

Correlation between environmental and biological monitoring of exposure to benzene in petrochemical industry operators

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ABSTRACT

The present work was aimed to study in petrochemical industry operators the correlation, if any, between environmental exposure to low levels of benzene and two biological exposure indexes in end-shift urine, i.e. trans, trans-muconic acid (*t,t*-MA) and S-phenylmercapturic acid (S-PMA). Exposure to benzene was assessed in 133 male subjects employed in outdoor operations in a petrochemical plant, using personal passive-diffusive air samplers worn at the breathing zone; adsorbed benzene was determined by GC-FID analysis. S-PMA was determined by a new HPLCMS/MS method, after (quantitative) acidic hydrolysis of the cysteine conjugate precursor. *t,t*-MA was measured by an HPLC-UV method. Smoking habits were assessed by means of a self-administered questionnaire.

Both environmental and biological monitoring data showed that benzene exposure of petrochemical industry operators was low (mean values were 0.014 ppm, 101 µg/g creat, and 2.8 µg/g creat, for benzene, *t,t*-MA, and S-PMA, respectively) if compared with the ACGIH limits. Cigarette smoking was confirmed to be a strong confounding factor for the urinary excretion of both metabolites: statistically significant increases of *t,t*-MA and S-PMA levels were recorded in smokers when compared to non-smokers ($p < 0.0001$). The best correlation found was that between exposure to benzene and S-PMA levels, particularly in non-smokers. This was partly due to the hydrolysis of the S-PMA precursor *N*-acetyl-S-(1,2-dihydro-2-hydroxyphenyl)-L-cysteine, a crucial step of the new analytical method used, which indeed reduced the variability of the results by means of an improved standardization of this critical preanalytical factor. A weaker correlation was found between exposure to benzene and *t,t*-MA, possibly explained by the fact that the latter is also a metabolite of sorbic acid, a common diet component.

In summary, even at such low levels of exposure, urinary metabolites proved to be a useful tool for assessing individual occupational exposure to benzene, S-PMA appearing to be a more specific biomarker than *t,t*-MA, particularly in non-smokers.

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1. Introduction

Human biomonitoring provides an efficient and cost effective mean of measuring human exposure to occupational and environmental chemicals. It accounts for all sources of exposure and all routes of uptake proving in most cases to be an excellent instrument for risk assessment and risk management (Angerer et al., 2007). It is particularly useful to assess exposure to those chemicals, such as benzene, that are absorbed to a significant extent through the skin and are, therefore, priority candidates for the allocation

of a biological limit value (BLV). Benzene is a known human carcinogen (IARC group 1) and has been assigned the "skin" notation by the American Conference of Governmental Industrial Hygienists (ACGIH), who recommends for the biological monitoring of benzene the determination of trans, trans-muconic acid (*t,t*-MA) and S-phenylmercapturic acid (S-PMA) (ACGIH, 2008). Both S-PMA and *t,t*-MA are minor metabolites of benzene, excreted in urine, which have been demonstrated to be suitable biomarkers for monitoring benzene exposure in occupational and environmental settings at levels as low as and even below 1 ppm (Boogaard and Van Sittert, 1996; Melikian et al., 2002; Qu et al., 2003). Other authors, however, did not always find a significant correlation between exposure to benzene and *t,t*-MA or S-PMA excretion data at such low levels of exposure (Carrieri et al., 2006; Manini et al., 2006). A number of reasons are envisaged for these conflicting results.

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Recent studies showed the existence in urine of *N*-acetyl-S-(1,2-dihydro-2-hydroxyphenyl)-L-Cysteine, a precursor of S-PMA (i.e. pre-S-PMA) that can be turned into S-PMA by acid hydrolysis (Inoue et al., 2000, 2001). The amount of S-PMA actually measured in urine also depends, therefore, on the degree of hydrolysis of its precursor, that changes as a function of both the pH and the storage conditions of the urine sample. This is one of the possible causes for the mis-correlation between airborne benzene concentration and levels of S-PMA measured in the urine of the exposed workers in some studies. The determination of urinary *t,t*-MA as a biomarker of benzene has also been questioned, due to the interference of sorbic acid, a common food preservative of which *t,t*-MA has been reported to be a metabolite too (Pezzagno and Maestri, 1997; Renner et al., 1999; Weaver et al., 2000; Negri et al., 2005).

Another important, probably the single main confounding factor in the study of occupational benzene exposure is smoking habit, as cigarette smoking causes the inhalation of significant amounts of benzene, comparable to those of low occupational exposure (Polzin et al., 2007).

