

EXPOSURE AND EFFECT BIOMARKERS IN SHOE MANUFACTURING PERSONNEL AND THE SIGNIFICANCE OF THEIR CHANGES

Felicia Grădinariu¹, Carmen Croitoru¹, Brigitte Scutaru¹, Valeria Hurduc¹, V Cazuc¹,
Al Maftel¹, Doina Hăvârneanu¹, Irina Alexandrescu¹, Mădălina Bohosievici¹,
Micaela Mărgineanu¹, Mirela Ghițescu¹, Liliana Găină²

1. Institute of Public Health Iași, România
2. Territorial Work Inspectorate Iași, România

Abstract: Health monitoring in small and medium enterprises (SMEs) and particularly in shoe industry is an obvious necessity in current conditions of Romanian economy in transition, in spite of the difficulties implied by this task. The **aim** of this paper was to analyze the relationship between different exposure and effect markers and other biochemical indices with the occupational risk factors and to detect if and how they reflect in the health status of the exposed workers. **Material and methods.** The exposed group (61 workers from shoes manufacturing factory from which 82% female) was investigated by a complex protocol including biochemical, immunological (IgE), and cytogenetic tests (oral mucosa micronucleus). Post-shift urinary acetone, hippuric and methylhippuric acid, total phenols and sulphate index were assayed. **Results and discussions.** The results were statistically analyzed and were compared to a control-matched unexposed group (n=58). The most frequent changes were found in sulphate index ratio and in micronucleus test. Serum IgE correlated with urinary acetone ($r=0.39$, $p<0.05$, $n=35$) in exposed with more than 5 years of service in footwear industry, showing the link of exposure with the frequent allergic outcomes diagnosed in these workers. Urinary sulphate index correlated negatively with the length of exposure in this industry ($r = -0.52$, $p<0.01$), showing the time dependence of this marker. Urinary hippuric acid correlated with total phenols ($r=0.78$, $p<0.001$) and also with urinary creatinine ($r=0.57$, $p<0.01$) suggesting the organism's intense effort for the detoxification of the solvents inhaled. **Conclusions.** We assume that the health status of the investigated workers seemed to be affected by working conditions. Our results demonstrate the need for further monitoring of these workers, in order to avoid harmful occupationally-induced health consequences. We recommend some measures for the reduction of workplace risk factors impact upon workers health.

Key words: solvent, occupational exposure, marker, effect

Rezumat: Supravegherea sănătății în întreprinderile mici și mijlocii (IMM) și în mod deosebit în cele de încălțăminte, reprezintă o necesitate imperioasă în condițiile economiei de tranziție din România, în pofida dificultății pe care o presupune un asemenea demers. **Scopul** acestei lucrări a fost acela de a analiza relațiile dintre diferiți markeri de expunere, de efect și a altor indicatori biochimici, cu factorii de risc ocupaționali și de a decela dacă și cum se reflectă aceștia în starea de sănătate a muncitorilor expuși. **Material și metodă.** Grupul de salariați expuși (61 muncitori din industria încălțămintei, din care 82% femei) a fost investigat printr-un protocol complex, incluzând teste biochimice, imunologice (IgE), citogenetice (testul

EXPOSURE AND EFFECT BIOMARKERS IN SHOE MANUFACTURING PERSONNEL

miconucleilor în celulele epiteliale bucale). În urina prelevată după încheierea programului de lucru s-au determinat acidul hipuric, metilhipuric, acetona, fenolii totali și sulfat indexul. **Rezultate și discuții.** Rezultatele analizelor efectuate s-au prelucrat statistic și s-au comparat cu cele ale unui lot martor (n=58). Cele mai frecvente modificări s-au găsit în testul miconucleilor și al sulfat indexului. IgE serice s-au corelat cu acetona urinară ($r=0,39$, $p<0,05$, $n=35$) la expunții cu peste 5 ani vechime în branșă, sugerând posibila legătura între expunere și fenomenele alergice frecvent diagnosticate la acești salariați. Sulfatul index s-a corelat invers cu durata expunerii ($r = -0,52$, $p<0,01$), ilustrând dependența de timp a acestui indicator. Acidul hipuric urinar s-a corelat direct cu fenolii totali ($r=0,78$, $p<0,001$) și cu creatinina urinară ($r=0,57$, $p<0,01$) fapt ce dovedește intensitatea efortului de metabolizare al organismului. **Concluzii.** Apreciem că starea de sănătate a salariaților investigați este afectată de condițiile de muncă. Rezultatele noastre demonstrează necesitatea continuării monitorizării biologice a salariaților, la toate nivelele, pentru a se evita efecte nedorite asupra sănătății. Sunt recomandate în final câteva măsuri pentru diminuarea impactului factorilor de risc ocupaționali asupra organismului.

