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Assessment of Environmental Exposure to Benzene: Traditional and New Biomarkers of Internal Dose

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1. Introduction

In view of the widespread ubiquity of benzene in the environment and of its carcinogenic effects on man, this toxicant poses a public health problem that has prompt nations to undertake active measures to contain environmental concentrations below the limit judged to be an acceptable risk threshold for the general population (World Health Organization [WHO], 2000).

In the occupational field, until the 1950s benzene was the solvent most commonly employed in some industrial processes, and especially in rubber, printing and shoemaking industries, because of its chemico-physical properties and low cost. This caused exposure of these workers to high benzene concentrations that induced toxic effects and acute non lymphocytic leukemia (Agency for Toxic Substances and Disease Registry [ATSDR], 2007; WHO, 1993). Due to these adverse effects, its use in industrial processes was then abandoned, replacing benzene firstly by hexane, but this proved to provoke peripheral neuropathies, and then by less toxic solvents such as heptane. In Italy the use of benzene as a solvent is banned by Law 245/1963, although traces below 2% are permitted in solvents of a different chemical nature (Italian Parliament, 1963).

Benzene is still used as a raw material or intermediate product in the chemical industry, mainly to synthesize ethylbenzene, cumene and cyclohexane, and to a limited extent as a

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chemical reagent in the laboratory. It is contained in crude oil, that is its main source of production nowadays, and is formed as a result of the incomplete combustion of fossil fuels such as coal and, to a lesser extent, wood.

Moreover, fuels derived from crude oil contain benzene not only because it is already present in the raw material but also because it is formed during the refining process (Brief et al. 1980; Holmberg & Lundberg 1985). For this reason, occupational exposure to benzene, albeit at concentrations of 2-3 orders of magnitude below the occupational limit values, such as the threshold limit value-time weighted average (TLV-TWA) of 1600 μg/m^3 proposed by the American Conference of Governmental Industrial Hygienists (ACGIH) , and the European limit value of 3250 μg/m^3 issued by the European Directive 1999/38/CE , is still present in oil refining and petrochemical industries, as well as in fuel tanker drivers and filling station attendants, workers in cokeries and in chemical laboratories (ACGIH, 2010; European Parliament, 1999).

The natural sources of emission into the atmosphere, originally volcanoes and fires, play a negligible role while human activities are the main source of benzene released in the environment (ATSDR, 2007). Among human activities one of the principal forms of emissions of benzene in the environment is in automobile exhaust. Evidence in literature reports higher concentrations of this toxicant in urban and dense traffic areas than in less busy traffic and rural areas.

Another source of environmental pollution by benzene is evaporation during filling operations at gasoline stations or during loading and unloading of fuel tanker lorries, even if the use of aspiration systems to retrieve the vapors during such operations has significantly reduced these emissions, by up to 75% (Duarte-Davidson et al., 2001). In addition, higher concentrations of benzene than the background outdoor levels are present inside vehicles, including buses and private cars, due not only to the penetration inside the body of the vehicle of exhaust fumes from other vehicles, but also to leaks from gasoline tanks and gasoline leads and circuits (Geiss et al., 2009; Li et al., 2009). The levels of benzene emitted by vehicles depend on the type and age of the vehicle, the type of traffic and the ventilation within the body of the car (Duarte-Davidson et al., 2001). Finally, in some geographic areas, apart from road traffic, other sources of benzene emission into the atmosphere, such as factories, hazardous waste dumps and domestic wood fires, can play a significant role in causing outdoor pollution (Barrefors & Petersson, 1995; Edgerton & Shah, 1992).

In western nations, the pollution of urban areas by benzene has gradually declined in recent years, thanks to the legislation introduced to reduce the content of benzene in fuels, currently limited in Italy to concentrations below 1% in volume (law n. 413/97), and to the application in the EU of the norms targeting an annual average exposure for benzene of 5 μg/m^3 as from 2010 (European Commission, 2000; Italian Parliament, 1997). Airborne benzene concentrations are proportionally lower than the true quantity of emissions thanks to the rapid chemical degradation of this toxicant, largely as a reaction to hydroxyl radicals. This limits the persistence of volatile benzene in the atmosphere to a few days or hours.

Active cigarette smoking is another important source of exposure to benzene in the general population, as mainstream smoke contains quantities of 28.0-105.9 μg/cigarette, and sidestream smoke no less than 70.7–134.3 μg/cigarette (International Agency for Research on Cancer [IARC], 2004). Wallace (1989) estimated a benzene intake of 1.8 mg/day with an average consumption of 32 cigarettes/day, equal to at least 10-fold the intake of a non-smoker, while Duarte-Davidson et al. (2001) estimated a dose of 400 μg/day of benzene

retained within the organism by a smoker of 20 cigarettes/day. Thus, cigarette smoking can induce a similar or higher benzene intake than that occurring in most occupationally exposed workers in western nations, and certainly higher than the intake caused by environmental exposure to airborne benzene levels near the upper limits for air quality. Moreover, the role of smoking as a source of benzene has become progressively more important as urban pollution levels have declined (Duarte-Davidson et al. 2001; Hattemer-Frey et al. 1990). In fact, in the period 1989-1997 Fruin et al. (2001) observed a marked reduction in the benzene quota derived from the environment (12%) and thus a proportional increase in the quota due to cigarette smoking (78%). Passive smoking can also induce significant exposure to benzene, equal to about 10% of the total intake in non smokers, and higher than the quota contributed by the entire set of US industrial emissions in the atmosphere (Duarte-Davidson et al., 2001; Wallace, 1995). Confirming this, higher benzene concentrations have been reported in homes with one or more smokers among the inhabitants (median 10.6 $\mu g/m^3$) than in non smoker homes (7.0 $\mu g/m^3$) (Wallace 1989).

