Environmental and biological monitoring of personal exposure to air pollutants of adult people living in a metropolitan area

Andrea Cattaneo[a1], Laura Campo[b1*], Simona Iodice[c], Andrea Spinazzè[a], Luca Olgiati[b], Francesca Borghi[a], Elisa Polledri[b], Laura Angelici[c,d], Domenico Maria Cavallo[a], Silvia Fustinoni[b,c], Valentina Bollati[c]

[*] Corresponding author at Environmental and Industrial Toxicology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via San Barnaba 8, 20122 Milan, Italy

[1]The authors contributed equally to the manuscript

[a]Department of Science and High Technology, Università degli Studi dell'Insubria, 22100 Como, Italy

[b]Environmental and Industrial Toxicology Unit Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, 20122 Milan, Italy

[c]EPIGET Laboratory, Department of Clinical Sciences and Community Health, Università degli Studi di Milano, 20122 Milan, Italy

[d]Department of Epidemiology, Lazio Regional Health Service, ASL Roma 1, Roma, Italy.

Corresponding author:

Laura Campo

Environmental and Industrial Toxicology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico

Via San Barnaba, 8-20122 Milano

Tel. 0039-02-50320116

Mail: laura.campo@policlinico.mi.it

Abstract

Background: Human exposure to air pollutants, and specifically to particulate matter (PM) and volatile organic compounds (VOCs), may pose a relevant risk on human health. **Aim:** To evaluate the personal exposure of adults living and working in Milan (Italy) by environmental and biological monitoring. **Methods**: Personal exposure of 51 volunteer adults to $PM_{2.5}$, $PM_{2.5-10}$ and selected VOCs, including benzene, toluene, ethylbenzene, o-xylene, m+p-xylene, methyl tert-butyl ether, naphthalene, hexane, cyclohexane, heptane, and limonene was assessed along a 24-h period via personal cascade impactors and radial diffusive samplers. Urine spot samples were collected to investigate the corresponding urinary biomarkers. Time-activity patterns were filled in by participants to explore the performed activities. Multiple regression models were applied to investigate the association between personal exposure, biomarker levels, and tobacco smoke, traffic exposure, commuting mode, cooking activities, and personal characteristics. **Results**: Median personal exposure to $PM_{2.5}$, $PM_{2.5-10}$, benzene, toluene, ethylbenzene o-xylene, m+p-xylene, methyl tert-butyl ether, naphthalene, hexane, cyclohexane, heptane, and limonene were 36.1, 7.8, 2.3, 7.8, 2.1, 1.8, 4.7, 0.8, 0.3, 1.4, 2.5, 1.6, and 59.9 $\mu g/m^3$, respectively. Median levels of urinary benzene, toluene, ethylbenzene o-xylene, m+p-xylene, naphthalene, hexane, and heptane were 78.0, 88.1, 21.5, 15.2, 43.9, 21.0, 11.0, and 22.5 ng/L, respectively. For personal exposure, multiple regression models explained up to 67% ($PM_{2.5}$) and 61% (benzene) of variability, with major contribution from commuting mode and environmental exposure. For biological monitoring, multiple regression analysis explained up to 74% of urinary benzene, with a major contribution given by creatinine, and secondary contributions by commuting mode, personal exposure to airborne benzene and smoking. **Conclusions:** Personal exposure to air pollutants was lower than that measured in the past in Milan. Personal exposure was mainly driven by traffic variables, while internal dose was mainly driven by personal characteristics and smoking habit.

Keywords

56 Particulate Matter, Volatile Organic Compounds, Exposure, Biological monitoring, Benzene,
57 Traffic

1. Introduction

Human exposure to air pollutants, and specifically to airborne particulate matter (PM) and some volatile organic compounds (VOCs), may pose a relevant risk on human health (Brook, Rajagopalan et al. 2010, WHO 2010, Cohen, Brauer et al. 2017). A fundamental point in assessing the impacts of air pollution on the general population is evaluating the exposure variations of people residing in different cities (Dockery, Pope et al. 1993, Pope, Ezzati et al. 2009) or in different areas within the same city (Raaschou-Nielsen, Andersen et al. 2013, Beelen, Raaschou-Nielsen et al. 2014).

Differences in exposure for subjects living in urban areas are mainly related to local and personal factors, such as the distance between subjects and local emission points, the time spent in microenvironments with specific sources, the time-activity patterns (Buonanno, Stabile et al. 2014, Spinazze, Cattaneo et al. 2015), the personal behaviors and lifestyle (Broich, Gerharz et al. 2011), as well as demographic and socio-demographic factors (Spinazzè, Cattaneo et al. 2014).

Indoor environments (house, office, etc.) also play a crucial role in contributing to the total daily exposure or dose, more than anything else, for the high proportion of time spent there (Morawska, Afshari et al. 2013). Indoor PM levels derive from different sources, including the infiltration from outdoors, cooking activities, tobacco smoke, wood burning and indoor photochemistry (Urso, Cattaneo et al. 2015). Indoor VOCs may derive from the infiltration of outdoor air, building material emissions, photochemical reactivity (e.g. ozone-initiated reactions) and human activities (e.g. the use of cleaning products) (Campagnolo, Saraga et al. 2017). It should also be noted that VOCs commonly reach higher indoor concentrations compared to the corresponding outdoor values (Mandin, Trantallidi et al. 2017).

In weekdays, adults working in metropolitan areas typically spend part of the day commuting (Tan, Roth et al. 2017). During commuting, the proximity to traffic-related sources is one of the major determinants of exposure to PM and VOCs. For some pollutants, as PM and benzene, the exposure to traffic exhausts depends on mode of transport, time of the day, and source characteristics (Adams, Nieuwenhuijsen et al. 2001, Rank, Folke et al. 2001, Spinazze, Cattaneo et al. 2013, Spinazze, Cattaneo et al. 2015, Borghi, Fanti et al. 2020, Borghi, Spinazzè et al. 2020). Despite the relatively limited amount of time (6-8%) usually spent by individuals in these environments (Kaur, Nieuwenhuijsen et al. 2007), they can greatly contribute to the total daily exposure (Buonanno, Fuoco et al. 2013, Spinazzè, Cattaneo et al. 2014),

As the spatial and temporal variability of exposure can be very high, the personal sampling of airborne pollutants in the breathing zone of individuals provides the best estimate of the actual exposure

especially in heterogeneous environments and for small, specific and selected sub-populations, while the use of other proxies of exposure (i.e. fixed monitoring stations) may be inadequate for an accurate assessment of risks for human health.

Biological monitoring, that is the monitoring of exposure to toxic pollutants and their effects in the biological fluids of exposed subjects, is a valuable tool in exposure assessment as it allows assessing the total absorbed (internal) dose of xenobiotic chemicals taking into account not only individual characteristics such as age, sex, and physiological conditions, but also the different sources of exposure deriving from the environment and personal habits (i.e. cigarette smoking and diet). In particular, the quantification of chemicals in urine has been shown as a valid tool to obtain profiles of exposure at low environmental levels using a non-invasive matrix (Fustinoni, Rossella et al. 2010).

The purpose of this study was to evaluate the personal exposure of a subset (n=51) of adult people living and working in Milan (Italy), one of the most urbanized and air-polluted metropolitan areas in Europe, by environmental and biological monitoring. The environmental monitoring was focused on determining the personal exposure to $PM_{2.5}$ (i.e. airborne particles with aerodynamic diameters below 2.5 μm), $PM_{2.5-10}$ (i.e. airborne particles with diameters between 10 and 2.5 μm, representing the coarse fraction of PM_{10}) and some selected VOCs (benzene, toluene, ethylbenzene, xylenes, methyl tert-butyl ether, naphthalene, hexane, cyclohexane, heptane, and limonene) of individuals, while performing their usual activities along a 24-h period. Urine spot samples were collected at the beginning and at the end of the monitored period to investigate the corresponding urinary biomarkers. The study is based on the analysis of time-activity patterns and is then intended to provide also an updated 24-h picture of the main determinants of personal exposure to air pollutants in a highly populated urban area.