Cuvinte cheie: solvent, expunere ocazională, marker, efect

INTRODUCTION

Health monitoring in shoe industry is an obvious necessity in the numerous small and medium enterprises (SMEs) with this profile, spread on the whole N-E territory of the country. These SMEs have a rather short lifetime because they appear and disappear after a few number of years or even months of activity. They have relatively small numbers of employees and encounter a high personnel mobility, because of their poor stability. Consequently, they often lack the financial and technical support to apply preventive measures for occupational risks. The complex mixture of hazards in the workplace of these SMEs is a real problem into evaluating them, as well as their combined health effects. In such conditions, health monitoring in this kind of workshops is not at all a simple and easy task.

The aim of this paper was to reveal the relations between work conditions and the health status of footwear workers,

as reflected through the exposure/effect biomarkers investigated.

MATERIALS AND METHODS

A group of 61 workers (82 % women) from a shoes manufacturing factory was investigated. They had an average age of 35.3 ± 9.3 years, total service length of 12.3 ± 8.9 years and 10.3 ± 8.9 years of service in footwear industry. A matched control group, having the similar average age and length of service, but unexposed to occupational hazards, was investigated according to the same protocol including clinical, biochemical, immunological and hematological exams. Post-shift urinary acetone, hippuric and methylhippuric acid, total phenols and sulphate index were assayed by commercial kits, as markers of exposure to organic solvents. Blood IgE changes as effect of allergens exposure were assessed by ELISA technique. Micronucleus test in oral mucosa was carried out as effect marker consecutive to the exposure to clastogenic substances.

Statistical analysis was performed using the Student's *t* test.

RESULTS AND DISCUSSIONS

Workplace characterization

The complex mixture of solvents in the glues used included: acetone, benzene, cyclohexane, ethyl acetate, ethyl-methyl-ketone, n-heptane, n-hexane, toluene, 3-methylpentane, n-pentane, o-xylene, p-xylene.

Workplace air contained also adhesives, dust particles (e.g., leather, rubber, polymers), additives in shoe materials (e.g. dyes, antioxidants) and heat degradation products of materials and additives, because leather pieces were thermally fixed together before

being sewed. Inadequate working microclimate was due to the noise, heat, poor lightening and improper ventilation of the rooms.

Workplace air measurements showed the presence of 12 different organic compounds. Four major solvents were quantified, as shown in table 1. Excepting ethyl acetate, exposure intensity did not exceed the allowable threshold limit values according with Romanian's legislation (1). We estimate that in spite of the moderate individual concentration, the overall cumulative effect of 8 hours daily exposure can not be neglected, due to the possible long term health effect of low-level exposure to these numerous compounds (2, 3).