In man, benzene has myelotoxic and carcinogenic effects. As regards the former environmental exposure to benzene is unlikely to cause the myelosuppression effects previously observed in occupational contexts, since a NOAEL of 1790 $\mu g/m^3$ has been individuated for these effects, that will probably never again be reached in the human living environment (Collins et al., 1997). The carcinogenic effect manifests in the form of a greater prevalence of acute non lymphocytic leukemia, as stated above. Benzene has been classified carcinogenic to humans by the IARC, ever since the first classification published in the 1980s (IARC, 1982). A review conducted in 2009 confirmed benzene in the same group, associated with acute non lymphocytic leukemia. Instead, there is still only limited evidence of a causal relationship with the onset of acute lymphocytic leukemia, chronic lymphocytic leukemia, multiple myeloma and non-Hodgkin lymphoma (Baan et al., 2009).

Exposure to benzene is considered to induce the onset of leukemias because benzene acts through a genotoxic carcinogenic mechanism, whereby a dose-response relationship of a linear type has been proposed, with no threshold. Although this hypothesis has recently been cast in doubt, the level of exposure that can be considered "safe" has not been identified, but only a LOAEL of 32000-80000 $\mu g/m^3$ (Bolt et al., 2008; Duarte-Davidson et al., 2001). As to the carcinogenic risk posed by environmental exposure, the concentration of this toxicant judged to pose an acceptable risk in the general population, exposed over a lifetime, has been estimated on the basis of epidemiological studies of workers occupationally exposed to medium-high concentrations of benzene. The WHO estimate, in particular, predicts an excess risk of 6 cases x 10^{-6} for chronic exposure to 1 $\mu g/m^3$ throughout life (WHO, 2000).

Absorption of benzene in the general population occurs virtually exclusively by inhalation, that accounts for more than 99% of the daily intake of this toxicant, whereas oral intake plays only a minor role, since the quantities of benzene present in water, foods and consumer products are minimal and skin intake is negligible (Hattemer-Frey et al., 1990). After being absorbed, benzene is partly eliminated as is in exhaled air, accounting for 16.8 % of the quota absorbed, and in urine, albeit only for 0.1% of the absorbed quota (Ghittori et al., 1993; Nomiyama & Nomiyama, 1974). The remainder is rapidly distributed throughout the organism and undergoes a biotransformation process, prevalently in the liver. This occurs very similar in man and experimental animals, and involves the initial formation of benzene oxide catalyzed by microsomial cytochrome P450 and especially isoenzyme 2E1

(Snyder & Heidli, 1996). After the formation of this reactive intermediate, many different metabolites are then formed in succession through different metabolic pathways, namely phenol, catechol and hydroquinone by oxidation, again catalyzed by cytochrome P450, S-phenylmercapturic acid (SPMA) through the action of the glutathione transferase system, 1,2-benzene dihydrodiol through the action of epoxide hydrolase and the subsequent oxidation to catechol and *t,t*-muconic acid (*t,t*-MA) through the opening of the benzene ring (Snyder et al., 1993). All these metabolites are then excreted in the urine in the form of sulfate or glucuronate derivatives.

For the progressive reduction of the benzene workplace concentrations to which workers are exposed, ever more sensitive and specific biomarkers have needed to be identified, that maintain their validity even in conditions of low or extremely low benzene concentrations. This is why urinary phenol is no longer used in routine practice, because it has poor validity for exposure to less than 16250 μg/m^3, a value that is still one order of magnitude greater than the TLV-TWA of the ACGIH (Boogard & van Sitter, 1995; Ong et al., 1995). The biological markers currently recommended by the ACGIH are *t,t*-MA and SPMA, that reveal an increased urinary excretion already at levels of exposure to airborne benzene concentrations of 65 μg/m^3, while the relation becomes unambiguous at concentrations of more than 650 μg/m^3 (Kim et al., 2006). A new marker of internal dose is now under study, namely the determination of benzene as is in the urine (urinary benzene) that may prove particularly useful to monitor exposure to very low concentrations of airborne benzene, since a correlation has been shown with benzene concentrations ranging from 6 to 478 μg/m^3 (Fustinoni et al., 2005).

Despite the ample volume of studies in the occupational field, the behavior and significance of the above-described biomarkers of internal dose are still little known when used to monitor environmental exposure to airborne benzene concentrations near the upper limit of 5 μg/m^3 for air quality. In particular, the validity of these markers needs to be confirmed as a means of excluding environmental exposure beyond this limit, and hence the presence of a carcinogenic risk above what is considered acceptable. The behavior of these biomarkers of internal dose also needs to be assessed in relation to the relative contributions made to the overall intake of benzene by cigarette smoking and urban pollution, the main sources of environmental exposure to the toxicant.

Aim of the present study was to assess the significance and limits of *t,t*-MA, SPMA and urinary benzene for biological monitoring of subjects with non occupational exposure to very low concentrations of benzene as those found in the general environment, as well as to study the influence of the different sources of environmental exposure on these biomarkers.

2. Materials and methods

2.1 Subjects

The study sample included 123 adult males resident in the cities and hinterland of Bari and Foggia (Apulia - Italy), all in good health and with no occupational exposure to benzene. In the geographic area where this study has been conducted there are no major industries or waste incinerators that could produce benzene emissions. All participants filled out a questionnaire posing questions about personal data, job at the time of the study, smoking habit with particular reference to the number of cigarettes smoked during the environmental sampling, alcohol consumption, personal medical history, time spent in

urban traffic during the environmental sampling, and hobbies. All subjects gave prior written consent to take part in the study.

