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## Determination of free and total *S*-phenylmercapturic acid by HPLC/MS/MS in the biological monitoring of benzene exposure

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### Abstract

Urinary *S*-phenylmercapturic acid (SPMA) is a biomarker suggested by the American Conference of Governmental Industrial Hygienists (ACGIH) for assessing occupational exposure to benzene. A possible cause of the miscorrelation between environmental monitoring and biological monitoring for benzene exposure, which many authors complain about, is the existence of a urinary metabolite that turns into SPMA by acid hydrolysis. Forty urine samples were tested to determine which concentration value would correspond to the ACGIH Biological Exposure Index (BEI) of  $25 \mu\text{g g}^{-1}$  creatinine if exposure assessment was based on the determination of SPMA after quantitative hydrolysis of its precursor. An aliquot of each sample was hydrolysed with 9 M  $\text{H}_2\text{SO}_4$ , a second one was brought to pH 2 and a third one was used as it was (free SPMA). SPMA was determined by high-performance liquid chromatography/tandem mass spectrometric technique (HPLC/MS/MS) using an internal standard. The analytical method was validated in the range  $0.5\text{--}50 \mu\text{g l}^{-1}$ . The average SPMA in pH 2 samples is 45–60% of the total, while free SPMA varies from 1% to 66%. The hydrolysis of pre-SPMA reduces the likelihood of variability in the results by reducing pH differences in urine samples and increasing the amount of measured SPMA. The BEI limit value would be about  $50 \mu\text{g g}^{-1}$  creatinine.

**Keywords:** *Biological monitoring, benzene exposure, S-phenylmercapturic acid, method validation, metabolite precursor, human urine*

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### Introduction

One of the biomarkers suggested by the American Conference of Governmental Industrial Hygienists (ACGIH) for assessing the professional exposure to benzene is the amount of *S*-phenylmercapturic acid (SPMA) in the urine of end-shift workers, for which the Biological Exposure Index (BEI) gives a limit value of  $25 \mu\text{g g}^{-1}$  creatinine (ACGIH 2005).

Some authors (Carrieri et al. 2006, Fustinoni et al. 2005) complain of poor correlation between the environmental monitoring of benzene and the results of the

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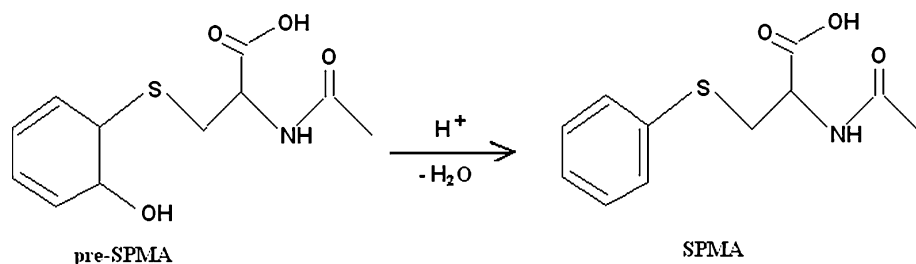


Figure 1. Hydrolysis of pre-S-phenylmercapturic acid (SPMA) to SPMA.

biological monitoring. A possible cause is the existence in the urine samples of N-acetyl-S-(1,2-dihydro-2-hydroxyphenyl)-L-cysteine, a precursor of SPMA (pre-SPMA) that can be turned into SPMA by acid hydrolysis (Figure 1) (Inoue et al. 2000, Inoue et al. 2001, Sabourin et al. 1988).

The amount of measured SPMA, which depends on the degree of hydrolysis of its precursor, therefore changes as a function both of the pH and of the storage conditions of the urine sample. In the analytical methods more commonly used for the determination of SPMA in human urine, the pH is not considered a critical factor. In Boogard & Van Sittert (1995) and in derived modifications, the urine sample is acidified around pH 2 in order to stabilise it, while Melikian et al. (1999), and others, use 10% acetic acid in the sample purification by solid-phase extraction (SPE).

