EXPOSURE TO AIR POLLUTION IN GENERAL POPULATION LIVING IN URBAN AND RURAL ENVIRONMENT

Urinary benzene as biological index of exposure

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ABSTRACT

Evidence is needed regarding air pollutant exposure in general population. One of the most important contributors to air urban pollution is benzene, a widespread air pollutant present both in outdoor and indoor environments, and a well known human carcinogen. The aim of our study was to investigate the use of urinary (u) unmodified benzene (UB) as a biomarker of air environmental pollution for general population. u-UB and u-cotinine were measured in urine samples of 243 Italian children (5-11 years) recruited in a cross-sectional study. Urine samples were collected at the end of the day, an analytical determination of benzene was performed by solid-phase micro-extraction (SPME) – GC/MS. Analytical results were compared with data obtained from questionnaires about participants' main potential exposure factors. The main findings were that u-UB levels were influenced by secondhand smoke (SHS) exposure and urbanization of residence areas. In addition, data showed that, excluding children exposed to SHS, u-UB concentrations were about 2-fold higher in subjects living in urban areas than in those in the rural environment (medians=210.50 and 92.50 ng/L, respectively). These results were confirmed by multivariate linear regression model. In conclusion, we found that u-UB is a good biomarker of benzene exposure in general population. In addition, u-UB could be considered as a synthetic biological index for the assessment of population exposure to atmospheric pollution.

1 INTRODUCTION

In recent decades, the potential adverse effects on human health of air pollution have caused great concern worldwide and, although the progressive enhancements of air quality in numerous countries, many outdoor air quality problems still exist both in the developed and developing world (WHO, 2006).

Many studies were performed to evaluate the link between outdoor air pollution and adverse health effects, and the results showed that pollutants can affect different systems, in particular respiratory and cardiovascular systems, but also immunological, hematological, neurological and reproductive/developmental systems. In addition, some researchers evidenced a significant correlation between exposure to outdoor air pollutants and development of some kind of cancer (Curtis et al., 2005).

Besides that, a recent report of World Health Organization showed that the global burden of disease due to ambient air pollution is still very high, increasing about 3% of mortality from cardiopulmonary disease, about 5% of mortality from cancer, and about 1% of mortality from acute respiratory infections in children under five years, worldwide (Cohen et al., 2005).

All cited adverse effects are related to various air pollutants, including many gas and particles ingredients, major of which are nitrogen dioxide, sulphur dioxide, particulate matters, carbon monoxide, benzene and ozone. These chemicals (except ozone) mainly originate from fuel combustion of motor vehicles, power stations and factories, while ozone is one of the most important constituent of photochemical smog, and it is formed by a series of complicated photochemical reactions of oxygen, nitrogen oxides and volatile organic compounds in the presence of sunlight and warm temperature (Ko et al., 2009).

Despite of the great number of publications concerning outdoor air pollution and relative adverse outcomes on human health, new evidences are still needed about risk analysis, and the assessment of human exposure is a critical step of the process.

The most important difficulty of human exposure assessment arise from the heterogeneity of air pollution, both in physical and chemical characteristics. As anticipated, air pollutants included many substances; thus, a synthetic indicator of outdoor air pollution could be very useful.

In this context, one substances that will be used for human exposure evaluation is benzene, defined as one of the most important health-based European Union priority substances (Bruinen de Bruin et al., 2008). Benzene is a well-known human carcinogen classified in group 1 (carcinogenic to humans) by the International Agency for Research on Cancer since 1982 (IARC, 1982), and a widespread air pollutant, diffused in outdoor and indoor environments (both occupational and general ambient). Major sources of benzene for non-occupational exposure are fuel combustion of motor vehicles and cigarettes smoke (Johnson et al., 2007).

Several different methods are actually used to assess benzene exposure in general population, generally classified in two groups: environmental and biological monitoring.