To our knowledge, literature studies on exposure assessment of urban populations to particulate and vapor pollutants using a combined environmental and biological approach are very limited and this study is the first investigating the association of several contributing factors on such a broad spectrum of environmental and urinary VOCs.

2. Materials and Methods

2.1 Study design and study population

The study population consisted of 51 individuals, living and working in the metropolitan area of Milan (Bonzini, Pergoli et al. 2017). The subjects were monitored in the period November 2014-March 2015. The subjects were recruited in the frame of the Sphere project (http://users.unimi.it/sphere) (Bollati, Iodice et al. 2014). The inclusion criteria were living and/or working in the metropolitan area and being not occupationally exposed to the investigated chemicals. Recruited subjects were workers with sanitary, administrative, or research jobs. The project was approved by the Ethical Committee of the University of Milan. Written informed consent was collected from each participant at the study. Each subject was asked and trained to wear personal sampling devices to measure their personal exposure to airborne VOCs, $PM_{2.5}$ and $PM_{2.5-10}$ during a 24-h period, beginning at 9 a.m. (day 1). On the following day at 9 a.m. (day 2), each subject returned the sampling device at the study center. During the 24-h personal sampling period, subjects were invited to perform their routine occupational and leisure activities and to continue their habits. No restriction to personal behavior during the sampling time was prescript to the subjects, including smoking.

2.2 Questionnaires and time-activity diary

Data regarding personal characteristics and habits were collected by two self-administered questionnaires that were checked by trained interviewers at the end of the monitoring period. The first questionnaire was aimed to collect detailed personal data, including anthropometric characteristics, education, area of residence, perceived traffic near home, job position and location, present and past smoking habits, and environmental tobacco exposure (ETS). The second questionnaire was aimed to collect specific information about petrol vapor exposure at filling stations and both active and passive tobacco smoke exposure during the investigated 24-hours.

Each participant completed a time/activity diary during the monitoring day. The recorded data included details on time activity patterns (time spent working, commuting, cooking, performing home activities, time spent in crowded places, leisure time), as well as specific activities that could affect exposure levels (e.g., number of printed pages at office), commuting mode (i.e., on foot, by tram, bus, car, bike, and motorbike), and the number of cigarettes smoked during the monitored period.

2.3 Personal air sampling and analysis

2.3.1 Personal airborne VOC exposure

Personal exposure to airborne benzene (BEN-A), toluene (TOL-A), ethylbenzene (EtBen-A), o-xylene (o-XYL-A), m+p-xilene (m+p-XYL-A) (all together BTEX), naphthalene (NAP-A), methyl tert-butyl ether (MTBE-A), hexane (HEX-A), cyclohexane (CYHEX-A), heptane (HEP-A), and limonene (LIM-A) was monitored during a 24-h period (starting at 9:00–9:30 a.m. at day 1). Air was sampled using the passive sampler Radiello equipped with a 35-50 mesh charcoal cartridge (Supelco, Sigma-Aldrich, Milano, Italy). Subjects wore the sampler in the respiratory zone. At the end of the sampling period, the cartridge was sealed in the proper glass tube, and kept in a clean box at room temperature until analysis, which occurred within 30 days from the collection, according to the manufacturer's instruction.

Airborne VOCs were measured by gas chromatography coupled to mass spectrometry (GC-MS) (Fustinoni, Rossella et al. 2010). Quantification limit (LOQ) was 8 μg/L for all analytes. Considering the average sampling time and the uptake rates of each analyte, this concentration was estimated to correspond to airborne levels of 0.5 $\mu g/m^3$ for all the analytes. Detailed information regarding VOC analysis and quantification is reported in the supplemental material.

2.3.2 Personal PM exposure

The subjects' personal exposure to PM (24-h average exposure) was monitored simultaneously with VOCs. PM sampling was performed by a personal cascade impactor sampler (PCIS; SKC Inc., Eighty Four, PA, USA) used in combination with personal sampling pump (Leland Legacy; SKC Inc., Eighty Four, PA, USA) at a calibrated flow rate of 9 L/min. PCIS is a miniaturized impactor that was developed for the sampling of size-fractionated PM ($PM_{0.25}$, $PM_{0.5}$, PM_1, $PM_{2.5}$, PM_{10}) (Misra, Singh et al. 2002). In the present study, the original configuration of PCIS was modified (by excluding the 1.0, 0.5 and 0.25 μm stages), to obtain a two-stage device, which allowed the sampling of $PM_{2.5}$ and $PM_{2.5-10}$. A good level of agreement (Pearson correlation coefficients were 0.940 with $p<0.001$ and 0.963 with $p<0.001$ for $PM_{2.5}$ and PM_{10}, respectively) between measurements performed simultaneously with the original and the modified PCIS, thus allowing the performances of these two devices to be classified as comparable, was recently proven (Spinazze, Fanti et al. 2017). More detailed information regarding the PM sampling and gravimetrical analysis methods is reported in the supplemental material.

2.4 Environmental VOC and PM levels

The Regional Agency for Prevention and Environment of Lombardy (ARPA) routinely monitors atmospheric pollution levels using a network of fixed monitoring stations in the urban environment, with daily averaged measurements being made available upon request. For the purposes of this study, daily concentrations of PM_{10}, $PM_{2.5}$, and benzene were obtained from the ARPA regional air quality monitoring network, and in particular from the three measurement stations located in downtown Milan (Milano Senato, Milano Verziere, Milano Zavattari). These stations were selected as a proxy of outdoor air contamination in the center of Milan, as most of the study subjects (78%) resided and/or worked within 2 km from the city center. The hypothesis was that urban PM and VOC concentrations could roughly represent the contribution of outdoor contamination to personal PM exposure. For each subject, environmental exposure to benzene and PM measured by air monitoring stations (average daily concentrations measured at the three stations at day 1) were then included among the possible determinants of subjects' personal exposure.

2.5 Urine sample collection and analysis

Urine spot samples were collected immediately before the beginning of air sampling at day 1 (BS) and within 10 min of the end of the air sampling at day 2 (ES). Urine was collected in disposable polyurethane bottles, and then a 7 ml aliquot was immediately poured into a pre-evacuated storage vials for the determination of urinary VOC using a disposable syringe (Fustinoni, Mercadante et al. 2007). A 20 ml aliquot was stored in a polyethylene tube for cotinine analysis, to measure smoking habit, and for creatinine analysis. After collection, the specimens were immediately stored at −20 °C and analyzed, according to their stability, within 60 days.

Urinary benzene (BEN-U), toluene (TOL-U), ethylbenzene (EtBen-U), m+p-xylene (m+p-XYL-U), o-xylene (o-XYL-U), naphthalene (NAP-U), hexane (HEX-U), and heptane (HEP-U) were determined by headspace solid-phase microextraction (HS-SPME) followed by GC-MS analysis according to published methods (Fustinoni, Giampiccolo et al. 1999, Fustinoni, Rossella et al. 2010) with some modifications (Fustinoni, Campo et al. 2010). The LOQ was 10 ng/L for all analytes. Detailed information regarding urinary VOC analysis and quantification is reported in the supplemental material.

Urinary cotinine (COT-U), a biomarker of tobacco smoking, was measured in BS and ES samples by LC-MS-MS (Fustinoni, Campo et al. 2013) and the mean value was obtained. The LOQ was 0.1 µg/L. Subjects with mean COT-U ≥ 30 µg/L were classified as smokers, subjects with COT-U in the 1.8 -

30 µg/L range as passive smokers, and subjects with COT-U ≤ 1.8 µg/L as non-smokers (Campo, Polledri et al. 2016). The use of the mean value of COT-U to classify subjects is supported by the relatively long half-life of urinary cotinine (6-22 hours) that makes this biomarker quite stable along the day in daily smokers (Benowitz, Jacob et al. 2002). The CV% of mean cotinine values in smokers was 14.4%.