In the present study, an analytical method for the quantitative determination of total SPMA was validated by high-performance liquid chromatography/tandem mass spectrometric technique (HPLC/MS/MS), with deuterated SPMA as an internal standard, and 40 urine samples collected from workers exposed to benzene were tested. For each sample, the percentage of SPMA measurable at pH 2 and without pH correction (free SPMA) was calculated with respect to the SPMA measured after quantitative hydrolysis, in order to determine which concentration value corresponded to the BEI value of  $25 \mu\text{g g}^{-1}$  creatinine established by the ACGIH, if the assessment of the exposure was made on the basis of total SPMA.

## Materials and methods

### *Chemicals and supplies*

The analytical reference standard of DL-SPMA was purchased from Tokyo Kasei Cogio Ltd. (Tokyo, Japan). The internal standard, deuterated DL-SPMA-3,3- $d_2$ , was obtained from CDN Isotopes Inc. (Pointe-Claire, Quebec, Canada). Glacial acetic acid (100%; Merck, Darmstadt, Germany) was used for the mobile-phase preparation, for the SPE and, together with 25%  $\text{NH}_3$  (Merck, Darmstadt, Germany), for urine pH adjustment, after dilution with purified water obtained from a Milli-Q Plus system (Millipore, Milford, MA, USA). Sulphuric acid 95% and NaOH solution 50–52% used for hydrolysis were provided by Fluka (Sigma-Aldrich, Germany). Methanol for LC/MS was provided by J.Y. Baker (Deventer, Holland). Control human urine samples, used to prepare standard calibration curves and quality control samples, were obtained from non-smoking healthy volunteers. SPE Vacuum Manifold and Sep-Pak Plus C18 (360 mg) cartridges were supplied by Waters (Milford, MA, USA). Anotop 10 LC<sup>®</sup> syringe filter devices (0.2  $\mu\text{m}$  pore size, 10 mm diameter) were

purchased from Whatman Inc. (Maidstone, UK). A Supelco Discovery C18 HPLC column (150 × 4.6 mm, 5 µm film thickness) was supplied by Sigma-Aldrich (Bellafonte, PA, USA) and used throughout the study.

#### *Urine sample preparation*

End-shift urine samples were collected from 26 non-smoking and 14 smoking workers at an oil refinery plant and stored frozen at -20°C until analysis.

An aliquot of 3 ml of each urine sample was treated with 1 ml of 9 M H<sub>2</sub>SO<sub>4</sub> for 10 min in order to perform quantitative hydrolysis of pre-SPMA, then 0.75 ml NaOH 50% in water was added to reach a pH of about 2. The stability of SPMA in these conditions was previously verified by adding deuterated SPMA to two aliquots of the same urine before and after hydrolysis, that gave the same analytical results. A second aliquot of 3 ml was brought to pH 2 with glacial acetic acid, while a third aliquot was analysed without pH correction.

After this step, 30 µl of a solution of 0.5 mg l<sup>-1</sup> of internal standard (SPMA-d<sub>2</sub>) dissolved in methanol was added to each aliquot in order to reach a final concentration of 5 µg l<sup>-1</sup>.

SPE purification was performed on Sep-pack C18 cartridges preconditioned with 3 ml methanol followed by 3 ml CH<sub>3</sub>COOH 0.1%. After loading the sample the cartridge was washed with 3 ml CH<sub>3</sub>COOH 0.1% and eluted with 3 ml methanol. The eluate was filtered on a 0.2-µm filter device and 20 µl injected into the HPLC/MS/MS system.

#### *Preparation of standard solutions in methanol*

A total of 10 mg standard SPMA were weighed and dissolved in 100 ml methyl alcohol in order to obtain a stock standard solution of 100 mg l<sup>-1</sup>, which was stored at 4°C. From this solution two further dilutions were prepared at the concentrations of 1 mg l<sup>-1</sup> (A) and 0.1 mg l<sup>-1</sup> (B).

Five calibration standards in methanol were prepared in the range 0.5–50 µg l<sup>-1</sup>.

The concentrations of 0.5 and 2 µg l<sup>-1</sup> were prepared by diluting 50 and 200 µl, respectively, of solution B in 10 ml, while in order to obtain 10, 20 and 50 µg l<sup>-1</sup> 100, 200 and 500 µl, respectively, of solution A were used. These solutions were analysed by HPLC/MS/MS in order to determine recovery and matrix effect.