The objective of the present study, therefore, was to study the correlation between personal exposure to airborne benzene and concentration of the urinary metabolites *t,t*-MA and S-PMA taking into account those confounding factor by excluding the effect of smoking and using a new validated analytical method for the determination of S-PMA after acid hydrolysis of its precursor.

2. Experimental

2.1. Chemicals and supplies

The analytical reference standard of DL-S-PMA was purchased from Tokyo Kasei Coggio LTD (Tokyo, Japan). The internal standard, deuterated DL-S-PMA-3,3- d_2 , was obtained from CDN Isotopes Inc. (Pointe-Claire, Quebec, Canada). Glacial acetic acid (100% Merck, Darmstadt, Germany) has been used for the mobile phase preparation, for the solid phase extraction (SPE) and, together with 25% NH_3 (Merck; Darmstadt, Germany), for urine pH adjustment, after dilution with purified water obtained from a Milli-Q Plus system (Millipore, Milford, MA, USA). Formic acid (98%, purity) used for SPE, sulphuric acid (95% purity), and the 50–52% (vol:vol) NaOH water solution used for hydrolysis have been purchased by Fluka–Sigma–Aldrich (Germany). Methanol has been purchased from J.Y. Baker (Deventer, Holland). Control human urine samples, used to prepare standard calibration curves and quality control samples (QC), were obtained from non-smoking healthy volunteers. SPE Vacuum Manifold and Sep-Pak Plus C18 (360 mg) cartridges for S-PMA analysis were supplied by Waters (Milford, MA, USA). SPE cartridges for *t,t*-MA analysis were supplied by Varian (EA Middelburg, The Netherlands). Anotop 10 LC[®] syringe filter devices (0.2 μ m pore size, 10 mm diameter) were purchased from Whatman Inc. (Maidstone, England). A Supelco Discovery C18 HPLC column (150 mm \times 4.6 mm, 5 μ m film thickness) was purchased by Sigma–Aldrich (Bellafonte, PA, USA) and an Ultrasphere C18 HPLC column (250 mm \times 4.6 mm, 5 μ m film thickness) was purchased by Beckman Coulter (Fullerton, CA, USA) for S-PMA and *t,t*-MA analysis, respectively.

2.2. Study population

The study was carried out from June to July 2006 on 29 workers employed in outdoor operations at a petrochemical plant in Northern Italy. The environmental and biological monitoring were carried out throughout 9 consecutive days (two to seven times for each worker), collecting a total of 145 environmental and biological samples. The investigated subjects were all healthy males, 9 smokers (48 samples) and 20 non-smokers (97 samples). Each worker provided one spot fresh urine sample at the end of the work-shift. Information about smoking habits, diet, and lifestyle was obtained by a self-administered questionnaire. An identification number was assigned to each completed questionnaire. The smoking habits and the ingestion of alcohol were reported in Table 1.

2.3. Analytical methods

Benzene exposure during the entire work-shift (approximately 8 h) was measured at the breathing zone level in all subjects, using personal diffusive samplers containing an active carbon cartridge (Radiello[®]). Analysis was performed by GC-FID after desorption of benzene from the active carbon with carbon disulfide. On the same day of personal airborne benzene monitoring, an urine sample was collected from all workers for the measurement of the metabolites. The concentration of S-PMA was determined according to a new HPLC-MS/MS method, previously validated in our laboratories, that comprises a (quantitative) acidic hydrolysis of its urinary precursor pre-S-PMA (Paci et al., 2007). The detection was in negative ions,

Table 1
Characteristics of the study population.

Parameter	Group and subgroup	n	%
Smoking	Non-smokers	20	66.9
	a. Ex smokers	6	17.2
	Smokers	9	33.1
	a. ≤ 10 cigarettes/day	6	24.1
	b. 11–20 cigarettes/day	2	7.6
	c. > 20 cigarettes/day	1	1.4
Alcohol ingestion	Non-drinkers	8	27.6
	Occasional drinkers	11	35.9
	Regular drinkers ^a	10	36.5
Total		29	100

^a < 60 g ethanol/day in all cases.

MRM mode, and the transitions were the following: $-238.1 \rightarrow -109.1$ for S-PMA and $-240.1 \rightarrow -109.1$ for the deuterium labelled internal standard.

Urinary *t,t*-MA analysis was carried out by HPLC-UV with detection at 264 nm, after SPE by an analytical method described elsewhere (Carrieri et al., 2006). Values measured for both S-PMA and *t,t*-MA were normalized for the concentration of urinary creatinine.