Urinary creatinine (crt) was determined using Jaffe's colorimetric method (Kroll, Chesler et al. 1986). No criteria of acceptability based on urine dilution was applied.

2.6 Statistical analysis

Statistical analysis was performed using the SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA). The raw values calculated from the integration of analytical peaks were used unchanged instead of applying substitution methods (e.g. using fractions of the quantification limit) to avoid substantial bias by substitution (Helsel 2006). The arithmetic mean of two determinations (BS and ES) was used to calculate the average level of urinary chemicals for each subject, and then used to describe each subject's exposure as this parameter reflect better the body burden of these compounds in comparison with each of the single measurement. For descriptive analysis, results are presented as percentiles of the distribution expressed as $\mu g/m^3$ for environmental data and ng/L for urinary data. For further statistical analysis, data of airborne chemicals and urinary biomarkers were decimal log-transformed except for $PM_{2.5-10}$ that was normally distributed. Student's t-test was applied to compare two independent groups (i.e., smokers vs. nonsmokers). Pearson's correlations were used to measure the associations between quantitative variables. A p value <0.05 was considered statistically significant.

Preliminary univariate regression analyses were performed to identify covariates that may exert an effect on external and internal pollutant concentrations. The following variables (as declared in questionnaire), were tested as dichotomic variables: ETS exposure, commuting on foot, by tram, bus, car, metro, train, bike, and motorbike, using gas cooker, using oven, cooking by boiling, by frying, and by grilling. The following variables were tested as 3-category variables: traffic near home (scarce, normal, and high) and smoke classification by U-COT (smoker, ETS exposed, and non-smoker). The following variables were tested as continuous variables: smoke exposure as COT-U (µg/L), time (minutes) spent commuting, cooking, printing in office, and in crowded places. A p-value of <0.1 was chosen as cut-off for inclusion in the following multivariate models including all possible determinants of exposure or internal dose.

Finally, two multiple regression models were used: the first, investigating the association between personal exposure to the selected urban air pollutants (log-transformed except for $PM_{2.5-10}$, $\mu g/m^3$) taken as dependent variable and the covariates selected in the preliminary univariate analysis, the second investigating the effect of personal environmental exposure and covariates of traffic exposures on the urinary level of a specific pollutant (log-transformed, ng/L), taken as dependent variable. In the second regression model, urinary creatinine (log-transformed, g/L), smoking habit (log COT-U, μg/L), age (years), gender (male=0, female=1), and body mass index (Kg/m^2) were also introduced as covariates, independently from their significance. The use of creatinine as independent variable in the multiple regression model allows the urinary analyte concentration to be adjusted appropriately for urinary creatinine and the statistical significance of other variables in the model to be independent of effects of creatinine concentration (Barr, Wilder et al. 2005). For multivariate models, results were expressed as determination coefficient (R^2) and significance, while for each predictive variable, estimates (β) and significance were reported.

For each model, the percentage increase of the dependent variable was calculated from the beta values as follows: the regression slopes of the resulting linear equations were anti-log10 converted to obtain the geometric mean ratio (GMR). The (GMR-1) x 100 gives the percentage increase or decrease of a selected variable (i.e. $PM_{2.5}$ or BEN-A) for each 10-fold increase of each continuous covariates (i.e. commuting time).

3. Results

3.1 Study population

Table 1 reports the main characteristics of the study population. Of the 51 participants, 21 were males (41%), the mean age was 49 (min 38-max 73) years, and the mean BMI was 24.4 (18.6-29.6) kg/m^2. As one subject refused to wear the PM sampling apparatus, data for PM were available for 50 individuals, while data for VOC were available for the entire population.

Based on the cotinine excretion value, 10 subjects were classified as active smokers, 11 as ETS-exposed, and 30 as non-smokers. In the questionnaire, 10 subjects self-classified as active smokers and 11 as ETS-exposed. A complete agreement was found between the self-classification of active smokers by questionnaires and that based on the cotinine value. For ETS, the self-classification did not agree with the cotinine-based classification: among 11 subjects who self-classified as ETS, only 3 were confirmed by the COT-U values, while 8 subjects who did not claim ETS exposure actually had COT-U higher than the cut-off value. Smokers declared to smoke a mean (min – max of 10.5 (1

– 30)) cigarettes/day and to have smoked a mean number of cigarettes of 12.3 (2–30) during the monitored period. Among smokers, the median (5^{th}-95^{th} percentile) cotinine excretion was 2211 (48–4216) μg/L.

Most of the subjects (59%) lived in the suburban area (Figure S1) and 67% of subjects worked in the urban area of Milan during the sampling day. Based on the time/activity diary, subjects spent, on average, 126 (10-360) minutes commuting, 26 (0-340) minutes in crowded places, and 16.4 (0-60) minutes cooking at home. Most of the subjects used multiple commuting modes of which travelling by metro, on foot, by car, bike or bus were predominant. Only few subjects (< 10) travelled by train, tram or motorbike. Also for cooking, subjects used multiple type of cooking, of which gas cooking and boiling were predominant.

3.2 Personal exposure to PM and VOC (environmental monitoring)

The levels of personal and environmental exposure to VOC and PM are reported in Table 2.

As regards personal exposure to VOC, data were above the LOQ in, at least, 80% of sample for all analytes but MTBE-A (67% above the LOQ) and NAP-A (43%). Limonene was characterized by the highest median levels (59.9 μg/m^3), followed by toluene (7.8 μg/m^3). Median personal exposure to benzene was 2.3 μg/m^3, only two individuals had benzene exposures above 5 μg/m^3 (6.0 and 10.1 μg/m^3, respectively). The median personal exposure to benzene was lower than the environmental levels measured by the fixed monitoring stations (median 4.2 μg/m^3).

Personal exposure to PM resulted in median levels of 36 and 7.8 μg/m^3 for $PM_{2.5}$ and $PM_{2.5-10}$, respectively. The results of the personal exposure measurements were comparable with the environmental concentrations of $PM_{2.5}$ (34 μg/m^3) and $PM_{2.5-10}$ (12 μg/m^3), with slightly higher values for fine particles and slightly lower levels for coarse particles.

3.3 Urinary levels (biological monitoring)

Urinary BTEX and VOC results are summarized in Table 2. Urinary samples were available for all subjects. Analytes were above the LOQ in at least 98% samples for BTEX-U, and NAP-U, while 59 and 51% of samples were above the LOQ for HEP-U and HEX-U.

Higher levels were found in smokers than in non-smokers for most analytes (BEN-U, p=0.083; m+p-XYL-U, p=0.040; o-XYL-U, p=0.001; EtBen-U, p=0.042; HEX-U, p=0.052). No differences between smokers and non-smokers were found for TOL-U, NAP-U and HEP-U.

3.4 Pearson's correlations

Significant correlations were found among variables describing personal exposure (Table S1). All BTEX-A, but EtBen-A were positively correlated each other ($0.727<r<0.791$; $p<0.001$); aliphatic hydrocarbons were correlated each other ($0.242<r<0.660$; $0.001<p<0.1$) but not with BTEX-A; MTBE-A was correlated with BEN-A and TOL-A ($r=0.298$ and $r=249$, respectively; $0.001<p<0.1$) and with all the aliphatic hydrocarbons ($0.325<r<0.675$; $p<0.05$); NAP-A was correlated only with HEX-A ($r=0.362$; $p<0.05$), while LIM-A was not correlated with any compound.

$PM_{2.5}$ and $PM_{2.5-10}$ were positively, but not significantly, correlated ($r=0.205$, $p>0.1$). $PM_{2.5}$ was correlated only with BEN-A and m+p-XYL-A ($r = 0.389$ and 0.280, respectively; $p<0.05$), while $PM_{2.5-10}$ was correlated only with m+p-XYL-A ($r=0.266$; $p<0.1$).

Considering personal exposure and fixed-station measurements, high correlations were found for benzene and $PM_{2.5}$, with Pearson's r 0.880 ($p<0.001$) and 0.761 ($p=0.02$), respectively, but a low correlation for $PM_{2.5-10}$ ($r=0.241$, $p=0.533$).

