Effect of occupationally phthalate exposure on pulmonary functions in Slovakian plastic industry

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ABSTRACT

Phthalates have adverse effect on human endocrine or reproduction system, but there is still lot of questions about their potential activity in human physiological functions. Number of papers indicates respiratory symptoms associated with possible phthalate exposure. Especially presence of MBP in human urine has been associated with decrease of FVC and FEV1 values. The aim of this study was to assess, by biological monitoring, worker's exposure to phthalates in the flexible-PVC industry in Slovakia to provide additional occupational exposure data, which are particularly scarce. Additionally, parameters of pulmonary functions and anthropometric values were obtained and analysed with exposure data. In response to determine human exposure to phthalates, we used high performance liquid chromatography (HPLC) and tandem mass spectrometry (MS/MS) analysis to quantitate trace levels of four phthalate metabolites, monobutyl phthalate (MBP), monoethylhexyl phthalate (MEHP), mono-n-octyl phthalate (MnOP) and monoisononyl phthalate (MiNP) in human urine. Urine samples, somatometric measures and spirometric values were collected from group of workers in plastic manufacture (n = 15; average age 44.8 ± 11.34). Lower values of FEV1 and FEV1/FVC point to potential airways obstruction in 13.3 % of probands (n = 2, average p/y = 12.5). We also observe overweight in 53.3 % of probands (n=8) indicated by BMI ≥ 25. Phthalate metabolites were detected in all urine samples. We suppose that occupationally increased exposure to phthalates has potential adverse effect on observed pulmonary parameters. However, to prove this assumption, we need more data to be analyzed.

***Key words**: anthropometry, HPLC/MS/MS, occupational health, phthalates, spirometry*

INTRODUCTION

Advances in materials science and engineering in recent decades have led to the widespread and diverse use of plastics to provide cheaper, lighter, stronger, safer, more durable and versatile products and consumer goods that serve to improve our quality of life. (1,2). Whereas every inhabitant in Europe and North America used 6.5 kg plastic each year in1980, the use had increased to around100kg in 2006 and continues to increase. In year 2005, the worldwide production amounted to 245 million tonnes a year and the industry employed more than 1.6million people (1,2). Components used in plastics, such as phthalates, bisphenol A (BPA) and others are released from plastic products and are also known as endocrine-disrupting compounds (EDCs) owing to their ability to modulate the human health (3). The esters of 1,2-benzene dicarboxylic acid, commonly called phthalates, are group of man-made chemicals used in large variety of industrial applications. As plasticizers, phthalates are additives which improve the flexibility, processability and softness of vinyl. (4) Phthalates can be also used for nonpolymeric application as fixatives, detergents, lubricating oils, and solvents (5). It is proved that phthalates exhibit endocrine disrupting activity and they can affect reproductive system a cause developmental anomalies (3,6-8). But there are lot of suggestions that they also play role in development of respiratory system disorders associated

with obstructions of airways. In studies (9-11) based on questionnaire data about plastic materials in homes was potential exposure to the phthalates associated with respiratory symptoms in young children. In adult studies (12-15) was assessed relationship between risks of respiratory disorders related to asthma and occupational exposure to PVC degradation products – potentially to phthalates. Urinary phthalate monoesters are considered good biomarkers for assessing phthalate exposure because of low contamination risk during processing and analysing samples (5). Despite of available analytical methods to assess human exposure via urinary levels of phthalate metabolites, there is lack of studies, which are using these methods in association with pulmonary function tests. There are basically three ways of exposure to these chemicals: by ingestion, inhalation and dermal contact (8). Because of presence of phthalates in big amount of materials and products of common use, there is high opportunity of exposure in everyday life. This risk is much higher in the process of production because workers are exposed to the fumes of the processed materials. Hence, we focused on assessment of phthalate exposure of workers in plastic factory by urine analysis and evaluation of pulmonary function of these workers.