Tab. 1 Solvent content in workplace air compared to Romanian TLVs

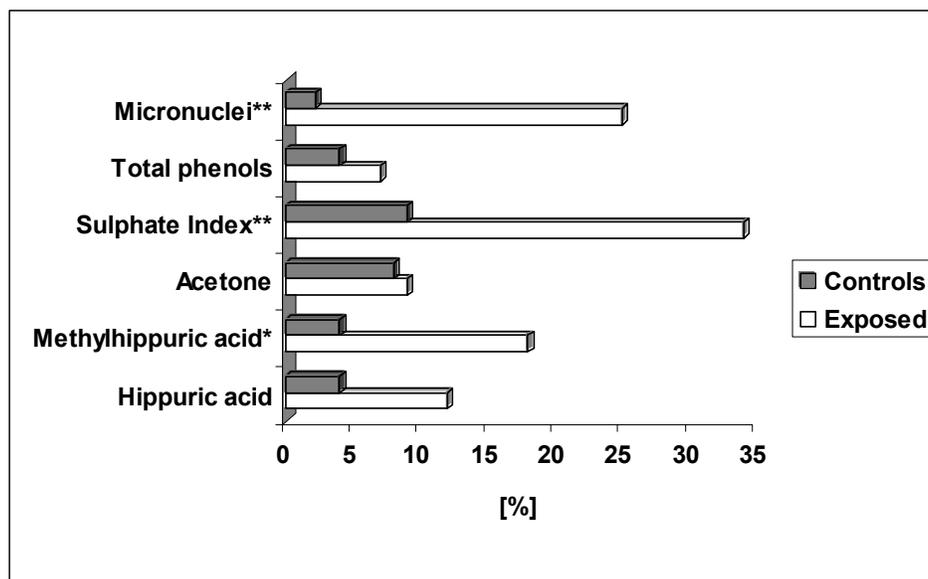
Curr. No.	Compound	Workplace air level mg/m ³ (15 min.)	TLV mg/m ³	
			15 min.	8 hours
1	Ethyl acetate	730	500	400
2	Acetone	240	-	1210
3	Xylene	66	442	221
4	Toluene	4	384	192

Health impact

65.5% of exposed subjects had at least the value of one biological marker over the normal limit, compared to 13.6% in the case of controls ($p < 0.001$). Fig. 1 shows the frequency of abnormal values for each biological marker for exposed, compared to controls. The most frequent changes were found in sulphate index ratio and in micronucleus test, confirming the fact, that the appearance of

micronuclei in the population of exfoliated cells in oral mucosa could be an early sign of clastogenic effect of toxic substances inhaled from workplace air (4, 5).

The higher frequency of micronuclei in buccal cells of exposed can not be attributed to smoking habit, because the average value of micronuclei in the 16 exposed smokers is significantly smaller than the non-smokers (1.93 vs. 3.38 of MN%, $p < 0.001$).



(** p<0.001; * p<0.01)

Fig. 1. The frequency of abnormal values of biomarkers in exposed group versus controls

In exposed workers with more than 5 years of service length in footwear industry, serum IgE level correlated slightly with the level of urinary acetone ($r=0.39$, $p<0.05$, $n=35$) (fig. 2). This relation confirms the link between the great number of allergic episodes encountered in these subjects, accompanied by elevated IgE and the intensity of exposure to organic solvents measured by high levels of urinary acetone, metabolite of ethyl acetate. Clinical examination revealed 30% cases of respiratory allergic syndrome and also 33.3% ocular allergic disturbances. In control group none of these complains were reported. These epidemiological findings are in concordance with recent experimental results which showed that mice, exposed

to low-level toluene, increased blood IgE and aggravated airway inflammatory responses by modulating neuro-immune signal (6, 7).

Urinary sulphate index correlated negatively with the length of exposure in footwear industry ($r = -0.52$, $p<0.01$) (fig 3). Sulphate index is the ratio between the anorganic sulphur eliminated through sulphate and the total sulphate consisting of the anorganic sulphate and the sulphate produced through metabolization by sulpho-conjugation. Aromatic solvents and also aliphatic alcohols are sulpho-conjugated to smaller and easier excreted molecules. The higher air content in such compounds the smaller is the value of sulphate index. This correlation clearly illustrates the

dependence of this marker of the length of solvents' exposure.