Correlations between urinary biomarkers were significant for BTEX-U, with Pearson's r ranging from 0.577 (BEN-U vs. TOL-U), to 0.846 (EtBen-U vs. m+p-XYL-U) ($p<0.001$). NAP-U was correlated only with HEP-U ($r = 0.243$, $p<0.1$), HEX-U was positively correlated to m+p-XYL-U and o-XYL-U ($r=0.307$ and $r=260$, respectively, $p<0.1$) (Table S2).

In all subjects, BEN-U, EtBen-U, HEP-U levels correlated positively with the respective airborne levels ($r= 0.370$, $p=0.007$; $r= 0.293$, $p=0.037$; $r= 0.633$, $p<0.001$, respectively); moreover, some positive correlations were observed between some urinary biomarkers and the different airborne pollutants (TOL-U vs. EtBen-A, $r=0.401$; m+p-XYL-U vs. EtBen-A, $r=0.368$; o-XYL-U vs. EtBen-A, $r= 0.282$; HEX-U vs.. EtBen-A, $r=0.243$; HEP-U vs. HEX-A, $r=0.484$; HEP-U vs. CYHEX-A, $r=0.245$). BEN-U was also correlated with PM2.5 ($r=0.287$, $p=0.043$) (Table S3).

In all subjects, BEN-U positively correlated with COT-U ($r= 0.508$, $p<0.001$). Significant and positive correlations with COT-U were also found for EtBen-U ($r= 0.298$, $p=0.034$), o-XYL-U ($r= 0.432$, $p=0.002$), m+p-XYL-U ($r= 0.373$, $p=0.007$) and HEX-U ($r= 0.306$, $p=0.029$) (Table S2). Smoking affected the correlations between the airborne and the urinary compounds. When only the non-smokers were considered, stronger correlations were found: BEN-U vs. BEN-A ($r=0.496$, $p=0.001$), EtBen-U vs. EtBen-A ($r=0.488$, $p=0.001$).

3.5 Exposure determinants

The univariate regression analysis among continuous variables showed that time spent in commuting activities was positively associated with personal $PM_{2.5}$ ($\beta = 0.0031$, $p < 0.001$), and BEN-A ($\beta =$

0.0018, p =0.077), and cooking time was associated with $PM_{2.5-10}$ (β = 0.080, p = 0.037), $PM_{2.5}$ (β = 0.0072, p = 0.093), BEN-A (β = 0.0086, p = 0.059), and m+p-XYL-A (β = 0.0015, p = 0.056).

The univariate regression analysis using dichotomic independent variables outlined a significant and positive relationship between secondhand smoking (as declared in the questionnaire) and personal exposure to $PM_{2.5}$ (β = 0.384; p = 0.022) and PM_{10} (β = 0.314; p = 0.043). When evaluating possible associations among routes of transport and personal exposure, cycling resulted to be significantly associated with exposure to BEN-A (β = 0.655; p <0.001), TOL-A (β = 0.712; p < 0.001), m+p-XYL-A (β=0.628; p = 0.006), o-XYL-A (β = 0.494; p = 0.003) and LIM-A (β= 1.00; p = 0.004), and marginally to $PM_{2.5}$ (β = 0.323; p = 0.064). Moving by metro resulted in a significant association with $PM_{2.5-10}$ (β= 3.26; p = 0.008); similarly, commuting by train resulted to be significantly related to exposure to $PM_{2.5}$ (β = 0.500; p = 0.005), $PM_{10-2.5}$ (β = 3.61; p = 0.024) and marginally BEN-A (β = 0.324; p = 0.084). Commuting by tram was associated only with HEP-A (β = 0.834; p = 0.038), while using motorbike was marginally associated with LIM-A (β = 0.994; p = 0.074). When considering personal habits, cooking meals for boiling was related to $PM_{2.5-10}$ (β = 3.46; p = 0.008) and marginally to m+p-XYL-A (β = 0.344; p = 0.094), while frying to $PM_{10-2.5}$ (β = 3.52; p = 0.088). The use of a domestic oven resulted in a positive association with $PM_{2.5}$ (β = 1.06; p = 0.002) and the use of a gas cooker was negatively associated with MTBE-A (β=-0.607, p=0.04).

The univariate regression analysis of subjects classified based on urinary cotinine as active- (COT-U≥ 30 μg/L), passive- (1.8 <COT-U<30 μg/L) or non-smokers (COT-U< 1.8 μg/L) resulted only in a significant and positive association of passive smoking with EtBen-A. No significant association was found between COT-U, used as a continuous variable of smoke exposure, and any variable of personal exposure.

The univariate regression analysis of subjects classified for self-reported traffic volumes (scarce, normal or high) outside home showed a positive association of "normal" traffic conditions with TOL-A, m+p XYL-A, and o-XYL-A.

Table 3 shows the details of the adjusted linear regression models. Multivariate regression models were performed for all analytes, except for HEX-A, CYHEX-A, HEP-A, and NAP-A as these compounds were not associated with any covariates tested in the univariate analysis. Environmental $PM_{2.5}$ was significantly associated with personal exposure to $PM_{2.5}$ (β=0.011, p<0.0001), while environmental benzene was associated with subjects' personal exposure to several VOCs (BEN-A, TOL-A, EtBen-A, and o-, m+p-XYL-A). Passive smoke positively influenced personal Et-Ben-A (β= 0.575, p=0.004) and marginally $PM_{2.5}$ (β= 0.225, p=0.066).The self-reported exposure to traffic

increased TOL-A (normal vs. scarce, β=0.590, p<0.0001) and m+p-XYL-A (normal vs. scarce, β=0.460,p=0.038). Commuting time was associated to increased exposure levels to $PM_{2.5}$ (β= 0.001, p=0.048) and marginally to BEN-A (β= 0.012, p=0.093), cycling increased BEN-A (β= 0.423, p=0.002), TOL-A (β= 0.369, p=0.040), LIM-A (β=0.901, p=0.009) and marginally o-XYL-A (β=0.299, p=0.068), metro increased $PM_{2.5-10}$ (β = 2.91, p=0.007), train increased $PM_{2.5}$ (β= 0.238, p=0.022) and $PM_{2.5-10}$ (β = 3.13, p=0.024), and walking decreased TOL-A (β= -0.294, p=0.032). As regards cooking, boiling food was associated to $PM_{2.5-10}$ (β = 2.88, p=0.017), while using an oven resulted marginally associated with MTBE-A (β=0.532).

As regards urinary biomarkers, in the univariate regression analysis using dichotomic independent variables, commuting by bike was associated with BEN-U (β=0.623, p=0.032), using motorbike was associated with NAP-U (β=0.295, p=0.027) and showed a borderline association with HEX-U (β=0.518, p=0.073) and BEN-U (β=0.833 p=0.062).Using car was associated with NAP-U (β=0.146, p=0.047), and showed a borderline association with BEN-U (β=0.421, p=0.086) and m+p-XYL-U (β=0.105, p=0.092), using tram was marginally associated with BEN-U (β=0.531, p=0.080) and HEP-U (β=0.453, p=0.076), and using train with HEP-U (β=0.486, p=0.056). As for personal habits, using a gas cooker was negatively associated with TOL-U (β=-0.227, p=0.047) and marginally with NAP-U (β=-0.133, p=0.083), using an oven was associated with BEN-U (β=1.08, p=0.033) and marginally with HEX-U (β=0.563, p=0.089), while grilling was associated with m+p-XYL-U (β=0.200, p=0.052).