In order to obtain a stock standard solution of 100 mg l<sup>-1</sup>, which was stored at 4°C, 10 mg of deuterated SPMA (SPMA-d<sub>2</sub>) were weighed and dissolved in 100 ml methyl alcohol. This solution was diluted with methanol in order to obtain a 0.5 mg l<sup>-1</sup> solution (C).

#### *Preparation of urine calibration standards and quality control samples*

Five different calibration curves were prepared, each one using the urine of a different healthy volunteer, non-smoker.

Each calibration curve contained: one blank urine sample, five standard samples at the concentrations of 0.5, 2, 10, 20 and 50 µg l<sup>-1</sup> and two independent replicates of quality control samples at the concentrations of 1, 5 and 25 µg l<sup>-1</sup>, each sample containing 5 µg l<sup>-1</sup> of internal standard. One of the calibrations contained five independent replicates of the quality controls in order to determine intra-day

variability. Samples were prepared by spiking 3 ml of urine with suitable amounts of solutions A and B and 30  $\mu\text{l}$  of solution C. All samples were purified on SPE as described for unknown urine samples (see Urine sample preparation).

#### Preparation of matrix standards

In order to determine the effect of the matrix on the instrumental response, five blank urine samples (3 ml) were submitted to the SPE procedure and the eluates spiked with suitable amounts of solutions A, B and C in order to reach the same final concentrations of the five urine calibration standards. This was repeated for each different urine sample.

#### HPLC/MS/MS analysis

The HPLC analyses of urine samples and calibration and matrix standards were performed on a Series 200 LC quaternary pump (Perkin Elmer, Norwalk, CT, USA) using a  $150 \times 4.6$  mm Discovery C18 analytical column.

The mobile phase, was as follows: 1 min equilibration with 30% methanol (A phase) and 70% 0.1 M acetic acid (B phase), 12 min with a linear gradient up to 90% A phase and 10% B phase, 2 min with a linear gradient back to the starting conditions, flow rate  $1.0 \text{ ml min}^{-1}$ . In these conditions, the retention times of SPMA and the internal standard are about 7.7 min. The total run time was 15 min (Figure 2).

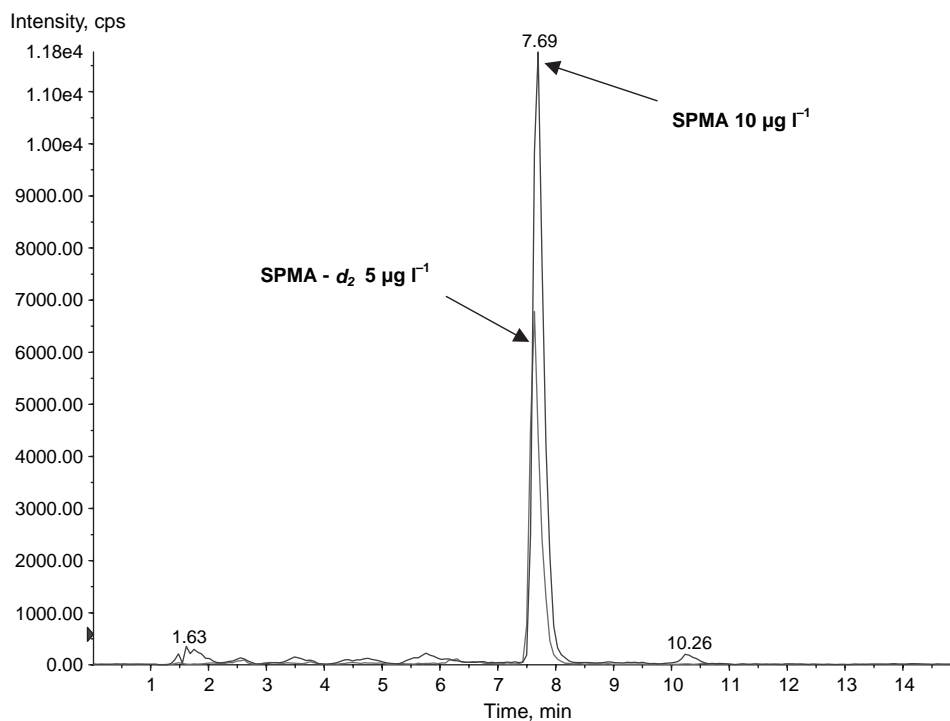


Figure 2. Typical multiple reaction monitoring (MRM) chromatogram of a urine calibration standard containing  $10 \mu\text{g l}^{-1}$  *S*-phenylmercapturic acid (SMPA) and  $5 \mu\text{g l}^{-1}$  internal standard (SPMA- $d_2$ ). cps: counts per second.