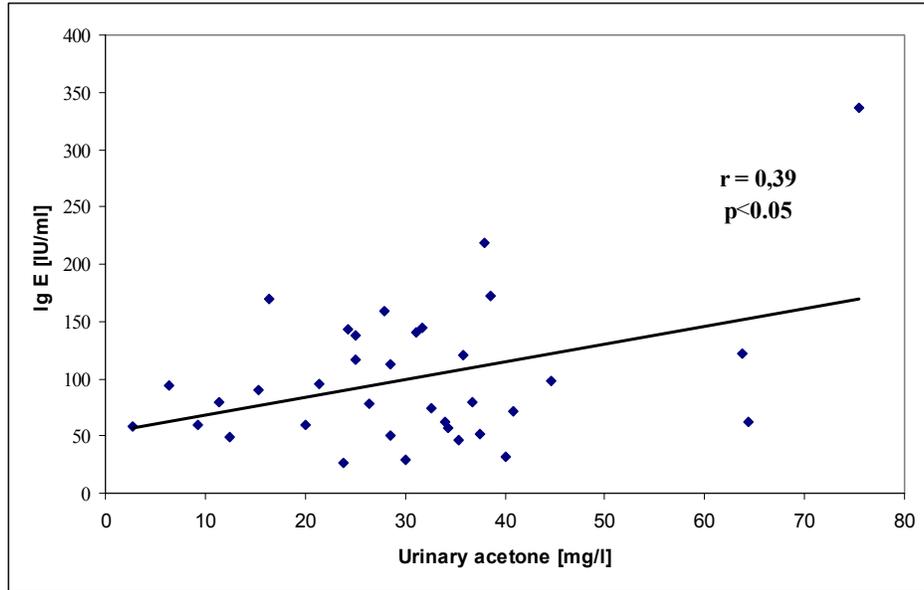


Fig. 2. The correlation between IgE and urinary acetone

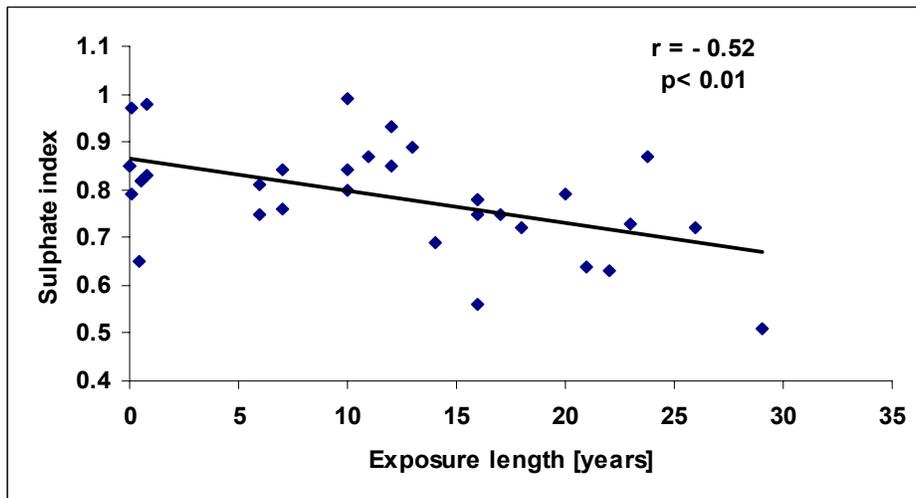


Fig. 3. The correlation between sulphate index levels and occupational exposure length

EXPOSURE AND EFFECT BIOMARKERS IN SHOE MANUFACTURING PERSONNEL

Methylhippuric acid level associated inversely with serum calcium level ($r=-0.25$) (fig 4). This could be an evidence for the occupational ethiology of the 13.3% cases of spasmohilya diagnosed in the predominantly feminine population of

these subjects. These disturbances are in line with recent *in vitro* experiments with sub-chronic low level of toluene, n-hexane and methyl ethyl ketone which induced comparable imbalance in intracellular calcium (8).