No significant association was found between secondhand smoking (as declared in the questionnaire) and urinary biomarkers. The univariate regression analysis of subjects classified based on urinary cotinine as active- (COT-U≥ 30 μg/L), passive- (1.8 <COT-U<30 μg/L) or non-smokers (COT-U< 1.8 μg/L) resulted in significant and positive association of active smoking with HEX-U (β=0.425, p=0.037), BEN-U (β=0,931, p=0.002), EtBen-U (β=0.205, p=0.047), m+p-XYL-U (β=0.170, p=0.034), and o-XYL-U (β=0.419, p=0.013). When smoke exposure was tested using COT-U as a continuous variable, positive associations were found between COT-U and again HEX-U (β=0.570, p=0.029), BEN-U (β=0,148, p<0.001), EtBen-U (β=0.028, p=0.033), m+p-XYL-U (β=0.028, p=0.007), and o-XYL-U (β=0.068, p=0.001).The univariate regression analysis among continuous variables showed that cooking time was negatively associated with HEX-U (β=-0.010, p=0.036) and marginally with CYHEX-U (β=-0.004, p=0.081). A borderline significant negative association was found between HEP-U and exposure to high traffic in subjects stratified for traffic exposure (scarce, normal, and high).

Table 4 shows the results of the multivariable regression models for the urinary biomarkers. BMI resulted significantly associated with BEN-U and EtBen-U ($p<0.05$) and marginally with TOL-U, and m+p-XYL-U ($0.05<p<0.1$), creatinine was significantly associated with BEN-U and EtBen-U and marginally with TOL-U, m+p-XYL-U, and o-XYL-U. Urinary cotinine was significantly associated with BEN-U, EtBen-U, m+p-XYL-U, and o-XYL-U. As regards personal exposure, BEN-U was associated with BEN-A ($\beta=0.392$, $p=0.017$), EtBen-U with EtBen-A ($\beta=0.105$, $p=0.087$), and HEP-U with HEP-A ($\beta=0.392$, $p=<0.001$). Commuting by car or by tram was associated with BEN-U ($\beta=0.386$, $p=0.026$, and $\beta=0.452$, $p=0.018$, respectively). Cooking time was negatively associated with HEX-U ($\beta=-0.010$, $p=0.040$).

4. Discussion

In this paper the exposure to PM and several VOCs was assessed by environmental and biological monitoring in a subset (n = 51) of adult people living and working in the metropolitan area of Milan, Italy. Moreover, the determinants of exposure, focusing on traffic exposure and personal habits, were studied. The investigation of a 24-h period gave us the opportunity to obtain a picture of real-life exposure, including both the so-called rush hours and the time spent indoors (office and home) in usual activities.

With the aim of protecting general population health, air quality guidelines (AQG), air quality limits and estimated thresholds for irritating and respiratory effects have been indicated by international agencies. For $PM_{2.5}$, the World Health Organization (WHO) indicates an AQG values of 25 $\mu g/m^3$ (24-h mean) with respect to short-term respiratory and cardiovascular effects (WHO 2006), and an AQG value of 10 $\mu g/m^3$ referred to an annual mean. Anyway, the WHO notes that it is unlikely that any standard or guideline value will lead to complete protection for every individual against all possible adverse health effects of PM, given that effect thresholds have not been identified and a substantial inter-individual variability in exposure and in the response to a given exposure exists (WHO 2006). Therefore, comparison with the WHO AQG should be cautious. In this context, the median 24-h $PM_{2.5}$ exposure level (36.1 $\mu g/m^3$; Table 2) found in this study was above the 24-h AQG and the 95th percentile (85.5 $\mu g/m^3$) was very high compared to this threshold. However, the study was conducted in winter, therefore these results cannot be representative of mean annual concentrations, and thus applied properly for a chronic risk assessment.

Among the investigated VOCs, a special attention should be regarded to benzene, a ubiquitous pollutant with known carcinogenic potential (IARC Group 1) (IARC 2018). Median levels in this study (2.3 $\mu g/m^3$), were lower than 5 $\mu g/m^3$, the level that has been enforced as a mean calendar year limit in the EU from 2010 (European Commission, 2000). Only two individuals were exposed to mean daily concentrations higher than 5 $\mu g/m^3$ (6.0 and 10 $\mu g/m^3$, respectively), showing that individuals may experience high exposure in their every-day life. As already reported for $PM_{2.5}$, a proper risk assessment for benzene cannot be performed due to the mismatch between the sampling period (24-h, in winter) and the reference period of EU limit (1-year).

For naphthalene, a compound classified as possibly carcinogenic to humans (IARC Group 2B) (Anttila, Bhat et al. 2002) the 24-h exposure median level was 0.3 mg/m^3, with a maximum of 3.5 mg/m^3, thus 3- to 30-fold lower than the WHO indoor AQG of 10 $\mu g/m^3$ as annual average (WHO 2010). Despite limonene concentrations were the highest among the investigated VOCs, median

levels (59.9 μg/m^3) were well below the EU Derived No Effect Level (DNEL) of 15 mg/m^3 for protecting the general population from long-term systemic effects via the inhalation pathway. The same holds for the other VOCs, whose levels were well below the specific DNELs for long-term systemic effects (e.g. the 15.0 mg/m³ for ethylbenzene - repeated dose toxicity; 56.5 mg/m³ for toluene - neurotoxicity; 65.3 mg/m³ for *o*-xylene - developmental toxicity / teratogenicity; 53.6 mg/m³ for MTBE - repeated dose toxicity) (ECHA 2020).

Our winter data (Table 2) were compared with those measured by personal monitoring in the last two decades in other countries. Personal exposure to $PM_{2.5}$ was noticeably lower than that measured in winter 2005/2006 gravimetrically and with the same size-selective sampler (median = 62 μg/m^3; (Schlitt, Cavallo et al. 2006)), comparable to that found in a large study in the US (median = 31 μg/m^3; Weisel et al. 2005) and substantially higher than that reported in winter in the US (range = 1.4 - 31 μg/m^3 (Brown, Sarnat et al. 2008)).

Considering studies in the area of Milan, median personal exposure to benzene, toluene, ethylbenzene, xylenes, naphthalene, and MTBE measured in this study were about half or lower than those previously measured in 108 individuals living and working in Milan and surrounding areas in the years 2007-2008 (benzene median 4.0 μg/m^3) (Fustinoni, Rossella et al. 2010), thus showing the decrease of VOC exposure along years. This could be explained in view of some risk management policies implemented in the last few decades either at European or at local level, such as the progressive improvement in vehicle emission standards (Euro3 came into force in 2005, Euro 5 in 2009, Euro 6 in 2014), and the congestion charge introduced in 2012 in the downtown area of Milan.

Personal exposure to benzene was similar to those reported in the U.K. in 2005-2007 (mean 2.2 μg/m^3) (Delgado-Saborit, Aquilina et al. 2011) and higher than those measured in Apulia region, Italy, in 2009 (median <2 μg/m^3) (Lovreglio, D'Errico et al. 2011). Personal levels of other VOCs, such as ethylbenzene, toluene, xylenes, hexane in South Korea and in the U.K. (Son, Breysse et al. 2003, Delgado-Saborit, Aquilina et al. 2011), naphthalene in the U.K. (Delgado-Saborit, Aquilina et al. 2011) and MTBE in the U.S. (Weisel, Zhang et al. 2005), were similar to those reported in literature. For limonene, a higher level was measured in our study than in a study conducted in the U.S. (Weisel, Zhang et al. 2005). To the best of our knowledge, this is the first time that personal exposure to cyclohexane and heptane is reported for urban people.

Multiple linear regression models allowed identifying the most important factors affecting the exposure to air pollutants in study individuals (Table 3). Personal exposure to $PM_{2.5}$ and BTEX was strongly associated with ambient levels of $PM_{2.5}$ and benzene (respectively) measured by fixed

stations located in downtown Milan. This was largely expected, though this association may vary across geographic areas and subpopulations (Monn 2001) also depending on the presence and intensity of $PM_{2.5}$ indoor sources (Urso, Cattaneo et al. 2015) and benzene spatial variability within urban areas (Cocheo, Sacco et al. 2000).

