing areas for all galvanisings, and for all areas in Galvanising 3. The classification of the three galvanisings in terms of health-related risks based on Fe and Cu is G3 > G2 > G1.

Table III presents the Cu and Fe elemental concentration determined in biological samples for non-exposed subjects, the Comparative Group, and for the Workers Group. It also shows the results for Reference Certified Materials for hair (GBW 0901) and urine (SRM 2670)—certified and experimental values—and those reported in the literature. For Cu and Fe in urine there are no Foreseen Biological Index (American Conference of Governmental Industrial Hygienists, 2000).The

Table I. Galvanisings: APM elemental concentrations (mg m^{-3})

	Element	TLV	Reception desk		Office		Bath area		Polishing area	
			Breathable fraction	Inhalable fraction	Breathable fraction	Inhalable fraction	Breathable fraction	Inhalable fraction	Breathable fraction	Inhalable fraction
G1	Cu fume	0.2	ND		ND		ND		0.24 ± 0.04	
	Dust and mist	1		ND		ND		ND		ND
	Fe fume and dust	5	ND	ND	0.030 ± 0.003	0.15 ± 0.08	1.2 ± 0.1	0.23 ± 0.03	15 ± 1	6 ± 1
G2	Cu fume	0.2	0.13 ± 0.01				0.03 ± 0.01		8.5 ± 0.3	
	Dust and mist	1		0.06 ± 0.02	No office	No office		0.15 ± 0.01		0.05± 0.01
	Fe fume and dust	5	0.3 ± 0.1	ND			0.4 ± 0.1	ND	167 ± 2	36 ± 1
G3	Cu fume	0.2	2.25 ± 0.04		0.05 ± 0.01		0.21± 0.07		4.7 ± 0.2	
	Dust and mist	1		0.50 ± 0.04		ND		0.33 ± 0.03		ND
	Fe fume and dust	5	31 ± 3	7 ± 2	10 ± 3	9 ± 4	229 ± 1	132 ± 5	ND	282 ± 15

ND, Not Detected; NF, Not Foreseen

Table II. Threshold Limit Values for Mixtures (TLVM), Cu and Fe, determined for the work areas

	TLVM			
	Reception desk	Office	Bath area	Polishing area
G1	<1	<1	<1	4.2
G2	<1	<1	<1	76
G3	17.5	7.5	46	23.5

results for the Comparative Group samples in general are in the same level of concentration presented in the literature. Observing Table III, it is easy to verify, that the majority of hair, toenail and urine elemental concentration results for workers were much higher than for the non-exposed.

Table IV shows results from the statistical analysis, for both Cu and Fe. It also shows the PCs, their percentage of the explained variance by each component of the total variation, the classes whose results, after applying the ANOVA, pointed out that the factories were different as well as the activities, groups and smoking. This table also shows the elements responsible for more positive (+) or negative (−) influence in each PC and the sub-class more influenced by this specific PC.

Concerning Table IV, Cu and Fe were present in several PCs showing their influence on highlighting the differences between the classes. Fe is among the group of elements which pointed out the smokers as the sub-class more influenced by this element compared to non-smokers. In fact all the Polishers presented the highest Fe concentration and all of them are smokers.

The multivariate analysis based on elemental concentrations determined in air filters, biomonitors and classes, showed that (i) the results obtained using different arrangements to group the data showed that the factories were different in spite of applying the same galvanisation processes, which is true as verified before because they offered different health-related risk; (ii) G3 was more influenced by several elements mainly Cu and Fe; (iii) toenail samples showed that the Worker and Comparative Groups were different population related to Fe.