2.2 Air sampling

Exposure to airborne environmental benzene was monitored in all subjects by passive personal sampling, using radial diffusion samplers (Radiello®) containing an active carbon cartridge, worn in the respiratory zone for 8 hours, typically from 8:30 a.m. to 4:30 p.m. After the sampling, the Radiello® vials were preserved at +4°C until the time of analysis.
Analysis of the vials was then performed by gas chromatography – flame iononization detector after desorption of the benzene from the active carbon with a carbon disulfide low benzene content according to the method reported by the manufacturer (Supelco, 2010). The detection limit of the procedure for benzene was 2 μg/m^3. All analyses were performed blinded.

2.3 Urine biomonitoring

Immediately after the environmental sampling, a urine sample was collected from all participants for assays of *t,t*-MA, SPMA and urinary benzene. Each urine sample was subdivided into two aliquots: 30 ml of urine were set aside to determine *t,t*-MA, SPMA and urinary creatinine, preserved in sterile containers, without the addition of preservatives or stabilizers, at -20°C until the time of analysis. The second aliquot, 10 ml of urine used to determine urinary benzene, was immediately transferred to a presealed 20 ml vial containing 4 g of NaCl and preserved at +4°C until the time of analysis. All analyses were conducted blinded.
Urinary *t,t*-MA analysis was carried out with the HPLC-UV method at 264 nm, after solid phase extraction (SPE) (SAX column-Varian) by an analytical method described elsewhere (Aprea et al., 2008). The limit of detection (LOD) of the procedure was 20 μg/L.
The analytical determination of urinary SPMA was performed following the application described in Sabatini et al. (2008). Briefly, after SPE and LC separation, samples were analyzed by a liquid chromatography/electrospray tandem mass spectrometry method (HPLC-ESI-MS/MS), operated in negative ion mode, using isotope-labeled analogs as internal standards. The LOD of the method was 0.03 μg/L.
The analytical determination of benzene in urine was performed by headspace analysis with automated solid phase micro-extraction (SPME), following a previously reported method (Barbieri et al., 2008). A gas chromatograph equipped with a split-splitless injector and coupled to a mass selective detector (GC/MS) was used for analysis. An autosampler (CTC Combi PAL system) was interfaced to GC/MS system for the SPME process. The LOD of the method was 0.02 μg/L.
Analyses of urinary creatinine were performed using the DCA 2000®+ analyzer. The creatinine assay is based on the Benedict/Behre test, and was performed on the same urine samples used for all the other analyses, collected at the endof the environmental sampling (Benedict & Behere, 1936).
All the laboratories conducting the analytical measurements conform to quality assurance procedures and participate in quality control programs.

2.4 Statistical analysis

Statistical analyses were done with the SPSS program (14.0 version, Chicago, IL, USA). A value corresponding to one-half of the LOD was assigned to measurements below the

analytical detection limit. A normal distribution of all the variables was checked with the Kolmogorov-Smirnov test. Non normally distributed variables were analyzed by parametric tests after logarithmic transformation or by non parametric tests. Correlation analyses were done with Spearman's test. Multiple linear regression models were applied to assess a dependency relation of the different biomarkers on the independent variables. The level of significance was set at $p< 0.05$.

3. Results

Of the 123 subjects, 55 were smokers and 68 non smokers, and the population sample featured a wide age (21-62 years) and BMI (19.4-44.4 Kg/m²) range (Table 1).
For all the biomarkers determined, the percentage of cases in the entire sample exceeding the LOD was significantly higher in smokers than non smokers. When the whole sample was subdivided according to exposure to urban traffic or not during the environmental sampling, a higher percentage of results above the LOD was found only for airborne benzene and urinary benzene (Table 2).

	N.	Mean±SD	Median	Range
Age (years)	123	41.5±11.8	44.0	21-62
Body mass index (Kg/m²)	123	26.8±4.8	25.8	19.4-44.4
Residence				
- urban	79	64.2%		
- rural	44	35.8%		
Alcohol consumption				
- Teetotal	20	16.2%		
- <10 g/day	51	41.5%		
- >10 g/day	52	42.3%		
Smoking habit				
- Smokers	55	44.7%		
- Non smokers	68	55.3%		
N. cigarettes/day*	55	15.8±8.6	15.0	5-40
N. cigarettes smoked during environmental sampling*	55	6.6±4.1	6.0	0-20
Time between last sigarette and urine collection (minutes)*	53	54.3±69.0	30.0	2-360
Exposure to urban traffic during environmental sampling				
- Yes	27	21.9%		
- No	96	78.1%		

*In smokers

Table 1. Personal data of the 123 subjects examined.

	Smoking habit				Exposure to urban traffic			
	Yes		No		Yes		No	
	$N_{>LOD}/N$	$N_{>LOD}/N\%$	$N_{>LOD}/N$	$N_{>LOD}/N\%$	$N_{>LOD}/N$	$N_{>LOD}/N\%$	$N_{>LOD}/N$	$N_{>LOD}/N\%$
Airborne benzene	14/55	25.5%	20/68	29.4%	18/27	66.7%[c]	16/96	16.7%
t,t-MA	55/55	100.0%[a]	62/68	91.2%	27/27	100.0%	90/96	93.7%
SPMA	44/53	83.0%[b]	2/66	3.0%	12/27	44.4%	34/92	37.0%
Urinary benzene	53/54	98.1%[b]	22/65	33.8%	25/27	92.6%[c]	50/92	54.3%

Smokers vs non smokers: [a]$p<0.05$; [b]$p\leq0.001$; Exposed vs not exposed to urban traffic: [c]$p<0.001$

Table 2. Percentage of determinations of airborne benzene, *t,t*-MA, SPMA and urinary benzene above the LOD in the sample, subdivided by smoking habit and exposure to urban traffic during sampling.