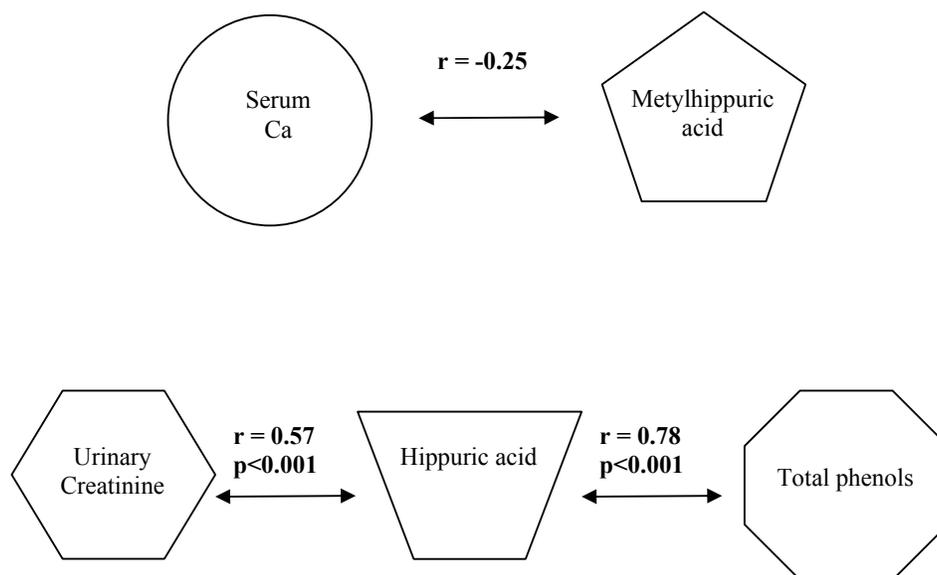


Fig. 4 Associations and correlation between different biochemical and exposure markers

Urinary hippuric acid level correlated with total phenols releases ($r=0.78$, $p<0.001$) and also with urinary creatinine ($r=0.57$, $p<0.01$) (fig 4). This double correlation suggests the organism's intense effort to detoxify the mixture of solvents inhaled from the workplace air. Data in the literature have recorded often similar results in workers employed in shoe industry (9, 10).

In order to avoid the deleterious effects of occupational exposure in shoe industry we recommend the implementation of new and safe technologies and the most appropriate measures of work security. It would also be beneficial to replace the common used adhesives with new water-based ones, which are already in use in several countries (11). We also recommend to set up and to apply preventive strategies for the detection and monitoring of the earliest signs of

affection, including the psychological and neurological tests, in order to avoid the onset of neurotoxic effects of solvent exposure (3).

CONCLUSIONS

The associations and correlations found between different assessed markers certify that the healthy status of these workers is affected by work conditions.

The complex matrix of occupational exposure in shoe manufacturing industry induced multiple adverse effects which have to be carefully monitored, at each biological level, in order to avoid harmful health consequences in exposed workers.

REFERENCES

1. HG 1218/2006 pub in *Monitorul Oficial* part. 1 nr. 854/13.10.2006
2. Herpin G, Gauchard GC, Vouriot A, et al: *Impaired neuromotor functions in hospital laboratory workers exposed to low levels of organic solvents* Neurotox Res. 2008, (3-4): 185-96.
3. Vouriot A, Hannhart B, Gauchard GC, et al: *Long-term exposure to solvents impairs vigilance and postural control in serigraphy workers* Int Arch Occup Environ Health. 2005, 78(6): 510-5.
4. Wetmore BA, Struve MF, Gao P, et al: *Genotoxicity of intermittent co-exposure to benzene and toluene in male CD-1 mice*. Chem Biol Interact., 2008, 173(3): 166-78.
5. González-Yebra AL, Kornhauser C, Barbosa-Sabanero G, Pérez-Luque EL, Wrobel K, Wrobel K: *Exposure to organic solvents and cytogenetic damage in exfoliated cells of the buccal mucosa from shoe workers* Int Arch Occup Environ Health, 2009, 82(3): 373-80.
6. Tin-Tin-Win-Shwe, Yamamoto S, Nakajima D, et al: *Modulation of neurological related allergic reaction in mice exposed to low-level toluene* Toxicol Appl Pharmacol. 2007, 222(1): 17-24.
7. Fujimaki H, Yamamoto S, Tin-Tin-Win-Shwe, et al: *Effect of long-term exposure to low-level toluene on airway inflammatory response in mice* Toxicol Lett. 2007, 68(2): 132-9.
8. McDermott C, Allshire A, van Pelt FN, Heffron JJ: *Sub-chronic toxicity of low concentrations of industrial volatile organic pollutants in vitro* Toxicol Appl Pharmacol. 2007, 219(1): 85-94.
9. Ikeda M, Ukai H, Kawai T, et al: *Changes in correlation coefficients of exposure markers as a function of intensity of occupational exposure to toluene* Toxicol Lett. 2008, 179(3): 148-54.
10. Ukai H, Kawai T, Inoue O, et al: *Comparative evaluation of biomarkers of occupational exposure to toluene* Int Arch Occup Environ Health. 2007, 81(1): 81-93.
11. Heuser VD, de Andrade VM, da Silva J, Erdtmann B: *Comparison of genetic damage in Brazilian footwear-workers exposed to solvent-based or water-based adhesive* Mutat Res. 2005, 583(1): 85-94.