2.4. Statistical analysis

Statistical analysis was carried out using the StatsDirect statistical software on log-transformed values. Parametric statistical tests were applied to \log_{10} -transformed values, in order to obtain a normal distribution, which was assessed by the Shapiro-Wilk test. Differences between groups were assessed using the *t*-test for independent samples. Correlations between variables were assessed by the Pearson's *r* coefficient. Any differences observed were also confirmed by nonparametric tests (Mann-Whitney *U*-test and Spearman's rho). To assess any contribution of smoking habits and alcohol consumption, multiple linear regression analysis models were used (software STATA Statistics/Data Analysis). In all tests, a *p* value lower than 0.05 (two-tailed) was considered as statistically significant.

3. Results

The present study shows that exposure to benzene of petrochemical industry operators, as assessed by both environmental and biological monitoring procedures, was low (overall mean values being 0.014 ppm for benzene, 101 μ g/g creat for *t,t*-MA, and 2.8 μ g/g creat for S-PMA), when compared to the ACGIH limit values (0.5 ppm for benzene, 500 μ g/g creat for *t,t*-MA, and 25 μ g/g creat for S-PMA). Airborne benzene concentration at the workers' airways level in no case was higher than 0.5 ppm. When considering the biomonitoring data, almost all the values were below the corresponding Biological Exposure Indices (BEIs[®]). The values, with few exceptions, were accounted for by smoking habits. Five values only (two for *t,t*-MA and three for S-PMA) were above the corresponding BEI[®] all of which were obtained from heavy smokers. The results are shown in Table 2 for all subjects and in Table 3 for smokers and non-smokers separately. In 12 cases (10 non-smokers and 2 smokers) the level of urinary creatinine was outside the range recommended by WHO for urine sample acceptability (0.3–3.0 g/L), and therefore these values were excluded from subsequent analysis. All variables followed a log-normal distribution; parametric statistical tests were applied on log-transformed data and the statistical results were confirmed by non-parametric tests.

Table 2
Environmental and biological monitoring data for all workers.

Analyte	n	Mean	Median	Range
Benzene in air (ppm)	145	0.014	0.003	< 0.001 –0.280
<i>t,t</i> -MA in urine (μ g/L)	145	130.57	97.41	< 8.0 –637.47
<i>t,t</i> -MA in urine (μ g/g creat)	133 ^a	100.98	68.49	< 6.86 –746.40
S-PMA in urine (μ g/L)	145	3.58	1.09	< 0.05 –33.32
S-PMA in urine (μ g/g creat)	133 ^a	2.83	0.89	< 0.06 –38.59

^a Twelve samples with a creatinine concentration outside the range 0.3–3.0 g/L have been excluded.

Table 3
Environmental and biological monitoring of benzene exposure for smokers and non-smokers.

Analyte	Smoking habit	n	Mean	Median	Range
Benzene in air (ppm)	Smokers	48	0.008 ^{NS}	0.003	<0.001–0.120
	Non-smokers	97	0.017 ^{NS}	0.004	<0.001–0.280
<i>t,t</i> -MA (μg/L)	Smokers	48	185.56	168.59	<8.00–637.47
	Non-smokers	97	106.52	77.03	<8.00–520.63
<i>t,t</i> -MA (μg/g creat)	Smokers	46 ^b	150.66 ^a	138.40	<8.00–746.40
	Non-smokers	87 ^b	74.72 ^a	52.15	<6.86–364.28
S-PMA (μg/L)	Smokers	48	7.25	5.40	0.16–33.32
	Non-smokers	97	1.77	0.73	<0.05–19.02
S-PMA (μg/g creat)	Smokers	46 ^b	6.02 ^a	3.69	0.13–38.59
	Non-smokers	87 ^b	1.14 ^a	0.48	<0.06–18.63

^{NS}Not statistically significant.

^a $p < 0.0001$ (two-tailed *t*-test for independent samples, smokers vs. non-smokers).

^b Samples with a creatinine concentration outside the range 0.3–3.0 g/L have been excluded.