To assess a simultaneous influence of smoking habit and exposure to urban traffic on the levels of airborne benzene and the urinary biomarkers, two-way analysis of variance was done, including in the model only the environmental and urinary values exceeding the LOD (Table 3). The results demonstrated an evident association between airborne benzene or *t,t*-MA or urinary benzene and cigarette smoking, whereas it was not possible to perform the analysis for SPMA because only 2 determinations were above the LOD in non smokers. In any case, there was no significant difference in the urinary concentrations of SPMA in smokers between subjects with or without exposure to urban traffic.

	Exposure to urban traffic	Smokers				Non smokers				ANOVA
		$N_{>LOD}$	Mean±SD	Median	Range	$N_{>LOD}$	Mean±SD	Median	Range	
Airborne benzene ($\mu g/m^3$)	Yes	10	7.4±3.7	7.3	3.9-16.3	8	4.7±1.0	4.5	3.3-6.2	Model: F=8.3[a] Smoke: F=8.4[a] Traffic: F=4.0
	No	4	6.2±3.9	5.7	2.0-11.5	12	3.4±1.1	3.0	2.0-5.7	
t,t-MA ($\mu g/g$ creat)	Yes	14	80.4±52.9	73.5	13.0-196.0	13	51.0±16.5	46.0	30.0-90.0	Model: F=13.0[b] Smoke: F=14.1[b] Traffic: F=0.0
	No	41	102.8±58.6	89.7	19.0-307.0	49	55.7±105.0	35.6	9.6-734.0	
SPMA ($\mu g/g$ creat)	Yes	11	1.47±1.30	0.99	0.38-4.48	1	-			-
	No	33	1.54±1.19	1.28	0.29-5.22	1	-			
Urinary benzene ($\mu g/L$)	Yes	14	2.24±3.62	0.41	0.04-11.40	11	0.07±0.02	0.06	0.04-0.10	Model: F=21.2[b] Smoke: F=58.7[b] Traffic: F=0.0
	No	39	0.97±1.00	0.60	0.06-4.27	11	0.07±0.03	0.06	0.04-0.12	

[a]$p<0.01$; [b]$p<0.001$

Table 3. Concentrations of airborne benzene, *t,t*-MA, SPMA and urinary benzene in the determinations above the LOD in the sample, subdivided by smoking habit and exposure to urban traffic.

In non smokers, a possible association between exposure to passive smoke during the environmental sampling and higher airborne benzene levels or urinary excretion of its metabolites was studied. No association was found in non smokers exposed or not exposed to passive smoke and airborne benzene and its urinary metabolites (data not shown).

Analysis of correlations between the general lifestyle of the participants, variables linked to smoking habit and exposure to urban traffic, the airborne benzene concentrations and the biomarkers studied was done on the entire sample and then subdivided into smokers and non smokers (Table 4). Airborne benzene was found to be correlated to the time spent in urban traffic during the sampling, both in the entire sample and when analyzing smokers and non smokers separately, whereas there was no correlation between airborne benzene and the number of cigarettes smoked per day or during the sampling time (Table 4). Among the biomarkers studied, airborne benzene was correlated only with urinary benzene, and only in the non smoker group. When analyzing the entire sample together, all the biological markers studied were found to be significantly correlated to the number of cigarettes smoked per day or during the sampling time. In smokers this correlation was significant only for SPMA. Moreover, *t,t*-MA and urinary benzene were correlated in non smokers with the time spent in urban traffic; when taking the group as a whole, this correlation was confirmed for urinary benzene only, albeit with a low rho value. Finally, the three biological markers were all mutually correlated in the entire sample taken as a whole (Table 4).

		Age (years)	BMI	N. cig. /day	N. cig./ sampling	Time since last cig.	Alcohol	Urban traffic (minutes)	Airborne benzene	*t,t*-MA	SPMA
BMI (Kg/m^2)	Total Smokers Non smokers	0.35[c] 0.36[b] 0.32[b]	-	-	-	-	-	-	-	-	-
N. cig. /day	Total Smokers Non smokers	-0.22[a] -0.08 -	-0.05 0.11 -	-	-	-	-	-	-	-	-
N. cig. / sampling	Total Smokers Non smokers	-0.21[a] -0.02 -	-0.04 0.08 -	0.94[c] 0.55[c] -	-	-	-	-	-	-	-
Time since last cigarette	Total Smokers Non smokers	- 0.23 -	- 0.08 -	- -0.48[c] -	- -0.22 -	-	-	-	-	-	-
Alcohol	Total Smokers Non smokers	0.37[c] 0.35[b] 0.38[c]	0.25[b] 0.14 0.37[b]	0.005 0.02 -	0.01 0.11 -	- 0.12 -	-	-	-	-	-
Urban traffic	Total Smokers Non smokers	0.14 0.46[c] -0.17	0.07 0.31[a] -0.14	0.04 -0.13 -	0.04 -0.04 -	- 0.21 -	0.11 0.25 -0.03	-	-	-	-
Airborne benzene	Total Smokers Non smokers	0.17 0.33[a] -0.01	0.09 0.26 -0.06	0.001 -0.03 -	0.03 0.17 -	- -0.01 -	0.11 0.13 0.10	0.51[c] 0.61[c] 0.40[c]	-	-	-
t,t-MA	Total Smokers Non smokers	-0.15 0.03 -0.12	-0.03 0.10 -0.12	0.57[c] 0.23 -	0.55[c] 0.07 -	- -0.11 -	-0.02 0.01 -0.08	0.04 -0.22 0.29[a]	0.04 -0.13 0.22	-	-
SPMA	Total Smokers Non smokers	0.05 0.32[a] 0.33[b]	-0.01 0.08 0.04	0.76[c] 0.42[c] -	0.76[c] 0.43[c] -	- -0.17 -	0.11 0.26 0.09	0.003 -0.13 0.02	0.02 0.04 -0.05	0.56[c] 0.28[a] 0.19	-
Urinary benzene	Total Smokers Non smokers	-0.30[c] -0.12 -0.19	-0.06 0.10 -0.08	0.77[c] 0.23 -	0.74[c] 0.17 -	- -0.18 -	-0.13 -0.38[b] 0.07	0.19[a] 0.01 0.50[c]	0.16 0.20 0.31[a]	0.44[c] 0.14 0.06	0.56[c] 0.23 -0.19

[a]$p<0.05$; [b]$p<0.01$; [c]$p\leq0.001$

Table 4. Spearman correlations among personal data, lifestyle, airborne benzene and biomarkers in the whole sample and subdivided by smoking habit.