MATERIAL AND METHODS

Study was conducted on workers of plastic factory (n=15), in which were used plastic injection molding machine to produce variety of products from PVC, PP and PET material. We obtained data about age, height, weight, pulmonary function parameters, smoking status and urine specimens. Control group consisted from probands of common population working outside of factory (n=25). All participants were physically healthy, without any acute symptoms. Probands' participation in this study was entirely voluntary and also had the possibility to withdraw their participation at any time during the study. Informed consent was required to be interviewed by the researcher, to provide samples of urine, complete questionnaires and allow the researchers to take measurements and also to process their medical and personal records and data. The anthropometric data was collected using standard anthropological methods; Body height was estimated by anthropometer (A 319 TRYSTOM, Ltd., Olomouc, Czech Republic). Body weight was estimated by digital weighting scale Omron BF510 (Kyoto, Japan) and body-mass index was classified by WHO (16). Spirometry was performed by Spirolab II (MIR S.r.l, Via Del Maggiolino, Rome, Italy) and Winspiro PRO 4.1 software. The European Respiratory Society predicted values (ERS) were used to calculate "normal" spirometry values. The best test result was determined following the ERS and ATS standards (American Thoracic Society), and FEV_1, FVC and PEF parameter reproducibility was also calculated.

For the phthalate analysis, urine samples were obtain from all volunteers and stored in transport box at 2-6 °C. In laboratory were all samples stored in deep freeze at the temperature -73°C until analysis (17). Four phthalate metabolites, monobutyl phthalate (MBP), monoethylhexyl phthalate (MEHP), monooctyl phthalate (MnOP), monoisononyl phthalate (MiNP) were measured in urine specimens by high performance liquid chromatograpy (HPLC) and tandem mass spectrometry (MS/MS). Urine analysis was made according to analytical method described by Silva (18) with use of manual solid phase extraction (SPE). Analytical standards were purchased from Cambrindge isotope laboratories (MA, USA). Briefly, 1ml of urine was thawed buffered with ammonium acetate and spiked with isotope labelled phthalate standards, β-glucuronidase enzyme (Roche, Germany) and incubated (37°C). After deconjugation were samples diluted with phosphate buffer (NaH_2PO_4 in H_3PO_4) and loaded on SPE cartridges (ABS Elut Nexus, Agilent). Cartridges were conditioned with acetonitrile followed by phosphate buffer before extraction. To remove hydrophilic compound were SPE cartridges flushed by formic acid and HPLC grade water.

Elution of analytes was performed by acetonitrile and ethylacetate. Eluate was dried by nitrogen gas and reconstituted with 200µl of H_2O. For HPLC purposed was used Agilent 1260 liquid chromatograph equipped with ZORBAX Eclipse plus phenyl-hexyl column. Separation was done using non-linear gradient program (Table 1). Agilent 6410 triplequad with electro-spray ionization was used for mass specific detection of phthalate metabolites. Instrumental settings were as follows: spray ion voltage (−3800 V), nitrogen nebulizer gas pressure (8 psi), nitrogen curtain gas pressure (7 psi), capillary temperature (430°C), and collision gas (nitrogen) pressure (1.5 mTorr). Precursor and product ions, collision energies, retention times and limits of detection (LOD) are showed in Table 2.

Table 1: Gradient program for HPLC separation

Time, min	0	4	6	8
A%	80	60	40	10
B%	20	40	60	90

Flow rate (0.3 $ml.min^{-1}$); mobile phase A (0.1% acetic acid in HPLC grade water) and mobile phase B (0.1% acetic acid in acetonitrile)

Table 2: Characterization of precursor, product ions, collision energies and retention times of phthalate metabolite and their labelled standards

Compound Name	Precursor Ion	Product Ion	Fragmentor (V)	Collision Energy (V)	RT, min	LOD, $ng.ml^{-1}$
MiNP	291,2	141,2	95	13	13,1	8.12
MEHP-C4	281,1	137,1	90	14	12,7	
MEHP	277,1	133,9	90	14	12,7	10.2
MnOP	277,1	127,2	90	10	12,9	8.52
MBP-C4	225,1	78,8	90	10	8,7	
MBP	221,1	76,9	90	10	8,7	3.47

RESULTS

For evaluation of pulmonary functions, we obtained anthropometric and spirometric data from 15 workers of plastic factory, three males and 12 females. Control group consisted of five males and 20 females approximately same age as target group. Values of anthropometric measurements, pulmonary function tests, age of both groups are showed in Table 3. Pack/year index (p/y) was calculated as a number of cigarettes smoked per day × number of years smoked/20 for participants with smoking history. We observed increased values of BMI in plastic factory workers and decrement of spiromteric parameters in plastic factory workers.