The mass spectrometer ion source of the PE Sciex API 4000 triple quadrupole mass spectrometer is able to work without split and therefore the HPLC column eluate was fed directly into the Turbo Ion Spray (TIS) probe. The instrument was calibrated using polypropylene glycol and the resolution was adjusted to a peak width (FWHM) of 0.7 Th over the  $m/z$  100–1000 range. The detection was carried out in negative ions multiple reaction monitoring (MRM) mode, and parameters were optimised for the analytes by means of the automated 'Infusion Quantitative Optimization' procedure and subsequently refined by means of the flow injection analysis (FIA) using the pure standards. The following  $m/z$  ion combinations (precursor  $\rightarrow$  product) were monitored, and the SRM transitions were as follows:  $-238.1 \rightarrow -109.1$  for SPMA and  $-240.1 \rightarrow -109.1$  for deuterated internal standard. All values are summarized in Figure 3.

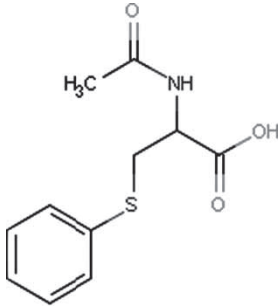
		
<b>DL-S-phenylmercapturic acid</b>		
<b>Precursor ion</b>	<b>Q1 Mass (m/z)</b>	<b>-238.1</b>
<b>Product ion</b>	<b>Q3 Mass (m/z)</b>	<b>-109.1</b>
<b>DL-S-phenylmercapturic acid - 3,3 d<sub>2</sub></b>		
<b>Precursor ion</b>	<b>Q1 Mass (m/z)</b>	<b>-240.1</b>
<b>Product ion</b>	<b>Q3 Mass (m/z)</b>	<b>-109.1</b>
<b>Common parameters</b>		
<b>CAD</b> (collisionally activated dissociation)	(arbitrary unit)	12
<b>CXP</b> (collision cell exit potential)	(ΔV)	-15
<b>CE</b> (collision energy value)	(ΔV)	-20
<b>DP</b> (cluster-breaking orifice voltage)	(ΔV)	-15

Figure 3. Mass spectrometry experimental conditions.

The version of 1.4 Analyst<sup>®</sup> software was employed for instrument control, using the section 'Quant Method Editor' to process quantitative data.

### Data processing

The peak areas generated by the samples were integrated by the 1.4 Analyst software. The area of the SPMA peak of the blank urine sample was subtracted from the areas of the urine calibration standards.

The five calibration curves were generated using a linear regression analysis according to the equation  $y = ax + b$ , where  $y$  is the peak area ratio of SPMA to internal standard,  $x$  is the concentration of analyte in the calibration sample,  $b$  is the intercept and  $a$  is the slope of the regression line. The concentrations of the analyte in the samples were calculated from the regression equation of the calibration curve and expressed as  $\mu\text{g l}^{-1}$  of urine. All the calibration equations are reported in Table I detailing  $a$  and  $b$  parameters and the correlation coefficient  $R^2$ .

## Results and discussion

### Analytical method validation

Five independent sets of calibration curve were analysed in five different days, three of which were not consecutive. Each calibration curve was prepared using urine from a different donor and analysed in duplicate (see Preparation of urine calibration standards and quality control samples). The results were used to determine the method performances.

**Linearity range.** Calibration curves show a linear response of the area ratio between SPMA and the internal standard obtained by means of HPLC/MS/MS analysis in the concentration range  $0.5\text{--}50 \mu\text{g l}^{-1}$  with a correlation coefficient always greater than 0.9994.