The dependency of *t,t*-MA, SPMA and urinary benzene on the variables age, BMI, number of cigarettes/day, urban traffic and airborne benzene was studied in the sample as a whole,

applying different multiple linear regression models. The results demonstrated a dependency of the urinary concentrations of *t,t*-MA and SPMA on the number of cigarettes smoked per day, and of urinary benzene both on the number of cigarettes/day and on the time spent in urban traffic (Table 5).
To verify the influence of cigarette smoking on the biotransformation of benzene, the urinary benzene/*t,t*-MA ratio was studied in smokers and non smokers, and showed significantly higher levels in smokers (median 0.0071 vs 0.0008; p≤0.001).

	t,t-MA			SPMA			Urinary benzene		
	t	p		t	p		t	p	
Age (years)	NS			NS			NS		
BMI (Kg/m²)	NS			NS			NS		
N. cigarettes/day	5.8	<0.001		11.9	<0.001		12.5	<0.001	
Urban traffic (minutes)	NS			NS			2.1	0.034	
Airborne benzene (µg/m³)	NS			NS			NS		
	F	p	R^2	F	p	R^2	F	p	R^2
Model	34.2	<0.001	0.22	141.0	<0.001	0.55	80.6	<0.001	0.59

NS= non significant

Table 5. Multiple linear regression analysis of the whole sample, taken as a single group, for the dependent variables *t,t*-MA, SPMA and urinary benzene.

4. Discussion

This research analyzed the contribution of both traditional and new biological markers of internal dose, namely *t,t*-MA and urinary SPMA as compared to urinary benzene, to the monitoring of environmental exposure to very low concentrations of benzene. A particular attention was paid to how much a smoking habit and exposure to urban traffic, the main non occupational sources of this toxicant, could condition the urinary excretion of the various biomarkers.
Environmental monitoring demonstrated that overall, the general population studied was exposed to only very low concentrations of benzene, with 72.4% of the air samples showing values of less than 2 µg/m³, a much higher percentage than in a recent study of a population with no occupational exposure resident in a large Northern Italian city with very dense road traffic, Milan. In that study, the 5th percentile of environmental benzene distribution was equal to 1.5 µg/m³ and the 25th percentile to 3.0 µg/m³ (Fustinoni et al., 2010). Thus, our results suggest that our study sample, resident in small-medium cities with a population of less than 500 thousand inhabitants, is exposed to generally similar or lower levels of environmental pollution by benzene than inhabitants of other industrial, urban and suburban/residential/rural areas in Italy and worldwide (Table 6). Apart from the lesser traffic volume, this difference is also due to climatic conditions, in particular the strong winds that commonly blow in this area and tend to prevent the stagnation of pollutants. Nevertheless, exposure to road traffic was in any case the main factor determining environmental benzene concentrations exceeding the LOD, even if no significantly higher benzene concentrations could be determined in the traffic-exposed group as compared to the non-exposed group when analyzing only the values obtained in excess of the LOD, possibly due to small sample sizes.

Although the airborne benzene levels measured were generally low, concentrations above the threshold limit for air quality of 5 μg/m^3 were observed in 13 cases, highlighting the fact that exposure to excess benzene levels in the daily environment is still possible in western nations even nowadays. In fact, the threshold of 5 μg/m^3 represents the mean annual exposure posing a carcinogenic risk considered to lie within acceptable limits for the general population with exposure over a lifetime. No definitive level of exposure below which the genotoxic carcinogenic effect of benzene is completely revoked has yet been identified, while adopting a threshold of 0, the only value that could guarantee the absence of risk, seems practically impossible bearing in mind the widespread ubiquity of benzene sources. It is now common practice to use the threshold value for air quality when interpreting the results of personal sampling for carcinogenic environmental agents in both occupational and non-occupational settings. All the same, it must be taken into account that the threshold value represents a mean value referred to annual measurements, and is thus less affected in the long term by the inevitably wide variability of benzene concentrations occurring over such a long period, whereas environmental samplings conducted as in our study refer to a short period, in our case 8 hours, and can therefore be affected by occasional peaks of exposure. Therefore, the finding of values in excess of the 5 μg/m^3 threshold during sampling lasting 8 hours does not necessarily indicate a raised health risk.

As regards the influence of a smoking habit, previous studies have demonstrated that the benzene concentrations measured with personal samplers do not reflect the true level of exposure to benzene induced by cigarette smoke (Fustinoni et al., 2005; Lovreglio et al., 2010). The results of the present study partly confirm reports in literature, since a smoking habit did not affect the percentage of benzene determinations exceeding the LOD, and no correlation was observed between airborne benzene and the number of cigarettes smoked during the sampling period. However, higher concentrations of airborne benzene were observed in smokers when only the values exceeding the LOD were analyzed, even after stratification for exposure to urban traffic.