Table 3: Mean±SD values of anthropometric and spirometric parameters

	Case		**Control**	
	♂ n=3	**♀ n=12**	**♂ n=5**	**♀ n=20**
Age	51.67±10.12	43.04±11.36	43.00±21.43	39.1±14.09
BMI	26.27±1.36	27.44±6.27	26.33±4.27	24.13±4.06
FVC	4.02±0.34	3.17±0.4	4.92± 0.78	3.53±0.52
FEV_1	3.66±0.10	2.85±0.45	4.16 ±1.09	2.89±0.58
FEV_1/FVC	78.7±3.62	78.17±7.72	79.9± 9.23	81.51±7.62
MEF 25-75	3.33±0.43	2.87±0.97	4.03± 1.86	3.65±0.49
VC	3.52±0.5	3.7±0.59	5.03±0.98	3.45±0.45
p/y	1.67±2.89	2.42±4.98	9.09±21.27	5.65±24.79

FEV_1/FVC value is one of crucial parameters for diagnosis obstructive disorders of airways. In 13.3% probands of target group and 8% of control was determined decrement of this parameter under value 0.7. Workers in plastic factory (46.6%) had decreased this value under 0.8 as compared with 36% of control group. Also other parameters of pulmonary functions were decreased in case group.

Table 4: Effect of smoking status on reduced FEV_1/FVC values

		Case				Control			
		♂ n=3		♀ n=12		♂ n=5		♀ n=20	
		n	%	n	%	n	%	n	%
FEV_1/FVC < 0.7	Smoker	0	0	2	16.6	0	0	0	0
	Ex-smoker	0	0	0	0	1	20	0	0
	Non-smoker	0	0	0	0	0	0	1	5
	All	**0**	**0**	**2**	**16.6**	**1**	**20**	**1**	**5**
0.7≤FEV_1/FVC<0.8	Smoker	0	0	1	8.4	0	0	4	20
	Ex-smoker	0	0	2	16.6	0	0	1	5
	Non-smoker	2	66.6	2	16.6	2	40	2	10
	All	**2**	**66.6**	**5**	**41.6**	**2**	**40**	**7**	**35**
0.8≤FEV_1/FVC	Smoker	1	33.3	0	0	1	0	0	0
	Ex-smoker	0	0	0	0	0	0	1	5
	Non-smoker	0	0	5	38.4	1	0	11	55
	All	**1**	**33.3**	**5**	**41.6**	**2**	**40**	**12**	**60**

We analysed urine samples of all plastic factory workers (n=15) and part of control group (n=10). Unfortunately, optimization of analytical procedure is not still over, hence we have no relevant concentration data about levels of phthalates in collected urine samples. Recoveries of phthalate metabolites are not satisfactory and vary from 32.4% to 68.5%. We were able to detect trace levels of phthalate metabolites in all urine samples of plastic factory workers (Figure 1). Qualitative analysis confirmed presence of MBP and MEHP in all urine samples. MiNP was determined in 66.6% of plastic factory workers in contrast with 28% of control group and MnOP in 40% of target group but in none of control.

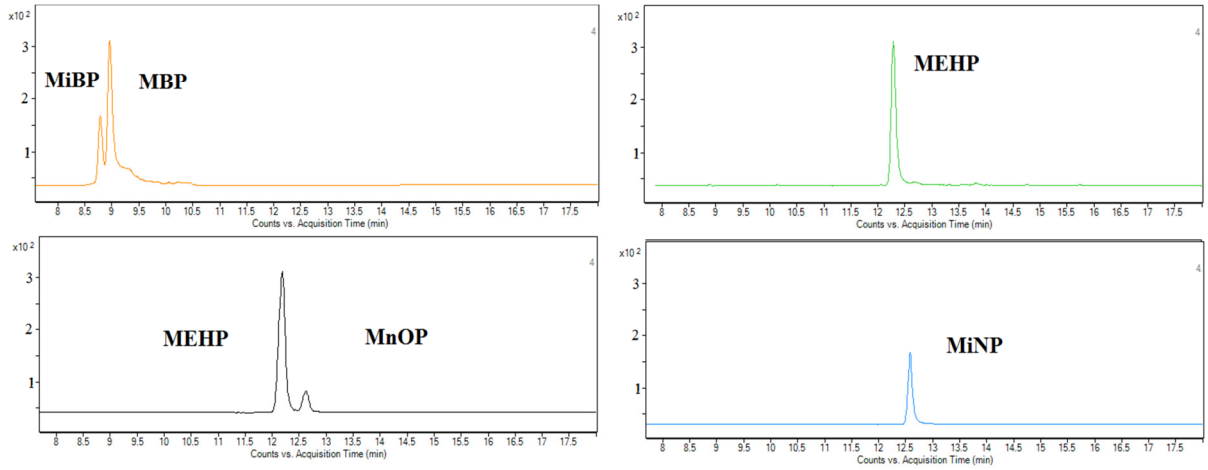

Figure 1: HPLC-MS/MS chromatogram of human urine sample with detection of all phthalate metabolites, retention times are showed in tab.2