5. Conclusions

This project revealed several elements present in workplace environment and various of them considered essential to human being as copper and iron were determined but in high concentrations, probably playing a role as toxic. The results from airborne particulate matter showed the high level of Cu and Fe which the workers were exposed to inside the galvanising factories and even at the office and reception desk, where there should be no pollution. The general assessment of risks related to the Breathable Fraction pointed out Galvanising 3 as the factory that offers the highest

Table III. Elemental concentration in biomonitors—Comparative and Workers Groups

	Hair (μg.g^{-1})	Toenail (μg.g^{-1})	Urine (μg.gcreat^{-1})	Hair (μg.g^{-1})	Toenail (μg.g^{-1})	Urine (μg.gcreat^{-1})
Reference material certified	GBW 0901 Human Hair	-	SRM 2670	GBW 0901 Human Hair	-	SRM 2670
	[a]20 ± 2	-	[a]140 ± 20	[a]ND	-	NA
	[b]23.0 ± 1.4	-	[b] 130 ± 20	[b]71.2 ± 6.6	-	NR
Literature	Saiki *et al.*, (1998)	Cheng *et al.*, (1995)	Iyengar, (1988)	Saiki *et al.*, (1998)	Cheng *et al.*, (1995)	Iyengar, (1988)
	4.0–56	1.71–34.8	17–300	7.2–37	8.2–207	180
Comparative group (22 individuals)	3–46 (21 individuals)	ND	<10	ND	78–323 (4 individuals)	<20
G1 (9 individuals)	23–590	ND–101	<10–36	75–3050	ND–430	IS
G2 (10 individuals)	45–882	40–101	<10–180	ND–12587	ND–420	<20–354
G3 (7 individuals)	ND–1637	29–144	17–112	ND–470	ND–316	60–993

NA, not analysed; NR, not reported; ND, not detected; IS, insufficient sample
[a]Experimental result; [b]Certified result

Table IV. Multivariate analysis results

Group	Matrix data	Principal component	Percent of explained variance (%)	Classes	Elements	Sub-classes
Workers	Hair	PC1	17.9	Activity	+ Al, Cu, Mn	Plater, owner polisher
		PC3	13.4	Galvanising	+ Co, Fe, La	G1
		$n = 5$			− Cl	
	Toenail	PC1	21.5	Activity smoking	+ Cl, Fe	Polisher smoker
					− Al, Au	
		PC2	20.5	Galvanising	+ Ag, Cu, Au, Cr	G3
		$n = 4$			− Fe, Mn	G1
	Urine	PC	57	Galvanising	+ Cr, Cu, Fe	G3
	Hair and toenail	PC2	22.5	Galvanising	+ Ag, Cu, Au, Cr, Zn	G3
					− Au	
		PC3	13.7	Smoking	+ Cl, Fe	Smoker
		$n = 4$			− Au	
	Hair, toenail and urine	PC	58.2	Galvanising	+ Cr, Cu, Fe	G3
Groups	Toenail	PC	47.8	Group	+ Au, Cr, Fe, Zn	Workers
Workers and air filter	Air filter	PC1	22.4		+ Cr, Zn, Fe, Na, Sb, Au	G3
		PC2	12.5	Galvanising	+ Ag	G3
		$n = 2$			− Mn, Cl, Cu	
	Air filter and workers' hair	PC1	51.3		+ Cr, Zn, Au, Fe	
		$n = 2$				

PC, principal component; *n*, number of principal components; G1, G2, G3, Galvanising 1, 2 and 3 health-risk to the workers, exactly the plant that had the most precarious conditions of work and where there was not physical separation between the processes.

Analysing hair, toenail and urine samples as biomonitors it was possible to verify the higher Cu and Fe levels at the workers when compared to the Comparative Group.

The statistical analysis applying the multivariate and ANOVA methods were useful. The methods demonstrated that through the elemental concentration results in the workers' hair, toenail and urine samples and airborne particulate matter samples it was possible to differentiate the factories, worker activities, group and smoking. The elements Cu and Fe were present in several PCs showing their influence on highlighting the differences between the classes.

The results of the study confirmed and reinforced the need of actions in order to minimise the hazardous work conditions. This first study alerts for the need of assessing the influence of a long-term exposure and will support the establishment of guidelines and data basis for the next occupational programme for this specific workplace. The results obtained evidence the efficiency of hair, toenail and urine as biomonitors suggesting endogenous contamination and the importance to carry out the airborne particulate matter sampling in parallel to these biomonitors mainly in occupational epidemiological studies.

The neutron activation analysis was applied to air filter, hair and toenail matrixes which confirms its status as one of the most versatile analytical techniques. The use of Graphite Furnace Atomic Absorption applied to urine as support technique was useful.

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