OCCUPATIONAL EXPOSURE						
Occupational settings	Sampling	Year	Exposure levels (μg/m^3) Mean ±SD	Median	Range	References
Chemicals manufacture Heavy truck drivers Crude petroleum extraction	Personal (8 h)^{NA}	1996-2007	357.5±1300.0 357.5±195.0 97.5±260.0	- - -	6.5-1300.0 81.2-487.5 3.25-975.0	Scarselli et al., (2011)
Fuel tanker drivers Filling station attendants	Personal (8 h)*	2006	306.7±266.7 23.5±17.4	246.6 20.9	7.4-1017.1 4.5-66.3	Lovreglio et al., (2010)
Petrochemical factories Gasoline service stations	Ambient (8 h)**	-	214.6±78.8 209.9±57.0	34.6 114.3	5.3-1766.2 5.6-773.6	Navasumrit et al., (2005)
Refinery blue collars	Personal (8h)****	-		190	60-2310	Fustinoni et al., (2011)
Sewage workplace	Personal (4 d)*	2008-2009	19.1±2.9			Al Zabadi et al.(2011)
Urban policemen	Personal (6 h)***	2004		9.6	5.4-22.5	Campo et al., (2011)

ENVIRONMENTAL EXPOSURE						
City	Sampling	Year	Exposure levels (µg/m³) Mean ±SD	Median	Range	References
INDUSTRIAL AREA						
Lin-Yuan TWN Ping-Tung TWN	Ambient $_{Outdoor}$ (1-2 h)****	2003-2004	25.8±34.7 5.9±3.4	8.4 5.0	3.7-120.6 ND-13.7	Hsieh et al., (2006)
La Plata ARG	Ambient $_{Indoor}$ (4 w)** Ambient $_{Outdoor}$ (4 w)**	2000-2002	19.1 16.1	18.0 13.4	max 59.5 max 37.2	Massolo et al., (2009)
Yokohama JPN	Ambient $_{Outdoor}$ ****	2007-2008	6.8±8.7			Tiwari et al., (2010)
Dunkerque FRA	Ambient $_{Outdoor}$ *	2007	1.9±2.1		0.8-6.7	Roukos et al., (2009)
URBAN AREA						
Bangkok THA	Ambient $_{Outdoor\ Roadside}$** Ambient $_{Outdoor\ School}$**	-	109.2±22.4 26.7±2.5	91.8 27.9	50.2-212.9 22.5-28.7	Navasumrit et al., (2005)
Naples ITA	Ambient $_{Outdoor}$ (24h)*	2006	9.8±4.4		4.4-17.2	Iovino et al., (2009)
Milano ITA	Personal (72 h)* Ambient $_{Indoor\ work}$ (1 w)* Ambient $_{Outdoor\ work}$ (1 w)*	2003	8.5±3.0 3.0±1.5 1.9±1.4			Bruinen de Bruin et al., (2008)
Catania ITA	Personal (72 h)* Ambient $_{Indoor\ work}$ (1 w)* Ambient $_{Outdoor\ work}$ (1 w)*		5.2±1.6 5.0±3.4 4.2±1.8			
Brussels BEL	Personal (72 h)* Ambient $_{Indoor\ home}$ (1 w)* Ambient $_{Indoor\ work}$ (1 w)* Ambient $_{Outdoor\ work}$ (1 w)*		3.2±1.4 2.7±1.2 2.1±0.2 1.9±0.6			
Helsinki FIN	Personal (72 h)* Ambient $_{Indoor\ home}$ (1 w)* Ambient $_{Outdoor\ work}$ (1 w)* Ambient $_{Indoor\ work}$ (1 w)*		2.0±0.9 1.7±1.0 1.0±0.3 1.0±0.2			
Bucharest ROM Madrid ESP Lisbon PRT Brussels BEL Ljubljana SVN Dublin IRL	Ambient $_{Outdoor}$ (1 d)*	2003 2003 2002 2002 2003 2004		7.1 4.5 3.8 2.5 3.1 1.1	max 18.2 max 15.0 max 7.9 max 6.2 max 5.4 max 2.9	Perez Ballesta et al., (2005)
Bari ITA	Personal (8 h)*	2006	4.6±2.6	4.3	<3.0-11.5	Lovreglio et al., (2010)
Milan ITA	Personal (5h)*	-		4.0	1.5-16.1	Fustinoni et al., (2011)
Beijing CHN	Ambient $_{Outdoor}$ (13 h) ****	2008	3.7±3.0			Liu et al., (2009)
LaPlata ARG	Indoor (4 w)** Outdoor (4 w)**	2000-2002	3.6 3.1	3.2 3.1	max 12.7 max 5.6	Massolo et al., (2009)
Bari ITA	Ambient $_{Indoor}$ (24h)*	2005	(weekly average) 1.3-14.8			Bruno et al., (2008)
Bari ITA	Ambient $_{Outdoor}$ (1 w)*	2008 Autumn 2008 Spring			1.3-9.0 0.8-2.7	Caselli et al., (2010)
Antwerp BEL	Ambient $_{Outdoor}$ (5 d) * Ambient $_{Indoor}$ (5 d) *	2002-2003	1.9 1.5		0.7-4.4 0.3-3.1	Stranger et al,. (2008)

ENVIRONMENTAL EXPOSURE						
City	Sampling	Year	Exposure levels (µg/m³) Mean ±SD	Median	Range	References
URBAN AREA						
Spain	Ambient $_{\text{Outdoor home}}$ (1 w)*	2004-2008	1.6±0.9			Estarlich et al.,(2011)
Toulouse FRA	Ambient $_{\text{Outdoor}}$ *	2001	1.1±0.3		2.0-0.7	Simon et al., (2004)
SUBURBAN/ RESIDENTIAL/ RURAL AREA						
Naples ITA	Ambient $_{\text{Outdoor}}$ (24h)*	2006	5.7±3.2		2.3-12.8	Iovino et al., (2009)
LaPlata ARG	Indoor (4w)** Outdoor (4w)**	2000-2002	4.7 1.6	3.1 1.7	max 13.2 max 1.8	Massolo et al., (2009)
Spain	Ambient $_{\text{Outdoor home}}$ (1 w)*	2004-2008	1.5±0.7			Estarlich et al., (2011)
Yokohama JPN	Ambient $_{\text{Outdoor}}$ ****	2007-2008	1.3±1.0			Tiwari et al., (2010)
Antwerp BEL	Ambient $_{\text{Indoor}}$ (5 d) * Ambient $_{\text{Outdoor}}$ (5 d) *	2002-2003	0.4		0.1-0.7	Stranger et al., (2008)
Auvergne FRA	Ambient $_{\text{Indoor}}$ (1w) *	-		0.8	0.3-9.8	Hulin et al., (2010)

* passive sampling by Radiello; ** passive sampling by 3M OVM 3500; *** passive sampling by Carbopack B; **** active sampling; NA not available; (sampling period): h= hour; d= day; w= week.

