Evaluation of benzene exposure in petrol pump attendants and in mechanics by urinary *trans, trans*-muconic acid (*t, t*-MA) determination

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Abstract

Occupational exposure to benzene in petrol pump attendants and in mechanics was studied by examining the benzene content in both the air breathed and in the urinary metabolite *trans*,*trans*-muconic acid (*t,t*-MA). Thirty petrol pump attendants and thirty mechanics (as exposed workers) and thirty adult male office workers (as non exposed workers) were involved in the study. Measures were taken at the begin and at the end of the working shifts. The benzene concentrations in the breathing air samples varied from 2 to 88 μg m^{-3}, lower than the EU acceptable limit for occupational environment. The average urinary *t,t*-MA in the petrol pump attendants at the begin and at the end of the working shifts ranged between 133 ± 69 and 255 ± 174 μg g^{-1} creatinine and in the mechanics between 204 ± 139 and 300 ± 211 μg g^{-1} creatinine, respectively. In all the participants the mean levels of urinary *t,t*-MA at the end of the working shifts were significantly higher than those at the beginning. In the exposed workers mean levels of urinary *t,t*-MA were significantly higher than in those of the non-exposed workers. The influence of the smoking was demonstrated by the urinary *t,t*-MA levels in smoking non-exposed subjects.

Key words: occupational exposure to benzene, trans,trans-muconic acid

Introduction

Benzene is an atmospheric pollutant of heavy toxicological relevance, because it is a mutagen and a carcinogen.[1-3]

The routes of human exposure to benzene are by inhalation, skin absorption and ingestion of contaminated food and water.[4] Urban environmental benzene pollution arises from combustion of oil derivates, particularly from road traffic and domestic heating. Indoor benzene sources include domestic fuel burning, products such as glues, solvents, paints and products for treating wood, but the main source is cigarette smoke, both active and passive. In the homes of smokers the concentration of benzene in the air can rise as high as 230 mg/die, with an average smoker inhaling about 1.8 mg benzene, which is 10 times higher than that of a non-smoker.[5,6] Accepted tolerable limits for benzene exposure in relation to carcinogenicity have not been established.[4] The occupational threshold limit value (TLV) of benzene in air (as 8 hours weighed average TWA_8) has been fixed in the EU Countries at 3.2 mg m^{-3} (1 ppm) as in USA, where the Occupational Safety and Health Administration (OSHA) has set the standard level for workplace exposure to benzene at 1 ppm, requiring employers to start monitoring when exposures reach 0.5 ppm[7,8]

Several studies have been undertaken to evaluate the issues of personal exposure levels and potential health damage for various categories of workers.[9-11] Petrol pump attendants and mechanics can be considered occupationally exposed to the inhalation of benzene through the release of gasoline vapours, but supporting data from biological monitoring is not currently available. Biomonitoring of benzene exposure is important in assessing its damaging effects and in the implementation of prevention strategies.As the urinary *trans,trans*-muconic acid (*t,t*-MA) is a benzene metabolite, which is considered a valuable indicator for the biomonitoring of occupational benzene exposure, the American Conference of Governmental Industrial Hygienists (ACGIH) proposed an urinary *t,t*-MA value limit of 500 μg g^{-1} creatinine in the people occupationally exposed.[12]

The present study has been carried out to evaluate the exposure to benzene in petrol pump attendants and in mechanics by the measuring of benzene levels in the breathing air during the working shift and of the urinary *t,t*-MA concentration.

Materials and methods

Thirty male petrol pump attendants and thirty male mechanics, aged between 18-55 years, were involved in the study. Thirty adult male office

workers were also studied as controls. All the participants appeared healthy. Sampling of breathing air was carried out by a radial diffusive passive sampler (Radiello Aquaria srl, Milan, Italy) worn by every participant during one complete working shift (8 hours), while levels of urinary *t,t*-MA and creatinine were obtained from urine samples individually collected at the begin and at the end of the working shift. At the end of the breathing air sampling the passive samplers were inserted in glass tubes with hermetic seals and stored at 4°C until analysis. The concentration of inhaled benzene was obtained by gas chromatography (GC-FID) desorbing the passive sampler by CS_2.