Environmental monitoring is carried out by the measurement of airborne concentrations of benzene, by personal or environmental sampling; area sampling approach involves the placing of the samplers in a stationary position for the entire time of sampling, while personal sampling strategy involves the use of a personal dosimeter placed near the breathing zone. Historically, area sampling procedure was the most common method used for collecting air pollutants samples, and quantifying human exposure on the basis of respiratory volumes and time activity patterns (Esmen et al., 2000). Besides, at today, in Italy, the stationary samplers are still used to control air quality according to law prescription (Ministerial Decree, 2002).

Despite of this, environmental researches demonstrated that personal sampling strategy provides more accurate estimates of the human exposure to pollutants, because it represents a better approximation of the contaminant levels in the breathing zone of body (Esmen et al., 2000).

The second method used to evaluate contaminants exposure is biological monitoring, that consists in the collection of biological samples for analytical determinations of the levels of the pollutants, their metabolites or specific biological effect parameters (Angerer et al., 2007). In particular, for benzene exposure assessment, different methods have been described, such as determination of unmodified benzene (UB) or its metabolites in biological fluids (breath, blood, urine), or the research of albumin adduct (e.g. benzene oxide albumin adduct and 1,4-benzoquinone albumin adduct) (Johnson et al., 2007).

When compared to environmental monitoring, biological approach offers additional information in exposure assessment because it represents the amount of the contaminant actually absorbed into the body, and it reflects the individual differences in absorption, metabolism and excretion (Manno et al., 2010).

As regard to benzene, recent studies conducted on general population, not professionally exposed to benzene, suggest urinary unmodified benzene (u-UB) as good exposure markers for benzene (Fustinoni et al.,

2005; Barbieri et al., 2008; Lovreglio et al., 2010), although its ability to discriminate different levels of exposure, especially at low environmental concentrations, are currently under evaluation.

The objective of the present research is to evaluate the possibility to use u-UB as a biological index of exposure to environmental pollution in general population. For this reason, we performed a survey on a particular group of general population, such as children. The choice of children is derived from the need to evaluate benzene exposure in a category of general population surely not exposed to benzene in occupational settings or from the habit to smoke.

2 MATERIALS AND METHODS

3.1 Study population and design

The research was conducted in two areas of central Italy, whose urbanization characteristics allowed us to classify one as urban and the other as rural. The choice of areas was based on relevant urbanization indicators from national databases (National Institute of Statistics, Italian Automobile Club) from 2007, the year in which the present study took place. The selected urbanization indicators were:

- Resident population: total number of persons who usually live in the area.
- Population density: number of individuals living in the area divided by its surface area.
- Green area density: percentage of green areas in relation to total municipal territory.
- Motorization rate: number of motor vehicles per 100 inhabitants.

Summary information about these urbanization indicators for the urban and rural areas is reported in Table 1.

Table 1. Summary information on relevant urbanization indicators of the selected urban and rural areas in 2007.

	Resident population (n)	Population density (persons per km^2)	Green area density (% of total municipal territory)	Motorization rate (number of vehicles per 100 inhabitants)
Urban area	32,886	395	< 85	76
Rural area	3,308	120	> 85	66

In each area, a district primary school was recruited; 150 children attended the urban school, and 166 children attended the rural school.

All of the students and their parents received information about the goals and plans for the research and were invited to take part in the cross-sectional study. The overall participation rate was 76% (urban: 81% and rural: 73%, respectively).

Study subjects were 243 apparently healthy children between 5-11 years of age who were presumably exposed to benzene as a pollutant.

Detailed information about socio-demographic characteristics, activities engaged in on the sampling day, living environment, and lifestyle factors of the investigated subjects was obtained from a questionnaire completed by their parents.

The measurement campaigns were conducted on Wednesdays during the winter of 2007.

Before the monitoring day, we conducted formation meetings for all children and their parents on the modalities to compile the questionnaire and to collect and store urine sample.

3.2 Sampling collection

One urine sample for each participant was collected in the evening (just before bedtime) in a benzene-free Polypropylene bottle with hermetic closure, and immediately stored in the refrigerator at 4°C. The next morning, the sample was placed into a polystyrene cooler containing an ice pack and was delivered to the research team.