Table 6. Levels of occupational and environmental exposure to benzene observed in the last 10 years.

Thanks to progressive improvements in the analytical techniques used to assay biomarkers since the 1970s, it has become possible to measure ever lower concentrations of chemical substances or their metabolites in human biological fluids after exposure to toxicants in the daily environment. For this reason, from being a tool used exclusively to assess occupational risks, biological monitoring has now become a valuable means of evaluating the risk of exposure to toxicants in the daily environment that integrates, but does not replace, environmental monitoring results. It thus could play a central role in public health and environmental medicine policies, helping to identify population groups at higher risk of exposure and/or effects, contributing to measure the internal dose and thus providing helpful information that can direct subsequent corrective measures (Angerer et al., 2007).

The results of biological monitoring in the general public are generally interpreted by comparison with reference values. These are obtained by statistical processing of the results of assays of the concentrations of a toxicant or its metabolites in biological fluids collected from a population or reference group with no occupational exposure to the substance (Apostoli & Minoia, 1999). It must be remembered, however, when assessing the risk of exposure to benzene, that unlike the threshold for air quality that derives from an estimate, albeit indirect, of the carcinogenic risk of exposure to benzene judged acceptable for the general population, the reference values do not bear any relation to the carcinogenic effect of benzene. Although the use of reference values does not therefore allow us to exclude a public health risk, it is important as a means of individuating specific population groups with higher levels of exposure to a toxicant than those of the reference population, due to residence in a highly polluted area, for instance, thus addressing one of the main objectives of biological monitoring in the non occupational field.

Many biological monitoring studies have been conducted to assess occupational exposure to benzene, but only few studies of the living environment. Moreover, these experiences have been gained largely in workers such as traffic wardens or public transport drivers who have occupational exposure to road traffic and so to generally higher concentrations of benzene than those affecting the general population (Barbieri et al., 2008; Campo et al., 2011; Fustinoni et al., 2005; Manini et al., 2008).

In this work the three main biological markers of internal dose currently in use, or under study for validation in biological monitoring of exposure to benzene, namely *t,t*-MA, SPMA and urinary benzene, were studied. Urinary *t,t*-MA is the only one of the three that is not entirely specific for benzene, since it can also derive from the metabolism of sorbic acid, an anti-mycotic preservative present in many foods, and so commonly absorbed in the diet, and in cosmetics. Although only a small percentage of the sorbic acid ingested is biotransformed to *t,t*-MA, the contribution of the diet can induce comparable concentrations of this metabolite to those observed in workers with occupational exposure to benzene levels equal to the TLV-TWA of ACGIH. Indeed, this phenomenon is growing proportionally as environmental exposure to benzene declines (Pezzagno et al., 1999). In 8 urine samples observed in the present population the concentrations of *t,t*-MA were above the upper limit of the reference range for the Italian population, equal to 15.2-163.1 µg/g creatinine (5^{th}-95^{th} percentile), recently defined in a multicentric national study (Aprea et al., 2010). However, the concentrations observed seem to be in line with the reference values for male subjects subdivided by smoking habit, since only 1 smoker and 3 non smokers had values exceeding the 95^{th} percentile established for the relative group (274.7 µg/g creatinine and 117.8 µg/g creatinine, respectively).

The concentrations of *t,t*-MA, unlike those of the other markers of internal dose studied, were nearly always above the LOD, even in the non smokers. Moreover, the highest concentration, 734.0 µg/g creatinine, that is, in fact, higher than the BEI of 500 µg/g creatinine recommended by the ACGIH to control occupational exposure, was observed in a non smoker without exposure to urban traffic. This result could be attributed to the intake of sorbic acid in the diet. However, assessment of the contribution of sorbic acid to the *t,t*-MA levels excreted in the population studied was not possible because no data were available on the population's diet in the days before the study. Despite the reduced specificity of *t,t*-MA, cigarette smoke can condition urinary excretion of this marker, that shows a dependency relation on the number of cigarettes/day characterized by a R^2 of 0.22. This finding is in agreement with those by Pezzagno et al. (1999), who estimated that only 25% of urinary *t,t*-MA can be attributed to the biotransformation of benzene.

This dependency of urinary *t,t*-MA on the dietary intake of sorbic acid and the quantity of benzene absorbed in cigarette smoke explains the absence of a relation between t,t-MA and the concentrations of airborne benzene, and hence the poor validity of this marker as a means of monitoring exposure to very low concentrations of benzene like those observed in this study. Nevertheless, a significant correlation was found, when considering only the non smokers, between urinary *t,t*-MA and the time spent in urban traffic, in agreement with the report by Bergamaschi et al. (1999).