A statistically significant correlation was found in all subjects between airborne benzene concentrations and levels of *t,t*-MA or S-PMA in urine ($p < 0.05$ and $p < 0.001$, for *t,t*-MA and S-PMA, respectively), although the correlation coefficients were low ($r = 0.18$ and 0.29 , for *t,t*-MA and S-PMA, respectively, results not shown). In non-smoking subjects only ($n = 87$), both the correlation's statistical significance and the correlation coefficient improve for either biomarker ($r = 0.31$ and 0.59 , for *t,t*-MA and S-PMA, respectively) (Figs. 1 and 2). In smokers, a good correlation was found, instead, between the level of the metabolites and the number of cigarettes smoked ($r = 0.32$ and $p < 0.05$ for *t,t*-MA; $r = 0.67$ and $p < 0.0001$ for S-PMA, results not shown). The correlation between urinary *t,t*-

MA and S-PMA for all workers was also statistically significant, as expected (Fig. 3).

A multiple linear regression analysis model was used in order to assess the influence of airborne benzene concentration, smoking habits and ingestion of alcohol on the levels of biomarkers, set as dependent variables. The factors found to influence the concentration of *t,t*-MA in urine were cigarette smoking and occasional alcohol consumption ($R^2 = 0.29$ and $p < 0.001$, for both factors), while the concentration of S-PMA in urine was found to be influenced only by cigarette smoking ($R^2 = 0.57$, $p < 0.001$), with a clear relation to the number of cigarettes smoked, as shown in Table 4.

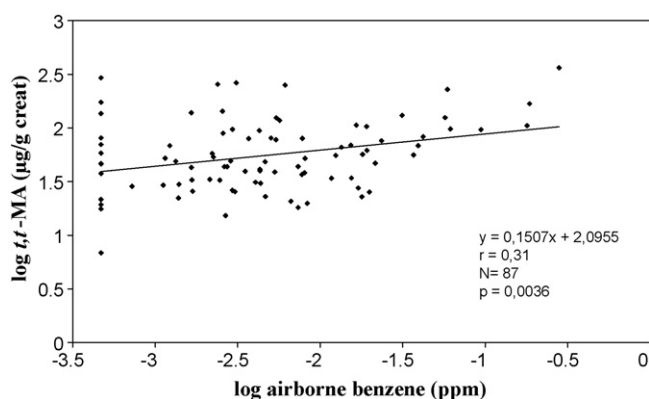


Fig. 1. Correlation between benzene exposure levels and urinary *t,t*-MA for non-smoking workers.

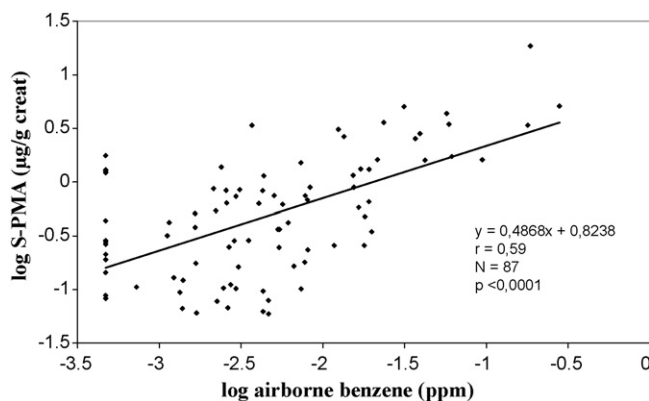


Fig. 2. Correlation between benzene exposure and urinary S-PMA for non-smoking workers.

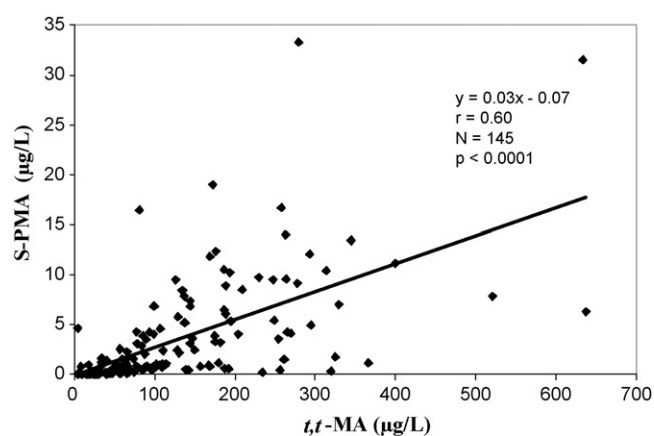


Fig. 3. Correlation between urinary *t,t*-MA and urinary S-PMA for all samples.

Table 4
Predictors of the urinary excretion of *t,t*-MA and S-PMA according to a multiple linear regression analysis.