Urinary creatinine was dosed by the immunoenzymatic method (DIACRON International. Srl, Grosseto, Italy). Urinary *t,t*-MA was detected according to the method described by Melikian et al. with only slight modifications.[13] Frozen urine samples were conditioned at ambient temperature (about 22°C) for 30 minutes and then centrifuged at 1400 g. The clean surnatant was adjusted to pH 4.5-5.5 with HCl after which 1 ml was extracted under vacuum (Visiprep SPE Vacuum Manifold, Supelco Inc., Bellefonte PA, USA) on a PrepSep SAX cartridge (Isolute International Sorbent Technology, Hengoed, UK), which had been preconditioned with 3 ml methanol and then 3 ml of water. After application of the urine, the cartridge was washed three times with 3 ml of 1% acetic acid and the eluates discarded. *t,t*-MA was eluted with 3 ml of 10% acetic acid and the eluate analysed by high performance liquid chromatography (HPLC) as described in a previous study.[14]

Personal data, information about the smoking habits and personal written informed consent were obtained by each participant in the study.

Results

In the petrol pump attendants the benzene concentration in the inhaled air was between 14 and 62 μg m^{-3} (mean 39 ± 12 μg m^{-3}); mean values of urinary *t,t*-MA varied from 133 ± 69 μg g^{-1} creatinine (range 47-300 μg g^{-1} creatinine), at the begin of the working shift, to 255 ± 174 μg g^{-1} creatinine, at the end. Among the mechanics the values of benzene in breathing air ranged from between 9 and 88 μg m^{-3} (mean 35 ± 13 μg m^{-3}), the mean urinary *t,t*-MA levels varied at the begin of the working shift, from 204 ± 139 μg g^{-1} creatinine (range 31-625 μg g^{-1} creatinine), to 300 ± 211 μg g^{1} creatinine (range 75-738 μg g^{-1} creatinine) at the end. In the control group from the air inhaled, the concentrations of benzene varied from 2 to 7 μg m^{-3} (mean value 4 ± 1 μg m^{-3}) while the mean urinary *t,t*-MA values were 119 ± 61 (range 44-280) μg g^{-1} creatinine and 184 ± 152 (range 51-630) μg g^{-1} creatinine, respectively at the begin and at the end of the working shifts (Table 1). All the values of benzene in the air inhaled were lower than the admissible limit of 3.2 mg m^{-3}[7]. The levels of benzene found in the breathing air were not

Table 1. Concentrations of benzene in the inhaled air (μg m^{-3}) and of t,t-MA (μ g g^{-1} creatinine) from the urine samples of the study participants. SD, Standard Deviation.

Workers	Benzene in the inhaled air μg m^{-3}	*t,t*-MA (at the begin of work) μg g^{-1} creatinine	*t,t*-MA (at the end of work) μg g^{-1} creatinine
	Mean ± SD *(range)*	Mean ± SD (*range)*	Mean ± SD *(range)*
Petrol pump attendants	39 ± 12 *(14-62)*	133 ± 69 *(47-300)*	255 ± 174 *(47-823)*
Mechanics	35 ± 13 *(9-88)*	204 ± 139 *(31-625)*	300 ± 211 *(75-738)*
Controls	4 ± 1 *(2-7)*	119 ± 61 *(34-280)*	184 ± 152 *(51-630)*

Table 2. Concentrations of benzene in the inhaled air (μg m^{-3}) and of t,t-MA (μg g^{-1} creatinine) from the urine samples of the smoking and nonsmoking study participants. SD, Standard Deviation.

			Benzene in the inhaled air μg m^{-3}	*t,t*-MA (at the begin of work) μg g^{-1} creatinine	*t,t*-MA (at the end of work) μg g^{-1} creatinine
Workers	**Smoking habits**	**n.**	Mean ± SD *(range)*	Mean ± SD (*range)*	Mean ± SD *(range)*
Petrol pump attendants	Smoking	17	39 ± 14 (21-62)	142 ± 68 (54-300)	288 ± 184 (108-824)
	Non-smoking	13	40 ± 10 (14-59)	125 ± 77 (47-297)	229 ± 178 (47-614)
Mechanics	Smoking	19	36 ± 17 (9-88)	224 ± 168 (31-625)	310 ± 228 (75-738)
	Non-smoking	11	32 ± 6 (25-45)	148 ± 65 (4-255)	279 ± 198 (75-737)
Controls	Smoking	13	4 ± 1 (2-7)	138 ± 71 (51-280)	259 ± 200 (57-630)
	Non-smoking	17	4 ± 1 (2-5)	99 ± 45 (34-176)	110 ± 56 (5-220)

significantly different among the two worker groups studied. The urinary *t,t*-MA concentrations at the beginning and at the end of the working shifts were very similar for both the petrol pump attendants and the mechanics.