**Sensitivity.** The limits of detection (LOD) and the lower limit of quantification (LLOQ) were, respectively, defined as three and ten times the standard deviation of the LC/MS/MS peak areas detected at the retention times of the analyte of interest in several blank urine samples. 'Blank' urine samples for SPMA were not available, and therefore peak areas were measured at retention times near to that of the analyte ( $8 \pm 1$  min) in the three different blank urine samples spiked with  $5 \mu\text{g l}^{-1}$  of internal standard. The corresponding concentrations were calculated by the area ratio with the

Table I. Parameters of regression lines of the five calibration curves (area ratio *S*-phenylmercapturic acid (SPMA)/ $d_2$ SPMA vs. SPMA concentration added to urine).

	a (slope)	b (intercept)	$R^2$
Day 1 – Urine donor 1	0.2121	0.0549	0.9995
Day 2 – Urine donor 2	0.2070	0.0307	0.9999
Day 3 – Urine donor 3	0.1998	0.0228	0.9997
Day 4 – Urine donor 4	0.2110	0.0492	0.9995
Day 5 – Urine donor 5	0.2073	0.0479	0.9994

internal standard on the relative calibration curve. LOD was found to be  $0.05 \mu\text{g l}^{-1}$ , while LLOQ was  $0.1 \mu\text{g l}^{-1}$ .

*Recovery.* The total recovery is the result of the contribution of two factors, the extraction recovery due to the sample preparation procedure and the matrix effect that can influence the instrumental response of the analyte in the negative ion mode in ESI-MS (Annesley 2003, Muller et al. 2002). A matrix effect lower than 100% indicates a phenomenon called ion suppression. The contribution of the matrix effect was evaluated with the procedure suggested by Matuszewski et al. (2003) analysing five 'matrix standards' prepared as described under 'Preparation of matrix standards'. The procedure was repeated for the five different urine samples (from different donors) as they give a different matrix effect. The SPE recovery was calculated by comparing the peak areas produced by the analysis of the urine calibration standards with those obtained for the matrix standards, and it was 90%, on average, over five independent experiments, each with five concentration levels (the values reported in Table II are the average of five results for each urine). The matrix effect is an ion-suppression effect, expressed as the ratio between the peak areas of the matrix standards and those of the same concentration of standards in methanol: in the five experiments values are between 30% and 97%, confirming the importance of using an internal standard as the instrumental response can change dramatically in different urine samples. All values are shown in Table II.

*Accuracy and precision.* The method was validated by analysing the quality control samples on five separate days. The intra-day precision was determined from the analysis performed on the same day of five independent replicates (five different SPE columns) at low, medium and high concentration levels ( $1.0$ ,  $5.0$  and  $25.0 \mu\text{g l}^{-1}$ ). The intra-day precision was expressed as the relative standard deviation (RSD) of the sample replicates over their mean values for each level, while the inter-day precision was calculated on five independent replicates tested over the 5 days of the validation study. The accuracy was determined by comparing the means of the measured concentration with the theoretical values in the quality control samples and presented as percentages. Results are summarised in Table III.

Table II. Recovery due to solid-phase extraction (SPE) and instrumental response (matrix effect).

	SPE recovery <sup>a</sup>	Matrix effect <sup>b</sup>	Total recovery <sup>c</sup>
Day 1 – Urine donor 1	105.24	43.03	44.87
Day 2 – Urine donor 2	92.23	30.20	27.85
Day 3 – Urine donor 3	73.36	92.65	61.26
Day 4 – Urine donor 4	89.40	97.16	86.56
Day 5 – Urine donor 5	89.71	96.97	86.89
Mean value	89.99	72.00	61.49

<sup>a</sup>Ratio between areas produced by urine samples in which standards have been added before and after SPE.

<sup>b</sup>Ratio between areas produced by urine samples in which standards have been added after SPE and same standards dissolved in methanol.

<sup>c</sup>Ratio between areas produced by urine samples in which standards have been added before SPE and same standards dissolved in methanol.

Table III. Intra- and inter-assay accuracy, precision and variability.