	Coef.	95% confidence interval	$p > t $
<i>t,t</i> -MA			
Airborne benzene	0.146	0.057, 0.234	0.001
Cig. smoking (≤ 10 cigarettes/day)	0.712	0.406, 1.018	<0.001
Cig. smoking (11–20 cigarettes/day)	1.305	0.828, 1.782	<0.001
Cig. smoking (> 20 cigarettes/day)	1.518	0.477, 2.559	0.005
Ingestion of alcohol (occasional)	0.440	0.112, 0.767	0.009
Ingestion of alcohol (regular)	0.119	-0.196, 0.434	0.457
S-PMA			
Airborne benzene	0.418	0.289, 0.546	<0.001
Cig. smoking (≤ 10 cigarettes/day)	1.788	1.373, 2.203	<0.001
Cig. smoking (11–20 cigarettes/day)	2.657	1.998, 3.316	<0.001
Cig. smoking (> 20 cigarettes/day)	4.621	3.139, 6.104	<0.001

4. Discussion

Our results showed clear statistically significant correlations between airborne benzene concentrations and levels of biomarkers. The observation that correlations between urinary metabolites and environmental benzene levels were better in non-smokers than in smokers confirms that cigarette smoking represents the most important confounding factor when biomonitoring occupational benzene exposure. The good correlation, found in this study, between the levels of metabolites in urine and the number of cigarettes smoked is another consistent observation and confirms the results reported by others (Feng et al., 2006). Nevertheless, no statistically significant differences in airborne benzene exposure were found between smokers and non-smokers. So, personal airborne benzene exposure does not seem to be significantly affected by cigarette smoking. Indeed benzene exposure in smokers is due to active inhalation of the cigarette smoke, not to exposure to passive smoking from the cigarette. Therefore, the higher airborne benzene levels found in non-smokers than in smokers are likely to be casual or due to some non-smokers having a higher occupational exposure. The metabolites, on the other hand, are produced by the combination of both occupational benzene exposure and cigarette smoking. A statistically significant increase in the levels of both urinary metabolites was recorded in smokers when compared to non-smokers ($p < 0.0001$), again confirming the high sensitivity of these biomarkers to cigarette smoking. In fact, the low positive correlation coefficients found between airborne benzene concentrations and levels of the urinary metabolites are probably due to the confounding effect of smoking. Indeed, when these data are considered separately on the basis of smoking habits, in non-smokers the statistical correlation ($p < 0.0001$) and the correlation coefficient are both significantly higher than in smokers where, on the other hand, there was a good correlation between the levels of metabolites and number of cigarettes/day.

When environmental and biological data in non-smokers are compared by regression analysis, the correlations found between airborne benzene levels and those of the metabolites in urine (see Figs. 1 and 2) indicate that exposure to levels of benzene equal to the current Threshold Limit Value (TLV[®]) of 0.5 ppm corresponds to a mean urinary excretion of *t,t*-MA and S-PMA equal to 444.8 and 18.4 $\mu\text{g/g creat}$, respectively, values not dissimilar to the corresponding current BEI[®] (ACGIH, 2008). The notation (B) for “background” reported by ACGIH and associated to the BEI[®] for both *t,t*-MA and S-PMA highlights the influence of non-occupational background exposure, especially from cigarette smoking and, particularly for *t,t*-MA, also from the diet. These two are probably the main factors affecting the interpretation of our as well as others’ results. In fact the background levels of urinary metabolites in non-occupationally exposed subjects have been reported to be almost as high as those observed in exposed subjects (Kim et al., 2006) and in some cases even higher than the BEI[®] (Aprea et al., 2008).

The significant correlation observed in this study, but not in previous studies, of our group nor by other authors, using an immunochemiluminescence method (Carrieri et al., 2006; Farmer et al., 2005; Fustinoni et al., 2005), between S-PMA in urine and low levels of benzene in air is probably due to the new analytical method used here for S-PMA determination. In the analytical methods more commonly used so far for the determination of S-PMA in human urine, the urinary pH (normal range 4–8) was not considered to be a critical factor. In Boogaard and Van Sittert (1995) the urine sample was acidified at around pH 2, while others (Melikian et al., 1999) used 10% acetic acid in the sample purification by SPE. We found instead (Paci et al., 2007) that the urine of subjects exposed to benzene contains a precursor of the metabolite, *N*-acetyl-S-(1,2-dihydro-2-hydroxyphenyl)-L-cysteine, that is transformed into “free” S-PMA by acidic pH. As a result, the amount

of “free” S-PMA actually present in the urine is variable and depends largely on urine pH. Indeed the S-PMA levels measured with quantitative acidic hydrolysis were found to be, in average, twice as high as those measured at pH 2 (Paci et al., 2007). In the present study, therefore, the quantitative hydrolysis of the S-PMA precursor indeed reduced the variability of the results by means of a better standardization of the critical preanalytical factor pH (results not shown) and this allowed a statistically significant correlation between S-PMA in urine and low levels of benzene in air to become apparent.