Unlike *t,t*-MA, SPMA is a highly specific metabolite of benzene, and in the occupational field it has been shown to be a valid biomarker of exposure to even low concentrations of this toxicant (Hoet et al., 2009). All the SPMA assays made in our sample, both adjusted and non adjusted for creatinine, were below the top limit of the reference values for the Italian population of 0.1-10 µg/L (5^{th}-95^{th} percentile) (Biolind, 2011), even if in 15 smokers and 2

non smokers the concentrations were higher than the 95th percentile of the values recently observed in a population with no occupational exposure resident in a northern Italian city (Milan) (Fustinoni et al., 2011). Cigarette smoke seems to be the only environmental factor causing such a high absorption of benzene as to condition the urinary excretion of SPMA, that was higher than the LOD practically only in smokers, yielding comparable results to those previously observed in a study of a population with no occupational exposure to benzene (Lovreglio et al., 2010). Unlike what was observed in subjects with occupational and non occupational exposure to higher concentrations of benzene, ranging from <3.0 to 66.5 μg/m^3, in which SPMA was found to be dependent not only on the number of cigarettes smoked per day but also on the levels of airborne benzene, in subjects exposed to concentrations of less than 16.3 μg/m^3, as in this study, SPMA was found to be conditioned only by a smoking habit (Lovreglio et al., 2011). Thus, urinary SPMA can be considered a very valid biomarker of internal dose when assessing exposure to low concentrations of benzene or excluding occupational exposure to benzene, but less useful for monitoring exposure to very low concentrations of benzene like those present in the living environment. Instead, thanks to its specificity and high sensitivity, urinary benzene has been proposed as a valid biomarker of even very low concentrations of benzene (Fustinoni et al. 2011). There are still some limits to the use of reference values to interpret the results of urinary benzene because the method for the pre-analytical phase of sample collection has not yet been standardized. This could explain why the top limit of the reference values for the Italian population of 0.05-1.45 μg/L (5th-95th percentile) was exceeded in 14 determinations (Biolind, 2011), all in smoker subjects, whereas comparison with other recent data on a non occupationally exposed population showed values exceeding the 95th percentile only in 7 determinations, again all smokers (Fustinoni et al., 2011). In agreement with the data in literature, cigarette smoking is confirmed to be the main factor conditioning urinary benzene concentrations. In addition, unlike SPMA, it was dosable in 1/3 of the non smokers and showed a higher percentage of determinations above the LOD in subjects exposed to urban traffic than in those with no such exposure. Therefore, urinary benzene seems to be able to reflect even exposure to benzene due to pollution in urban areas, in agreement with what was previously observed in a population with exposure to higher concentrations of benzene attributable to the pollution of urban areas (Fustinoni et al., 2010).

Moreover, urinary benzene was found to be the only biomarker correlated to the concentrations of airborne benzene after excluding the contribution of cigarette smoking, in agreement with the reports by Fustinoni et al. (2010) and Campo et al. (2011). The concentrations of airborne benzene in the latter two studies were higher than those in our study (median values 4.0 and 9.6 μg/m^3 vs. <2.0 μg/m^3). The different trend of airborne benzene–urinary benzene correlations in smokers versus non smokers seems to confirm a confounding effect of the relation between exposure to airborne benzene and the biomarkers of internal dose due to a compounding effect of the benzene absorbed with cigarette smoke. This aspect was already emphasized in our previous experience as an important point to be taken into account when interpreting the results of biological monitoring to assess non occupational exposure to benzene.

5. Conclusion

Overall, our results seem to highlight that even when assessing the risk of non occupational exposure to benzene to exclude the possibility that a given population is exposed to higher

concentrations of benzene than the reference population, biological monitoring with different biomarkers of internal dose can usefully integrate environmental monitoring results. In this sense, the use of reference values can have a great importance, especially if smokers and non smokers are considered separately. In fact, in agreement with the findings in previous studies, cigarette smoking was shown to be the main non occupational source of benzene in smokers, that conditions the urinary excretion of all the biomarkers studied. Moreover, our results seem to confirm that even in conditions of exposure to very low benzene concentrations there is a competitive inhibitory effect of cigarette smoking on the metabolism of this toxicant, that induces proportionally higher concentrations of urinary benzene in smokers than of the metabolites such as *t,t*-MA. This can be explained by the presence of high quantities of chemical substances in cigarette smoke, that are partly metabolized through the same biotransformation pathways as benzene, especially via the oxidation of CYP2E1 (van Vleet et al., 2001).

Among the biomarkers analyzed in this study, urinary benzene seems to be the most sensitive and so the only one with a dependency relation not only on cigarette smoking but also on the time spent in urban traffic, as well as being the only one found to be correlated with the concentrations of airborne benzene, after excluding the effect of cigarette smoking. Thus, urinary benzene can be considered the biomarker of choice for assessing exposure to benzene in the daily environment, especially in terms of the contribution of urban traffic, but it is essential to take a smoking habit into account when interpreting the results of such studies.

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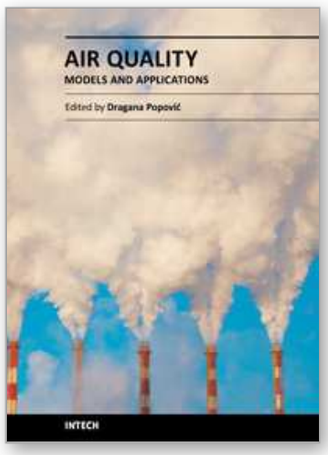

Air Quality-Models and Applications

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Air pollution has been a major transboundary problem and a matter of global concern for decades. High concentrations of different air pollutants are particularly harmful to large cities residents, where numerous anthropogenic activities strongly influence the quality of air. Although there are many books on the subject, the one in front of you will hopefully fulfill some of the gaps in the area of air quality monitoring and modeling, and be of help to graduate students, professionals and researchers. The book is divided in five sections, dealing with mathematical models and computing techniques used in air pollution monitoring and forecasting; air pollution models and application; measuring methodologies in air pollution monitoring and control; experimental data on urban air pollution in China, Egypt, Northeastern U.S, Brazil and Romania; and finally, the health effects due to exposure to benzene, and on the influence of air pollutants on the acute respiratory diseases in children in Mexico.

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