Exposure levels to $PM_{2.5}$ and to benzene, even with a lower significance, were also correlated to the time spent commuting, with an estimated increase of 0.14% and 0.12% $\mu g/m^3$ for each minute, respectively. The association between BEN-A and time spent commuting was in agreement with a previously reported association between personal exposure to benzene and time spent in traffic (Lovreglio, D'Errico et al. 2011).

Commuting by train resulted positively correlated with $PM_{2.5}$, while both metro and train transport modes were significant predictors of personal exposures to $PM_{2.5-10}$ (Table 3). The main explanation given for the high $PM_{2.5}$ and $PM_{2.5-10}$ levels in metro is the release of steel particles from friction phenomena between wheel and rail, wear of brakes, vaporization of metals due to sparking and the resuspension and airborne transport of aerosols due to the so-called "train piston effect", i.e. an intense air flow generated at the front of platforms (Lonati, Ozgen et al. 2011, Colombi, Angius et al. 2013). Other sources of airborne coarse particles inside railway carriages include passenger activities and personal clouds that may be particularly important during rush hours for the high density of occupants. Moving by bike was associated with an increased exposure to VOCs, such as benzene, toluene and limonene, and marginally o-xylene. Many studies have proved that higher exposures to traffic exhausts occur while cycling than walking (Bergamaschi, Brustolin et al. 1999, Kaur, Nieuwenhuijsen et al. 2007, McNabola, Broderick et al. 2008, de Nazelle, Fruin et al. 2012, Ragettli, Corradi et al. 2013). This may depend from the different distance and position with respect to vehicle exhaust pipes, as cyclists are closer and just behind them (Panis, de Geus et al. 2010). This interpretation is also suggested by the lower exposures to toluene monitored during walking.

Traffic volumes near home may also have an impact on indoor contamination by PM and VOCs (Urso, Cattaneo et al. 2015, Campagnolo, Saraga et al. 2017, Spinazzè, Campagnolo et al. 2020). However, this factor was a significant predictor of personal exposure only for toluene and m+p-xylenes (Table 3) owing probably to the fact that the categorical variable collected from questionnaires was self-reported and thus subjective, and not quantitative.

A limited impact of tobacco smoke was observed for the investigated pollutants. Personal exposure was never affected by active smoking and only $PM_{2.5}$ and ethylbenzene levels were affected by ETS. As regards cooking activities, only boiling was associated with an increase of $PM_{2.5-10}$ exposures.

Boiling is a predominant activity in the traditional Mediterranean cooking compared to other cooking modes, typically responsible of high emission of aerosols indoor such as frying and grilling (Olson and Burke 2006). Consistently, boiling activities were reported by 16 subjects, while frying and grilling only by 5 and 4 subjects, respectively. Boiling emits high amounts of salted water vapor and very low amounts of solid particles into the indoor atmosphere.

Multiple regression models explained up to 67% and 61% of variability for personal $PM_{2.5}$ and benzene, respectively, with major contribution given by commuting mode and environmental exposure (Table 3). For the other VOCs, the percentage of explained variability was lower (range 12 - 49% for MTBE and toluene, respectively), but with similar contribution by covariates of commuting and traffic exposure, suggesting that for these pollutants the models did not include the principal sources of exposure. As a matter of facts, other factors supposedly affecting VOC exposure which could contribute to explain the variability not captured by the proposed models, were not included in the analysis, including the refueling at petrol stations, traffic conditions at the work site and along the routes used for commuting, flooring at home and in the office, furnishing, cleaning agents, use of air fresheners and cosmetics, recent renovation, and dampness (Schlink, Thiem et al. 2010, Delgado-Saborit, Aquilina et al. 2011).

Biological monitoring of VOC exposure outside industrial settings has been very limited in the literature, and in particular, data on urinary VOCs are not readily available. Median levels of BTEX-U and NAP-U were lower than those we previously measured in Milan and surrounding areas (i.e. median BEN-U 122 ng/L in 2007 vs. 78.0 ng/L in the present study) (Fustinoni, Rossella et al. 2010), consistently with the environmental monitoring. BEN-U levels were also lower than those measured in 2013 in Cyprus (median 118 ng/L) (Tsangari, Andrianou et al. 2017) and in Sardinia (Italy) in 2006-2007 (median 99 ng/L) (Campagna, Satta et al. 2014), and similar to those found in Apulia region (Italy) in 2009 (median 80 ng/L) (Lovreglio, D'Errico et al. 2011). Levels of HEX-U were lower than those reported in two pioneering studies conducted in Italy in the early ’90 (median 39 and 550 ng/L) (Perbellini, Pasini et al. 1988, Brugnone, Perbellini et al. 1994), while to the best of our knowledge, this is first time that data on HEP-U are reported for urban people.

Urinary biomarkers, and in particular BEN-U, EtBen-U, and HEP-U were significantly associated with their respective airborne levels, with r= 0.370, 0.293, and 0.633, respectively. For BEN-U the association here found was similar to those found previously (r=0.329) (Fustinoni, Rossella et al. 2010). In general, the different biomarkers were well correlated among each other, pointing to the same source of exposure. Considering the low air and biomarker levels here found, it is expected that multiple confounders may affect their relationship, thus leading to low correlation coefficients. As a

source of variability, it should be considered that the personal air monitoring and the biological monitoring insisted on a different time-frame, i.e. the geometric mean of the BS (related to the exposure before the starting of the air monitoring) and ES determinations have been considered for the biological indicators, while the personal air sampling was performed for the 24 hours between these two collecting times. However, this study design was based on the reasonable assumption that the general population exposure is roughly constant along the days. Tobacco smoke is a known source of several chemicals and the results of this study confirm that it is a relevant contributor to urinary VOCs and in particular to BEN-U. Consistently, positive correlations were found between some urinary VOCs and COT-U, stronger association were found between airborne levels and urinary biomarkers in non-smokers, and finally, smoke was a significant determinant of BEN-U, EtBen-U, and XYL-U in multiple regression models (Table 4).

The association found in the univariate statistics between several biomarkers and the different commuting mode were confirmed by the multiple regression analysis only for BEN-U and using car (β=0.386, p=0.026) or tram (β=0.452, p=0.018). This shows that, among the investigated biomarkers, BEN-U is the most reliable biomarker of traffic exposure. Indeed, associations between BEN-U and different surrogates of traffic exposure were previously reported, such as time spent in urban traffic (Lovreglio, D'Errico et al. 2011), and residence in urban areas (Campagna, Satta et al. 2014).

However, the multiple regression models highlighted that personal characteristics played a major role on urinary VOC excretion. A positive association was found between BTEX-U and both urinary creatinine and BMI, confirming our previous observations (Fustinoni, Rossella et al. 2010, Campo, Cattaneo et al. 2011, Fustinoni, Campo et al. 2012).

The models used in multiple regression analysis allowed explaining up to 74% of BEN-U variability, with contribution given by personal characteristics, smoking habit, commuting mode, and personal exposure to airborne benzene. For the other VOCs, the percentage of explained variability was lower (range 21-47%, for HEX-U and HEP-U, respectively), suggesting that relevant sources of exposure to these pollutants were not identified, similarly to what observed for the respective airborne chemicals.

A limitation of this study is that many covariates used to identify traffic exposure and personal habits were not quantitative and were described as categorical variables. Moreover, some covariates, such as self-reported traffic exposure or ETS exposure, are affected by subjectivity, possibly leading to the observed low variability explained by multiple regression models. However, it should be considered that questionnaire and time-activity diary are low-cost and simple tools that enable collecting a lot of

information at a time. This allowed us to test several variables in the regression models and for some of them, such as smoking, different descriptors were used, comparing self-reported active and passive smoke and biological measurements. Another limitation is the study period (winter) that is not representative of the whole year. This may affect the concentration of air pollutants as well as some personal habits, such as the commuting mode. This does not allow a proper evaluation of the exposure on a long-range scale, but it was chosen to represent the worst case, as winter is the most polluted period of the year in the study area (Masiol, Squizzato et al. 2017). Finally, the low number of the enrolled individuals may have limited our ability to explain the variability of the investigated pollutants and their associated biomarkers. Participants were recruited on a volunteer base and not by a randomized design, so they could be not representative of the general population living in Milan and in the surrounding area.