Across the three groups of workers considered, the urinary *t,t*-MA levels were increasing significantly during of the daily working shifts ($p < 0.05$).The increasing ratio in the urinary *t,t*-MA levels from the begin to the end of the working was 70% in the petrol pump attendants, 97% in the mechanics and 91% in the control group.

Data disaggregated for smoking habits showed that the *t,t*-MA levels of the worker's urine samples varied significantly both for smokers and for non-smokers except in the non-smoking control group (Table 2). Considering the results obtained from the non-smoking exposed workers, the increases in the urinary *t,t*-MA excretion from the beginning to the end of the work shift show that under the studied conditions, the role of inhaled benzene in the workplace can be relevant.

The increasing ratio between the urinary *t,t*-MA levels at the begin and at the end of the working shifts, across the three groups considered, was almost the same for the smoking and non-smoking, petrol pump attendants (from 62% to 78%), mechanics (from 88% to 100%) and the control group (from 77% to 100%).

Even though the benzene levels found in the inhaled air were about one hundred times lower than the established limit, in the 8% of the urine samples (180 samples) the concentration of *t,t*-MA exceeded the fixed limit of ACGIH 500 μg *t,t*-MA g^{-1} creatinine, in both smokers and non-smokers. Further studies involving larger numbers of participants are needed to confirm and explain this observation. Such studies should also monitor individual trends in excreted urinary concentration levels of *t,t*-MA. Environmental exposure and diet must also be considered in relation to their potential role in increasing benzene intake.

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References

1) IARC, International Agency for Research on Cancer. Monographs on the evaluation of the carcinogenic risk of chemicals to humans. Some industrial chemicals and dyestuffs. Benzene. Vol 29. Lyon, France: IARC, 1982.

2) Aksoy M. Hematoxicity and carcinogenity of benzene. Environ. Health Perspect. 1989;82:193-97.

3) Snyder R, Wits G, Goldstein BD. The toxicology of benzene. Environ. Health Perspect. 1993;100:293-306.

4) WHO World Health Organization. Air quality guidelines for Europe. WHO Regional Publication European Series N° 23. WHO Publ. Geneva, 1987

5) Wallace L, Pellizzari E, Hartwell TD, Perritt R, Ziegenfus R. Exposure to benzene and other volatile compounds from active and passive smoking. Arch. Environ. Health 1987;42:272-79.

6) WHO World Health Organization.. Benzene. Environmental Health Criteria 150. IPCS International Programme on Chemical Safety. WHO Publ. Geneva, 1993. Available at: http://www.inchem.org/documents/ehc/ehc/ehc150.htm

7. EU Directives 97/42/CE and 99/38/CE.

8) Hricko A. Rings of controversy around benzene. Environ. Health Perspect. 1994;102 (3):276-81.

9) Hogstedt B., Holmén A, Karlsson A, Raihle G, Nillius K, Vestlund K. Gasoline pump mechanics had increased frequencies and size of micronuclei in lymphocytes stimulated by pokeweed mutogen. Mutat. Res.1991;263:51-5.

10) Carere A, Antoccia A, Cimini D, et al. Genetic effects of petroleum fuels: cytogenetics monitoring of gasoline station attendants. Mutat. Res. 1995;332:17-26.

11) Bukvic N, Bavaro P, Elia G, Cassano F, Fanelli M, Guanti G. Sister chromatid exchange (SCE) and micronucleus (MN) frequencies in lymphocytes of gasoline station attendants. Mutat. Res. 1998;415:25-33.

12) ACGIH American Conference of Governmental Industrial Hygienists. Threshold limits values and biological exposure indices for 1994-1995. Cincinnati, OH, USA.

13) Melikian AA, Prahalad AK, Secker-Walker RH. Comparison of the levels of the urinary benzene metabolite trans, trans-muconic acid in smokers and non-smokers, and effects of pregnancy. Cancer Epidemiol. Biomarkers Prev. 1994;3:239-44.

14) Amodio Cocchieri R, Del Prete U, Cirillo T, Agozzino E, Scarano G. Evaluation of benzene exposure in children living in Campania (Italy) by urinary trans, trans-muconic acid assay. J. Toxicol. Environ. Health A. 2001;63:78-87.