Good correlations between S-PMA in urine of exposed subjects and airborne benzene concentrations were reported in several studies, on German, Estonian or Chinese workers, where exposure levels were very high (maximum values of 2.6 mg/m^3 , 47 mg/m^3 and 31 ppm, respectively) (Popp et al., 1994; Kivisto et al., 1997; Waidyanatha et al., 2004). Another study carried out in residents living near a petrochemical plant in Korea (Choi et al., 2000) reported a good correlation between urinary S-PMA and personal benzene exposure (range 0.00–22.88 $\mu\text{g}/\text{m}^3$) but even in this non-occupational study the range of concentration for the metabolite (1.33–109.68 $\mu\text{g}/\text{g creat}$) was quite different (much higher) from that of our data, which is also found in the general Italian population (Maestri et al., 2005).

Regarding *t,t*-MA, the literature shows conflicting data. Some authors did not find any correlation between metabolite concentration and benzene exposure, while others found a significant correlation between the two parameters, although with a very low correlation coefficient of approx. 0.10 (Gobba et al., 1997; Fustinoni et al., 2005). One study only (Bergamaschi et al., 1999) found a significant correlation ($r = 0.59$) between *t,t*-MA values and environmental benzene levels in the range 0.005–0.083 mg/m^3 . In the present study a highly statistically significant correlation was found only for non-smoking subjects ($r = 0.31$), with a high line intercept and a great dispersion of data for very low levels of exposure. This may be explained by the fact that *t,t*-MA is also a metabolite of sorbic acid present in the diet and, therefore, the urinary level of *t,t*-MA in workers is influenced strongly by the diet. After a single oral administration of 447 mg sorbic acid, the average urinary *t,t*-MA concentration was found to increase by as much as 20 times (Pezzagno et al., 1999).

The correlation between *t,t*-MA and S-PMA levels reported in Fig. 3 shows, at the BEI[®] value for S-PMA of 25 $\mu\text{g}/\text{g creat}$, a level of *t,t*-MA of about 836 $\mu\text{g}/\text{creat}$, and not 500 $\mu\text{g}/\text{creat}$ as indicated by ACGIH. The approx. 40% excess amount of *t,t*-MA was probably due to the ingestion of food containing sorbic acid by the workers.

Another factor that in the present study might have contributed to the low correlation observed between environmental and biological data overall (subjects were monitored from three to seven times each) is interindividual variability of the biological data, due to the effect of the genetic polymorphism of the enzymes involved in benzene metabolism (Rossi et al., 1999; Manini et al., 2006). In fact, a strong statistically significant correlation was found between *t,t*-MA and S-PMA in urine in all subjects (see Fig. 3), and a good correlation was also found in the present study, for the first time, between the environmental benzene level and urinary S-PMA concentration when repeated measures from the same subject were considered, with correlation coefficients above 0.80 in 70% of subjects. Similar results were not found, however, for *t,t*-MA probably because of the confounding factor of the diet, which varies from one subject to another and, for the same subject, from 1 day to another, as discussed above.

In summary, even at such low levels of exposure as those found in the present study, the biological monitoring of the metabolites proved to be effective in detecting occupational exposure to benzene, at least on a group base. When the present modified method of S-PMA determination is used, S-PMA seems to be a more

specific biomarker of benzene exposure than *t,t*-MA, particularly in non-smokers. Finally, cigarette smoking is confirmed to be a strong confounding factor when assessing occupational exposure to benzene, both individually and as a group, and should always be considered in the interpretation of biomonitoring data, particularly for exposures to low airborne concentrations.

Conflict of interest

There is not any conflict of interest for all authors.