A very limited number of studies are present in literature dealing with general population exposure to particulate and vapor urban pollutants via personal exposure monitoring, and even less using a combined environmental and biological approach, such that used in this study. To our knowledge, this is the first time that such a broad spectrum of environmental and urinary VOCs has been investigated in the general population and interpreted in the light of several contributing factors.

In summary, this study shows that the personal exposure to a range of pollutants was lower than that measured in the past in Milan and in the surrounding area. Personal exposure was mainly driven by traffic variables, including commuting mode, commuting time, and traffic near home. On the other hand, internal dose was mainly driven by personal characteristics and smoking habit. The obtained information can be used to better understand the complex of exposure sources in real environments, for a science-based selection of effective interventions (i.e. choice of commuting routes, change of personal habits) for risk mitigation.

Acknowledgments

We greatly thank Luca Del Buono for the skillful help in PM sampling and laboratory work.

Funding

Valentina Bollati received support from the EU Programme “Ideas”, European Research Council (ERC-2011-StG 282413).

Captions for Tables

Table 1. Summary of selected characteristics of the study subjects.

Table 2. Subjects' personal and environmental exposure to airborne VOCs and PM (24-h sampling), and VOC urinary levels (as geometric mean of two determinations).

Table 3. Results (estimates - β) of multiple linear regression models for personal exposure to air pollutants ($\mu g/m^3$): only the associations significant in the univariate analysis were tested in the multiple linear regression models. Results are reported for significant associations (β values are shown only below the 0.10 significance level) and not significant associations (cells with the "ns" notations). Blank cells correspond to associations not tested in the multiple regression models, as they were not significant in the univariate analysis.

Table 4. Results (estimates - β) of multiple linear regression models for urinary excretion (ng/L) of air pollutants: only the associations significant in the uni-variate analysis were tested in the multiple linear regression models. Results are reported for significant associations (β values are shown only below the 0.10 significance level) and not significant associations (cells with the "ns" notations). Blank cells correspond to associations not tested in the multiple regression models, as they were not significant in the univariate analysis.

REFERENCES

Adams, H. S., M. J. Nieuwenhuijsen, R. N. Colvile, M. A. McMullen and P. Khandelwal (2001). "Fine particle (PM2.5) personal exposure levels in transport microenvironments, London, UK." Sci Total Environ **279**(1-3): 29-44.

Anttila, A., R. V. Bhat, J. A. Bond, S. J. Borghoff, F. X. Bosch, G. P. Carlson, M. Castegnaro, G. Cruzan, W. C. Gelderblom and U. Hass (2002). "IARC monographs on the evaluation of carcinogenic risks to humans: Some traditional herbal medicines, some mycotoxins, naphthalene and styrene." IARC monographs on the evaluation of carcinogenic risks to humans **82**.

Barr, D. B., L. C. Wilder, S. P. Caudill, A. J. Gonzalez, L. L. Needham and J. L. Pirkle (2005). "Urinary creatinine concentrations in the US population: implications for urinary biologic monitoring measurements." Environmental health perspectives **113**(2): 192-200.

Beelen, R., O. Raaschou-Nielsen, M. Stafoggia, Z. J. Andersen, G. Weinmayr, B. Hoffmann, K. Wolf, E. Samoli, P. Fischer, M. Nieuwenhuijsen, P. Vineis, W. W. Xun, K. Katsouyanni, K. Dimakopoulou, A. Oudin, B. Forsberg, L. Modig, A. S. Havulinna, T. Lanki, A. Turunen, B. Oftedal, W. Nystad, P. Nafstad, U. De Faire, N. L. Pedersen, C. G. Östenson, L. Fratiglioni, J. Penell, M. Korek, G. Pershagen, K. T. Eriksen, K. Overvad, T. Ellermann, M. Eeftens, P. H. Peeters, K. Meliefste, M. Wang, B. Bueno-de-Mesquita, D. Sugiri, U. Krämer, J. Heinrich, K. de Hoogh, T. Key, A. Peters, R. Hampel, H. Concin, G. Nagel, A. Ineichen, E. Schaffner, N. Probst-Hensch, N. Künzli, C. Schindler, T. Schikowski, M. Adam, H. Phuleria, A. Vilier, F. Clavel-Chapelon, C. Declercq, S. Grioni, V. Krogh, M. Y. Tsai, F. Ricceri, C. Sacerdote, C. Galassi, E. Migliore, A. Ranzi, G. Cesaroni, C. Badaloni, F. Forastiere, I. Tamayo, P. Amiano, M. Dorronsoro, M. Katsoulis, A. Trichopoulou, B. Brunekreef and G. Hoek (2014). "Effects of long-term exposure to air pollution on natural-cause mortality: an analysis of 22 European cohorts within the multicentre ESCAPE project." Lancet **383**(9919): 785-795.

Benowitz, N., P. Jacob, K. Ahijevych, M. Jarvis, S. Hall, J. LeHouezec, A. Hansson, E. Lichtenstein, J. Henningfield and J. Tsoh (2002). "SRNT Subcommittee on Biochemical Verification. Biochemical verification of tobacco use and cessation." Nicotine Tob Res **4**(2): 149-159.

Bergamaschi, E., A. Brustolin, G. De Palma, P. Manini, P. Mozzoni, R. Andreoli, S. Cavazzini and A. Mutti (1999). "Biomarkers of dose and susceptibility in cyclists exposed to monoaromatic hydrocarbons." Toxicology Letters **108**(2-3): 241-247.

Bollati, V., S. Iodice, C. Favero, L. Angelici, B. Albetti, R. Cacace, L. Cantone, M. Carugno, T. Cavalleri, B. De Giorgio, L. Dioni, S. Fustinoni, M. Hoxha, B. Marinelli, V. Motta, L. Patrini, L. Pergoli, L. Riboldi, G. Rizzo, F. Rota, S. Sucato, L. Tarantini, A. S. Tirelli, L. Vigna, P. Bertazzi and A. C. Pesatori (2014). "Susceptibility to particle health effects, miRNA and exosomes: rationale and study protocol of the SPHERE study." BMC Public Health **14**: 1137.

Bonzini, M., L. Pergoli, L. Cantone, M. Hoxha, A. Spinazzè, L. Del Buono, C. Favero, M. Carugno, L. Angelici, L. Broggi, A. Cattaneo, A. C. Pesatori and V. Bollati (2017). "Short-term particulate matter exposure induces extracellular vesicle release in overweight subjects." Environ Res **155**: 228-234.

Borghi, F., G. Fanti, A. Cattaneo, D. Campagnolo, S. Rovelli, M. Keller, A. Spinazzè and D. M. Cavallo (2020). "Estimation of the inhaled dose of airborne pollutants during commuting: Case study and application for the general population." International Journal of Environmental Research and Public Health **17**(17): 1-14.

Borghi, F., A. Spinazzè, G. Fanti, D. Campagnolo, S. Rovelli, M. Keller, A. Cattaneo and D. M. Cavallo (2020). "Commuters' personal exposure assessment and evaluation of inhaled dose to different atmospheric pollutants." International Journal of Environmental Research and Public Health **17**(10).

Broich, A. V., L. E. Gerharz and O. Klemm (2011). "Personal monitoring of exposure to particulate matter with a high temporal resolution." Environ Sci Pollut Res Int **19**(7): 2959-2972.

Brook, R. D., S. Rajagopalan, C. A. Pope, J. R. Brook, A. Bhatnagar, A. V. Diez-Roux, F. Holguin, Y. Hong, R. V. Luepker, M. A. Mittleman, A. Peters, D. Siscovick, S. C. Smith, L. Whitsel, J. D. Kaufman and C. o. t. K. i. C. D. American Heart Association Council on Epidemiology and Prevention, and Council on Nutrition, P.ysical Activity and Metabolism (2010). "Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association." Circulation **121**(21): 2331-2378.