Spot urine samples were divided into two aliquots: a 14-mL aliquot was poured into a 20-mL glass vial previously added with 4 g of NaCl, promptly closed with a rubber lid with a polyperfluoroethylene lining, and crimped with an aluminum seal; and about 2 mL of specimen was partitioned into plastic tubes for urinary cotinine (u-cotinine), and u-creatinine determinations. All samples were coded and then frozen at -20°C until analysis.

A total of 243 urine samples were collected; 18 samples were rejected due to unsatisfactorily closure; besides, the volume of some other samples was not enough to carry out the whole set of analyses. Consequently, analytical determinations were performed on 185 vials for u-UB and on 225 tubes for u-cotinine and u-creatinine. Samples were analyzed within 30 days from sampling.

3.3 Analytical determination

u-UB was determined by headspace solid phase micro extraction (SPME) followed by gas chromatography-mass spectrometry (GC-MS) according to procedures outlined in Vitali et al. (2006). We used a 5973 GC-MS operating in Selected Ion Monitoring (SIM) mode (Agilent, Santa Clara, CA, USA) equipped with a 30 m x 0.25 mm x 0.25 µm HP-VOC column (HP, Palo Alto, CA, USA). Pesticide-grade reagents, high-purity benzene and benzene d-6 were supplied by Carlo Erba (Milan, Italy); all standards were used without further purification. The SPME apparatus, fitted with a 75-µm carboxen/polydimethylsiloxane fiber, was purchased from Supelco (Bellefonte, PA, USA).

Before analysis, the vials were conditioned at room temperature and then maintained at 60°C for 1 hour. The SPME fiber was held in the headspace for 10 minutes to reach the partition equilibrium, and then it was retracted into the needle and immediately inserted into the GC injector for thermal desorption. No carry-over effects were observed.

The chromatographic conditions were as follows: splitless injection port (at 290°C) with purge valve closed for 3 min; helium carrier gas at 1 mL/min; column temperature was maintained at 50°C for 5 min and then increased at 15°C/min to 200°C; dwell time was set at 50 ms/ion; and monitored ions were 78 and 52 m/z for benzene and 84 m/z for benzene-d6.

Quantitative determination was conducted using benzene d-6 as the internal standard (IS). The linearity of the method was tested by spiking urine samples at 50, 100, 250, 500, 1,000 and 2,000 ng/L. The results showed good linearity, with a correlation coefficient of 0.998.

The coefficient of variation of the method (CV%) was below 9.8% for all intra- and inter-day determinations.

The limit of detection (LOD), calculated as the signal to noise ratio (S/N) > 3, was 8 ng/L. All analytical determinations were above the corresponding limits of detection.

u-cotinine and u-creatinine were analyzed using a methodology that has been previously described and extensively used in previous publications (Manini et al., 2008).

u-cotinine were adjusted for u-creatinine and expressed as µg/g creatinine. u-UB levels were not adjusted for u-creatinine because u-UB is excreted into urine through a concentration-dependent passive process that involves tubular reabsorption, while creatinine is eliminated through glomerular filtration and is not reabsorbed (Boeniger et al., 1993; Serdar et al., 2003).

3.4 Statistical analyses

Statistical analyses were carried out using SPSS software (version 14.0 for Windows, Chicago, IL).

The first data showed that the biomarkers' levels were not normally distributed. Therefore, parallel analyses were conducted with non-parametric techniques (Kolmogorov-Smirnov test and Mann-Whitney test) and corresponding parametric methods on natural log-transformed data (t-test for independent or paired samples).

Descriptive statistical elaborations were performed on all selected children and on children unexposed to secondhand smoke (SHS). All children were considered to be exposed to SHS if they lived in households where at least one person was a smoker. Simple linear regression analyses were used to assess the relationship between u-UB and u-cotinine in children exposed to SHS.

Forward multiple linear regression analysis was run on the entire sample to assess the role of residence area, SHS exposure status, and other independent variables on u-UB. In the model, the natural log-transformed values of u-UB was included as a dependent variable, and the covariates were as follows: residence area (0=rural area, 1=urban area), SHS exposure status (0=unexposed to SHS, 1=exposed to SHS), gender (male=0, female=1), and age (0=1st, 2nd, or 3rd grade of primary school, 1=4th or 5th grade of primary school).