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References

- ACGIH, 2008. 2008 TLVs and BEIs. Based on the Documentation of the Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. Cincinnati, Ohio.
- Angerer, J., Ewers, U., Wilhelm, M., 2007. Human biomonitoring: state of the art. *Int. J. Hyg. Environ. Health* 210, 201–228.
- Aprea, C., Sciarra, G., Bozzi, N., Pagliantini, M., Perico, A., Bavazzano, P., Leandri, A., Carrieri, M., Scapellato, M.L., Bettinelli, M., Bartolucci, G.B., 2008. Reference values of urinary *trans,trans*-muconic acid: Italian multicentric study. *Arch. Environ. Contam. Toxicol.* 55, 329–340.
- Bergamaschi, E., Brustolin, A., De Palma, G., Manini, P., Mozzoni, P., Andreoli, R., Cavazzini, S., Mutti, A., 1999. Biomarkers of dose and susceptibility in cyclists exposed to monoaromatic hydrocarbons. *Toxicol. Lett.* 108, 241–247.
- Boogaard, P.J., Van Sittert, N.J., 1995. Biological monitoring of exposure to benzene: a comparison between *S*-phenylmercapturic acid, *trans,trans* muconic acid and phenol. *Occup. Environ. Med.* 52, 611–620.
- Boogaard, P.J., Van Sittert, N.J., 1996. Suitability of *S*-phenylmercapturic acid and *trans,trans*-muconic acid as a biomarker for exposure to low concentrations of benzene. *Environ. Health Perspect.* 104, 1151–1157.
- Carrieri, M., Bonfiglio, E., Scapellato, M.L., Maccà, I., Tranfo, G., Faranda, P., Paci, E., Bartolucci, G.B., 2006. Comparison of exposure assessment methods in occupational exposure to benzene in gasoline filling-station attendants. *Toxicol. Lett.* 162, 146–152.
- Choi, Y., Shin, D., Park, S., Chung, Y., Kim, M., 2000. Biological monitoring of benzene in residents living near petrochemical industrial areas in Korea. *J. Occup. Health* 42, 31–37.
- Farmer, P.B., Kaur, B., Roach, J., Levy, L., Consonni, D., Bertazzi, P.A., Pesatori, A., Fustinoni, S., Buratti, M., Bonzini, M., Colombi, A., Popov, T., Cavallo, D., Desideri, A., Valerio, F., Pala, M., Bolognesi, C., Merlo, F., 2005. The use of *S*-phenylmercapturic acid as a biomarker in molecular epidemiology studies of benzene. *Chem. Biol. Interact.* 153–154, 97–102.
- Feng, S., Roethig, H.J., Liang, Q., Kinser, R., Jin, Y., Scherer, G., Urban, M., Engl, J., Riedel, K., 2006. Evaluation of urinary 1-hydroxypyrene, *S*-phenylmercapturic acid, *trans,trans*-muconic acid, 3-methyladenine, 3-ethyladenine, 8-hydroxy-2'-deoxyguanosine and thioethers as biomarkers of exposure to cigarette smoke. *Biomarkers* 11, 28–52.
- Fustinoni, S., Buratti, M., Campo, L., Colombi, A., Consonni, D., Pesatori, A.C., Bonzini, M., Farmer, P., Garte, S., Valerio, F., Merlo, D.F., Bertazzi, P.A., 2005. Urinary *t,t*-muconic acid, *S*-phenylmercapturic acid and benzene as biomarkers of low benzene exposure. *Chem. Biol. Interact.* 153–154, 253–256.
- Gobba, F., Rovesti, S., Borella, P., Vivoli, R., Caselgrandi, E., Vivoli, G., 1997. Inter-individual variability of benzene metabolism to *trans,trans*-muconic acid and its implications in the biological monitoring of occupational exposure. *Sci. Total Environ.* 199, 41–48.
- Inoue, O., Kanno, E., Yusa, T., Kakizaki, M., Watanabe, T., Higashikawa, K., Ikeda, M., 2000. Urinary phenylmercapturic acid as a marker of occupational exposure to benzene. *Ind. Health* 38, 195–204.
- Inoue, O., Kanno, E., Yusa, T., Kakizaki, M., Watanabe, T., Higashikawa, K., Ikeda, M., 2001. A simple HPLC method to determine urinary phenylmercapturic acid and its application to gasoline station attendants to biomonitor occupational exposure to benzene at less than 1 ppm. *Biomarkers* 6, 190–203.
- Kim, S., Vermeulen, R., Waidyanatha, S., Johnson, B.A., Lan, Q., Rothman, N., Smith, M.T., Zhang, L., Li, G., Shen, M., Yin, S., Rappaport, S.M., 2006. Using urinary biomarkers to elucidate dose-related patterns of human benzene metabolism. *Carcinogenesis* 27, 772–781.