DISCUSSION

Phthalates used in plastics for property enhancement are emerging environmental contaminants of concern. The limited human data, and in certain instances inconsistent data across studies, highlight the need for further epidemiological research on these classes of

chemicals (18). In our study, we monitored the environmental health status of workers in plastic industry. As shown by the results, lung volumes and flow rates were decreased in plastic factory workers as compared with control group. In accordance with GOLD criteria (19), we determined values of FEV_1/FVC and FEV_1 that can be associated with chronic obstructive pulmonary disease (COPD) in 13.3% probands of target group and 8% of control. Symptoms such as chronic cough, expectoration and normal spirometry ($FEV_1/FVC \leq 0.7 – 0.8$; $FEV_1 \geq 80$ %) are typical for cancelled 0 stage of COPD, which was reclassified (19) as simple and mucopuruluent chronic bronchitis with a higher risk of developing COPD. We identified this potential risk in 46.6% of plastic factory workers and in 36% of control group. We have also observed decrement of MEF_{25-75} parameters in plastic factor workers. This decline is associated with obstruction in small airways (19). As shown in table 3, risk of these respiratory disorders was higher in a group of plastic factory workers.

Smoking is considered to be most important risk factor in development of obstructive disorders of airways (19). Due to the small number of participants, we had to include to our study also active or ex-smokers. So decreased pulmonary function parameters might by partially due to the effect of smoking. However changes in pulmonary functions are also observed in non-smokers. Comparison of decreased values of FEV_1/FVC in both groups in dependence on sex and smoking status are shown in table 4.

The combined effects of smoking and work environment on the development of chronic respiratory disease were highlighted by the high percentage of subjects with symptoms of simple and mucopurulent chronic bronchitis in target group of our study.

Qualitative urine analysis confirmed presence of phthalate metabolites in all samples and there was no difference in detection of MBP and MEHP between case and control group. Parent phthalate compound of these two metabolites are one of the most common phthalates (18) and general population might be easily exposed to them. These findings correlate with other biomonitoring studies of phthalate exposure (4,20,21). Silva (20) and Blount (4) assessed presence of MnOP and MiNP in less than 20% of general population urine samples and Kato (21) did not even detect these metabolites. Their findings are in correspondence with our results for control group; nevertheless in urine of plastic factory workers was detected increased presence of these two phthalate metabolites. We suppose that this fact is affected by the work environment and not only presence, but also concentration levels of phthalates should be higher in plastic factory workers.

In addition, we detected levels of monoisobutyl phthalate (MiBP), which is isomeric form of MBP. As we see at the figure 1, there are two not fully separated compounds with fragmentation characteristics of MBP. According to Silva (17) MBP and MiBP have very similar fragmentation pattern, same precursor and product ions; hence they have to be qualified by chromatographic separation. Because of small side alkyl chain, retention times of these two analytes are shorter and they elute too close. For this reason, our gradient program needs to be modified to achieve better separation.

Exposure to MBP was associated with decrement of FVC, FEV1 and PEF in males (22). We determined presence of MBP in all urine samples. Unfortunately, without assessment of concentration of phthalate metabolites in urine, we cannot verify association between exposure to phthalates and impairment of pulmonary functions. Although there was contrast in presence MnOP and MiNP between groups, association between presence of these phthalates and spirometric measurements was not found. In studies based on presence of plastic material in homes (9-11) and workplace (12-15) were found similar obstructive disorders in association with potential exposure to phthalates.

CONCLUSION

We identified reduction of lung volumes and flow rates in plastic factory workers and detection of phthalate metabolites in urine samples confirmed exposure to unusual phthalates as in compare with control group. Presence of MBP and MEHP in all of analysed samples support assumption, that risk of exposure to phthalate is part of our everyday life. Following presented studies and our results, we suppose that exposure to phthalates play role in development of obstructive disorders of airways. However this assumption needs to be proved by quantitative analysis of phthalate.

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