The significance level for all tests was $p \leq 0.05$ (two-tailed). Linear regression analyses were run using a significance level of 0.05 for entry and 0.10 for removal from the model. The "goodness of fit" of the model was assessed using R^2 statistics.

3 RESULTS

Descriptive characteristics of the studied subjects are presented in Table 2.

Table 2. General characteristics of subjects analyzed in the present study.

		Total children	Urban area	Rural area
Gender (%)	Male	51.8	52.3	51.3
	Female	48.2	47.7	48.8
Grade of primary school (%)	1st	21.0	20.5	21.4
	2nd	18.8	19.6	17.9
	3rd	19.7	18.8	20.5
	4th	20.5	24.1	17.1
	5th	20.1	17.0	23.1
SHS exposure status (%)	Exposed	39.7	23.7	56.0
	Unexposed	60.3	76.3	44.0
Time (min) spent in different environments during sampling day until urine collection Mean ± SD	At school (indoor environment)	443.99 ± 75.85	461.54 ± 38.36	429.27 ± 90.38
	Other indoor environments	263.85 ± 97.79	240.09 ± 83.90	290.00 ± 102.29
	Outdoor environments	51.84 ± 60.83	58.47 ± 49.33	47.18 ± 68.53
	Motor vehicles	26.27 ± 43.68	21.69 ± 21.28	29.40 ± 52.00

The two groups were comparable with respect to gender and time spent in indoor and outdoor environments. The percentage of children who lived in a rural area who were exposed to SHS was greater than the percentage of SHS-exposed children in the urban group (56.0% versus 23.7%). In addition, Table 2 shows a wide range of time spent in motor vehicles on the sampling day between subjects, especially in rural children (mean=21.29 min; SD=52.00 min).

The impact of SHS exposure status on u-UB concentrations were determined both in urban and rural children; the results are presented in Table 3.

Table 3. Summary statistics for urinary analytes in children differentiated according to secondhand smoke (SHS) exposure.

		u-UB ng/L			u-cotinine µg/g creatinine		
		Median	IQ range[a]	*p*	Median	IQ range[a]	*p*
Urban area	Exposed to SHS	411.50	234.25 1,188.50	0.003[b]	4,36	2,72 7,08	<0.001[b]
	Unexposed to SHS	210.50	167.25 329.50	<0.001[c]	2,07	1,23 3,15	<0.001[c]
Rural area	Exposed to SHS	359.50	267.75 629.50	<0.001[b]	3,77	2,61 7,12	<0.001[b]
	Unxposed to SHS	92.50	51.25 141.50	<0.001[c]	2,64	1,51 3,65	<0.001[c]

u-UB: urinary unmodified benzene
u-cotinine: urinary cotinine
[a]IQ Range: Interquartile Range
[b]Mann–Whitney *U*-test was used to compare exposed and unexposed to SHS
[c]Unpaired t-test was used to compare exposed and unexposed to SHS (ln-trasformed data)

Table 3 shows that concentrations of u-cotinine, a sensitive biomarker for exposure to active and passive smoke (Gourlay et al., 1996), were significantly higher among the SHS-exposed group when compared with the unexposed group in both urban and rural children; this result confirms the reliability of the questionnaire to collect information on the smoking habits of the studied children's cohabitants.

In addition, data showed in Table 3 evidence that u-UB is strongly influenced by SHS exposure; this finding was confirmed by the significant positive relationship between u-UB and u-cotinine in all samples and in the subgroup exposed to SHS, respectively described by the equations (1) and (2) of simple regression models:

$$\ln \text{u-UB} = 5.087 + 0.259 * \ln \text{u-cotinine}; p < 0.01 \qquad (1)$$

$$\ln \text{u-UB} = 5.294 + 0.474 * \ln \text{u-cotinine}; p < 0.01 \qquad (2)$$

The impact of passive smoke on u-UB is in agreement with other previous studies. Minoia et al. (1996), for instance, evaluated u-UB as a biomarker of benzene exposure during childhood and found a significant increase of u-UB in the group exposed to SHS compared with unexposed subjects.