- Kivisto, H., Pekari, K., Peltonen, K., Svinhufvud, J., Veidebaum, T., Sorsa, M., Aitio, A., 1997. Biological monitoring of exposure to benzene in the production of benzene and in a cookery. *Sci. Total Environ.* 199, 49–63.
- Maestri, L., Negri, S., Ferrari, M., Ghittori, S., Imbriani, M., 2005. Determination of urinary *S*-phenylmercapturic acid, a specific metabolite of benzene, by liquid chromatography/single quadrupole mass spectrometry. *Rapid Commun. Mass Spectrom.* 19, 1139–1144.
- Manini, P., De Palma, G., Andreoli, R., Poli, D., Mozzoni, P., Folesani, G., Mutti, A., Apostoli, P., 2006. Environmental and biological monitoring of benzene exposure in a cohort of Italian taxi drivers. *Toxicol. Lett.* 167, 142–151.
- Melikian, A.A., O'Connor, R., Prahalad, A.K., Hu, P., Li, H., Kagan, M., Thomson, S., 1999. Determination of the urinary benzene metabolites *S*-phenylmercapturic acid and *trans,trans* muconic acid by liquid chromatography tandem mass spectrometry. *Carcinogenesis* 20, 719–726.
- Melikian, A.A., Qu, Q., Shore, R., Li, G., Li, H., Jin, X., Cohen, B., Chen, L., Li, Y., Yin, S., Mu, R., Zhang, X., Wang, Y., 2002. Personal exposure to different levels of benzene and its relationships to the urinary metabolites *S*-phenylmercapturic acid and *trans,trans*-muconic acid. *J. Chromatogr. A* 778, 211–221.
- Negri, S., Bono, R., Maestri, L., Ghittori, S., Imbriani, M., 2005. High-pressure liquid chromatographic-mass spectrometric determination of sorbic acid in urine: verification of formation of *trans,trans*-muconic acid. *Chem. Biol. Interact.* 153–154, 243–246.
- Paci, E., Pignini, D., Cialdella, A.M., Faranda, P., Tranfo, G., 2007. Determination of free and total *S*-phenylmercapturic acid by HPLC/MS/MS in the biological monitoring of benzene exposure. *Biomarkers* 12, 111–122.
- Pezzagno, G., Maestri, L., 1997. The specificity of *trans,trans*-muconic acid as a biological indicator for low levels of environmental benzene. *Indoor Built. Environ.* 6, 12–18.
- Pezzagno, G., Maestri, L., Fiorentino, M.L., 1999. *Trans,trans*-muconic acid, a biological indicator to low levels of environmental benzene: some aspects of its specificity. *Am. J. Ind. Med.* 35, 511–518.
- Polzin, G.M., Kosa-Maines, R.E., Ashley, D.L., Watson, C.H., 2007. Analysis of volatile organic compounds in mainstream cigarette smoke. *Environ. Sci. Technol.* 41, 1297–1302.
- Popp, W., Rauscher, D., Muller, G., Angerer, J., Norpoth, K., 1994. Concentrations of benzene in blood and *S*-phenylmercapturic acid and *t,t*-muconic acid in urine of car mechanics. *Int. Arch. Occup. Environ. Health* 66, 1–6.
- Qu, Q., Shore, R., Li, G., Jin, X., Chen, L.C., Cohen, B., Melikian, A.A., Eastmond, D., Rappaport, S., Rupa, D., Waidyanatha, S., Yin, S., Yan, H., Meng, M., Winnik, W., Kwok, E.S., Li, Y., Mu, R., Xu, B., Zhang, X., Li, K., 2003. Validation and evaluation of biomarkers in workers exposed to benzene in China. *Res. Resp. Health Eff. Inst.* 115, 1–87.
- Renner, T., Baer-Koetzle, M., Scherer, G., 1999. Determination of sorbic acid in urine by gas chromatography-mass spectrometry. *J. Chromatogr. A* 847, 127–133.
- Rossi, A.M., Guarnieri, C., Rovesti, S., Gobba, F., Ghittori, S., Vivoli, G., Barale, R., 1999. Genetic polymorphisms influence variability in benzene metabolism in humans. *Pharmacogenetics* 9, 445–451.
- Waidyanatha, S., Rothman, N., Li, G.L., Smith, M.T., Yin, S.N., Rappaport, S.M., 2004. Rapid determination of six urinary benzene metabolites in occupationally exposed and unexposed subjects. *Anal. Biochem.* 327, 184–199.
- Weaver, V.M., Buckley, T., Groopman, J.D., 2000. Lack of specificity of *trans,trans*-muconic acid as a benzene biomarker after ingestion of sorbic acid-preserved foods. *Cancer Epidemiol. Biomarkers Prev.* 9, 749–755.