Replicate	SPMA added to urine					
	1 $\mu\text{g l}^{-1}$		5 $\mu\text{g l}^{-1}$		25 $\mu\text{g l}^{-1}$	
	Found concentration	% Accuracy	Found concentration	% Accuracy	Found concentration	% Accuracy
Same day						
1	0.89	89.00	4.47	89.40	25.10	100.40
2	0.92	92.00	4.80	96.00	24.62	98.48
3	0.99	99.00	4.10	82.00	25.35	101.40
4	0.97	97.00	4.20	84.00	25.85	103.40
5	0.99	99.00	4.25	85.00	25.47	101.88
Mean ( $n=5$ )	0.95	95.20	4.36	87.28	25.28	101.11
Standard deviation (STD)		4.49		5.58		1.83
Intra-day CV (%) (STD/mean)		4.72		6.39		1.81
Different days						
1	0.96	95.96	4.06	81.19	23.50	94.01
2	1.00	99.50	4.71	94.10	25.54	102.22
3	0.96	96.00	5.10	102.00	25.08	100.03
4	0.95	95.20	4.36	87.28	25.28	101.11
5	0.98	98.66	4.50	90.00	25.83	103.31
Mean ( $n=5$ )	0.97	97.06	4.55	90.91	25.05	100.14
Standard deviation (STD)		1.89		7.77		3.64
Inter-day CV (%) (STD/mean)		1.95		8.55		3.63

*Results of workers' biological monitoring*

The concentration of SPMA was determined for each urine sample in the three different conditions:

- After acid hydrolysis (total SPMA)
- Adjusting urine pH at 2.0 (pH 2 SPMA)
- Without any pH correction (free SPMA).

The results are expressed as  $\mu\text{g l}^{-1}$  of urine and the ratios between free and total SPMA and between 'pH 2' and total SPMA have been calculated and expressed as percentages.

The mean values of these percentages were determined together with their % CV, and all results are reported in Tables IV and V for smokers and non-smokers.

The SPMA measured at pH 2 was 57.7% of the total for non-smokers with 29% CV, while for smokers it was 44.5% with 21% CV. Free SPMA is on average 25% of the total amount for non-smokers, with a 70% CV, and 9.6% for smokers with a 74% CV. This higher variability is due both to the urine pH variability and to the lower concentrations detected.

The difference between the ratio of free to total SPMA in smokers and non-smokers is statistically significant. Data from the two groups were compared by the non-parametric two-tailed Mann-Whitney U test (61.5;  $p = 0.001$ ).

One possible interpretation for this difference is that, at the physiological urine pH (5–7) when the total SPMA is high (smokers have a higher value as they are exposed to benzene from cigarette smoke), only a small quantity of the precursor is spontaneously transformed into free SPMA, whereas when the total amount is low, almost all the precursor is transformed into SPMA. The regression line for the ratio total SPMA/free versus total SPMA for all subjects put together (smokers and non-smokers) shows a positive linear trend with a correlation coefficient  $R^2 = 0.76$ .

This would mean that, when hydrolysis is not performed and only free SPMA is measured, the higher are the values of total SPMA (i.e. for significant benzene exposure), the higher is the possibility of underestimation of the benzene exposure.

## Conclusions

The analytical method validated in the present study allows sensitive and reliable determination of total SPMA in human urine after benzene exposure. The hydrolysis of pre-SPMA reduces the likelihood of variability in the results by reducing pH differences in urine samples and increasing the amount of measured SPMA. The limit value of  $25 \mu\text{g g}^{-1}$  creatinine for occupational exposure has been recommended on the basis of biological monitoring data obtained by using analytical methods that did not perform sample hydrolysis; in most of these studies urine pH was around 2 (ACGIH 2001, Boogaard & Van Sittert 1995, Ghittori et al. 1995) and therefore the ratio between SPMA determined at pH 2 and after quantitative hydrolysis can give an indication on the possible BEI limit value for total SPMA. On the basis of the present results this value should be about  $50 \mu\text{g g}^{-1}$  creatinine. This confirms the value of  $55 \mu\text{g g}^{-1}$  creatinine estimated by Inoue et al. (2001) on the basis of the exposure–excretion relationship.

Table IV. Values for free and total *S*-phenylmercapturic acid (SPMA) in the urine of smokers (n = 14).