For this reasons, the comparison between urban and rural groups were performed only on the group unexposed to SHS. The results were summarized in Table 4.

Table 4. Summary statistics for urinary unmodified benzene in children grouped according to residence area in unexposed to secondhand smoke (SHS) group.

	Mean	Standard deviation	Min - Max	Median	IQ range[a]	*p*
Urban area	311.54	303.98	36 - 1,950	210.50	167.25 - 329.50	0.003[b]
Rural area	107.10	67.58	37 - 296	92.50	51.25 - 141.50	<0.001[c]

u-UB: urinary unmodified benzene
[a]IQ Range: Interquartile Range
[b]Mann–Whitney U-test was used to compare urban and rural areas
[c]Unpaired t-test was used to compare urban and rural areas (ln-trasformed data)

Urinary levels of u-UB present a great variability. This finding is in line with data reported in other recent studies on children and general population (Johnson et al., 2007). In our study, levels varied from a low of 36 to high of 2,094 ng/L for u-UB, similar to the ranges of 27 - 2,060 ng/L for u-UB reported by other researchers for adults (Waidyanatha et al., 2001), and to the range of 50 – 1166 ng/L for children (Aprea, 2003).

Maximum levels resulted very higher for "worst cases" (urban children) compared to rural group concentrations (1,950 and 296, respectively). However, u-UB were found in all the analysed samples, confirming the ubiquitous diffusion of benzene even in rural environments.

Significantly larger values of u-UB levels were observed in children living in urban areas compared to the rural area group, with concentrations about 2-fold higher in urban groups than in rural ones.

These results are hardly surprising considering that benzene is a known traffic-related pollutant. It is clear, in fact, that children living in urban areas are exposed to higher levels of air pollutants, such as benzene, than are children in rural areas, who are exposed to much less traffic congestion; however, the comparison of data between urban and rural groups permitted us to confirm the sensitivity of u-UB as biomarkers of benzene exposure and its suitability for the assessment of environmental benzene exposure at and below ppm levels.

The final multiple linear regression models (Table 4) summarize how the weights of residence area, SHS exposure, age and gender explain the variability of u-UB.

Table 5. Significant predictors of urinary concentration of urinary unmodified benzene (natural log-transformed data) in forward multiple linear regression models.

Dependent variable	Independent variable	B[a]	SE[b]	β[c]	*p*	R^2 of the model
u-UB ng/L[e]	Constant [d]	4.684	0.110		< 0.001	0.337
	SHS exposure (exposed)	1.120	0.130	0.568	< 0.001	
	Residence area (urban)	0.685	0.127	0.357	< 0.001	

u-UB: urinary unmodified benzene
[a]B = unstandardized regression coefficients
[b]SE = standard error
[c]β = standardized regression coefficients
[d]Constant = estimated intercept value
[e]Variables considered: Residence area (urban vs. rural), SHS exposure status (exposed vs. unexposed), age (1st, 2nd, and 3rdgrade vs. 4th and 5th grade of primary school), gender (female vs. male)

Unlike previous research (Fustinoni et al., 2005; Manini et al. 2008), we prefer to use the questionnaire as indicator of exposure to SHS both for the different half-life of cotinine and benzene and the reliability of questionnaire to distinguish children exposed and unexposed to SHS.

Table 5 show that residence area and SHS exposure are significant contributors to benzene exposure; in particular u-UB levels increases of 56.7% in children exposed to passive smoking and of 35.7% in children that live in urban area.

4 CONCLUSIONS

In conclusion, we found that, using the strategy to collect urine sample at the end of the day and the analytical determination performed with SPME technique, u-UB resulted a good biomarker of benzene exposure in the general population.

Additionally, u-UB could be considered as a synthetic biological index in the process of exposure assessment to environmental pollution.

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