Smokers	Total SPMA <sup>a</sup> ( $\mu\text{g l}^{-1}$ )	pH 2 urine SPMA <sup>b</sup> ( $\mu\text{g l}^{-1}$ )	Free SPMA <sup>c</sup> ( $\mu\text{g l}^{-1}$ )	pH 2/total (%)	Free/total (%)
	3.59	1.515	0.33	42.20	9.19
	20.88	5.685	0.2	27.23	0.96
	18.8	11.835	0.33	62.95	1.76
	3.06	1.41	0.08	46.08	2.61
	1.09	0.66	0.31	60.55	28.44
	3.3	1.305	0.25	39.55	7.58
	1.54	0.675	0.08	43.83	5.19
	1.46	0.69	0.14	47.26	9.59
	3.19	1.215	0.42	38.09	13.17
	4.87	2.355	0.33	48.36	6.78
	6.59	2.085	0.43	31.64	6.53
	8.19	3.81	1.2	46.52	14.65
	1.51	0.66	0.22	43.71	14.57
	2.29	1.035	0.3	45.20	13.10
Mean	5.74	2.50	0.33	44.51	9.58
Standard deviation (STD)	6.32	3.04	0.27	9.46	7.11
CV (%) (STD/mean)	110.07	121.73	82.89	21.25	74.17
Min	1.09	0.66	0.08	27.23	0.96
Max	20.88	11.84	1.20	62.95	28.44

<sup>a</sup>Measurement made after hydrolysis.<sup>b</sup>Measurement made at urine sample pH = 2.<sup>c</sup>Measurement made at physiological urine pH.

Table V. Values for free and total S-phenylmercapturic acid (SPMA) in the urine of non-smokers (n = 26).

Non-smokers	Total SPMA <sup>a</sup> ( $\mu\text{g l}^{-1}$ )	pH 2 urine SPMA <sup>b</sup> ( $\mu\text{g l}^{-1}$ )	Free SPMA <sup>c</sup> ( $\mu\text{g l}^{-1}$ )	pH 2/total (%)	Free/total (%)
	0.92	0.525	0.2	57.07	21.74
	0.42	0.27	0.13	64.29	30.95
	0.89	0.54	0.32	60.67	35.96
	0.43	0.27	0.07	62.79	16.28
	0.33	0.21	0.07	63.64	21.21
	0.36	0.165	0.08	45.83	22.22
	0.48	0.21	0.09	43.75	18.75
	0.35	0.195	0.07	55.71	20.00
	0.33	0.285	0.23	86.36	69.70
	0.55	0.33	0.05	60.00	9.09
	0.78	0.405	0.3	51.92	38.46
	0.5	0.27	0.11	54.00	22.00
	0.83	0.375	0.1	45.18	12.05
	0.2	0.15	0.06	75.00	30.00
	0.23	0.165	0.09	71.74	39.13
	0.7	0.33	0.19	47.14	27.14
	0.59	0.3	0.13	50.85	22.03
	0.66	0.225	0.05	34.09	7.58
	3.77	2.895	0.42	76.79	11.14
	0.17	0.09	0.05	52.94	29.41
	0.38	0.15	0.02	39.47	5.26
	0.62	0.315	0.33	50.81	53.23
	0.16	0.09	0.01	56.25	6.25
	0.17	0.165	0.1	97.06	58.82
	0.68	0.315	0.05	46.32	7.35
	0.2	0.135	0.09	67.50	45.00
Mean	0.66	0.40	0.12	57.68	25.09
Standard deviation (STD)	0.89	0.70	0.11	16.75	17.57
CV (%) (STD/mean)	135.78	175.11	95.07	29.04	70.03
Min	0.16	0.09	0.01	34.09	5.26
Max	3.77	2.90	0.42	97.06	58.82

<sup>a</sup>Measurement made after hydrolysis.

<sup>b</sup>Measurement made at urine sample pH = 2.

<sup>c</sup>Measurement made at physiological urine pH.

The present experiment is part of the project 'Benzene exposure in work environments: development of biosensors for environmental monitoring', partly supported by Italian Health Ministry fundings. Biological monitoring will be performed on about 500 subjects and the results will allow a better estimation of a BEI limit value for total SPMA.

Further investigations will confirm whether or not the determination of total SPMA improves the correlation with airborne benzene concentrations.

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