Brown, K. W., J. A. Sarnat, H. H. Suh, B. A. Coull, J. D. Spengler and P. Koutrakis (2008). "Ambient site, home outdoor and home indoor particulate concentrations as proxies of personal exposures." J Environ Monit **10**(9): 1041-1051.
Brugnone, F., L. Perbellini, C. Giuliari, M. Cerpelloni and M. Soave (1994). "Blood and urine concentrations of chemical pollutants in the general population." Med Lav **85**(5): 370-389.
Buonanno, G., F. C. Fuoco, L. Morawska and L. Stabile (2013). "Airborne particle concentrations at schools measured at different spatial scales." Atmospheric Environment **67**: 38-45.
Buonanno, G., L. Stabile and L. Morawska (2014). "Personal exposure to ultrafine particles: the influence of time-activity patterns." Sci Total Environ **468-469**: 903-907.
Campagna, M., G. Satta, L. Campo, V. Flore, A. Ibba, M. Meloni, M. G. Tocco, G. Avataneo, C. Flore, S. Fustinoni and P. Cocco (2014). "Analysis of potential influence factors on background urinary benzene concentration among a non-smoking, non-occupationally exposed general population sample." International Archives of Occupational and Environmental Health **87**(7): 793-799.
Campagnolo, D., D. E. Saraga, A. Cattaneo, A. Spinazze, C. Mandin, R. Mabilia, E. Perreca, I. Sakellaris, N. Canha, V. G. Mihucz, T. Szigeti, G. Ventura, J. Madureira, E. D. Fernandes, Y. de Kluizenaar, E. Cornelissen, O. Hanninen, P. Carrer, P. Wolkoff, D. M. Cavallo and J. G. Bartzis (2017). "VOCs and aldehydes source identification in European office buildings - The OFFICAIR study." Building and Environment **115**: 18-24.
Campo, L., A. Cattaneo, D. Consonni, L. Scibetta, P. Costamagna, D. M. Cavallo, P. A. Bertazzi and S. Fustinoni (2011). "Urinary methyl tert-butyl ether and benzene as biomarkers of exposure to urban traffic." Environment International **37**(2): 404-411.
Campo, L., E. Polledri, P. Bechtold, G. Gatti, A. Ranzi, P. Lauriola, C. A. Goldoni, P. A. Bertazzi and S. Fustinoni (2016). "Determinants of active and environmental exposure to tobacco smoke and upper reference value of urinary cotinine in not exposed individuals." Environmental Research **148**: 154-163.
Cocheo, V., P. Sacco, C. Boaretto, E. De Saeger, P. P. Ballesta, H. Skov, E. Goelen, N. Gonzalez and A. B. Caracena (2000). "Urban benzene and population exposure." Nature **404**(6774): 141-142.
Cohen, A. J., M. Brauer, R. Burnett, H. R. Anderson, J. Frostad, K. Estep, K. Balakrishnan, B. Brunekreef, L. Dandona, R. Dandona, V. Feigin, G. Freedman, B. Hubbell, A. Jobling, H. Kan, L. Knibbs, Y. Liu, R. Martin, L. Morawska, C. A. Pope, H. Shin, K. Straif, G. Shaddick, M. Thomas, R. van Dingenen, A. van Donkelaar, T. Vos, C. J. L. Murray and M. H. Forouzanfar (2017). "Estimates and 25-year trends of the global burden of disease attributable to ambient air pollution: an analysis of data from the Global Burden of Diseases Study 2015." Lancet **389**(10082): 1907-1918.
Colombi, C., S. Angius, V. Gianelle and M. Lazzarini (2013). "Particulate matter concentrations, physical characteristics and elemental composition in the Milan underground transport system." Atmospheric Environment **70**: 166-178.
de Nazelle, A., S. Fruin, D. Westerdahl, D. Martinez, A. Ripoll, N. Kubesch and M. Nieuwenhuijsen (2012). "A travel mode comparison of commuters' exposures to air pollutants in Barcelona." Atmospheric Environment **59**: 151-159.
Delgado-Saborit, J. M., N. J. Aquilina, C. Meddings, S. Baker and R. M. Harrison (2011). "Relationship of personal exposure to volatile organic compounds to home, work and fixed site outdoor concentrations." Sci Total Environ **409**(3): 478-488.
Dockery, D. W., C. A. Pope, X. Xu, J. D. Spengler, J. H. Ware, M. E. Fay, B. G. Ferris and F. E. Speizer (1993). "An association between air pollution and mortality in six U.S. cities." N Engl J Med **329**(24): 1753-1759.
ECHA. (2020). Retrieved 10/06/2020, 2020, from https://echa.europa.eu/it/brief-profile/-/briefprofile/100.002.591, from https://echa.europa.eu/it/brief-profile/-/briefprofile/100.003.297, from https://echa.europa.eu/it/brief-profile/-/briefprofile/100.002.203, from https://echa.europa.eu/it/brief-profile/-/briefprofile/100.015.140.
Fustinoni, S., L. Campo, R. Mercadante and P. Manini (2010). "Methodological issues in the biological monitoring of urinary benzene and S-phenylmercapturic acid at low exposure levels." Journal of Chromatography B **878**(27): 2534-2540.
Fustinoni, S., L. Campo, E. Polledri, R. Mercadante, L. Erspamer, A. Ranzi, P. Lauriola, C. A. Goldoni and P. A. Bertazzi (2013). "A validated method for urinary cotinine quantification used to classify active and environmental tobacco smoke exposure." Current Analytical Chemistry **9**(3): 447-456.

Fustinoni, S., L. Campo, G. Satta, M. Campagna, A. Ibba, M. G. Tocco, S. Atzeri, G. Avataneo, C. Flore, M. Meloni, P. A. Bertazzi and P. Cocco (2012). "Environmental and lifestyle factors affect benzene uptake biomonitoring of residents near a petrochemical plant." Environment International **39**(1): 2-7.
Fustinoni, S., R. Giampiccolo, S. Pulvirenti, M. Buratti and A. Colombi (1999). "Headspace solid-phase microextraction for the determination of benzene, toluene, ethylbenzene and xylenes in urine." Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences **723**(1-2): 105-115.
Fustinoni, S., R. Mercadante, L. Campo, L. Scibetta, C. Valla, D. Consonni and V. Foa (2007). "Comparison between urinary o-cresol and toluene as biomarkers of toluene exposure." Journal of Occupational and Environmental Hygiene **4**(1): 1-9.
Fustinoni, S., F. Rossella, L. Campo, R. Mercadante and P. A. Bertazzi (2010). "Urinary BTEX, MTBE and naphthalene as biomarkers to gain environmental exposure profiles of the general population." Science of the Total Environment **408**(14): 2840-2849.
Grammer, L. C., K. E. Harris and P. R. Yarnold (2002). "Effect of respiratory protective devices on development of antibody and occupational asthma to an acid anhydride." Chest **121**(4): 1317-1322.
Helsel, D. R. (2006). "Fabricating data: How substituting values for nondetects can ruin results, and what can be done about it." Chemosphere **65**(11): 2434-2439.
IARC (2018). "IARC Working Group on the Evaluation of Carcinogenic Risks to Humans - Benzene."
Kaur, S., M. J. Nieuwenhuijsen and R. N. Colvile (2007). "Fine particulate matter and carbon monoxide exposure concentrations in urban street transport microenvironments." Atmospheric Environment **41**(23): 4781-4810.
Kroll, M. H., R. Chesler, C. Hagengruber, D. W. Blank, J. Kestner and M. Rawe (1986). "AUTOMATED-DETERMINATION OF URINARY CREATININE WITHOUT SAMPLE DILUTION - THEORY AND PRACTICE." Clinical Chemistry **32**(3): 446-452.
Lonati, G., S. Ozgen, G. Ripamonti, S. Cernuschi and M. Giugliano (2011). "Pedestrian exposure to size-resolved particles in Milan." J Air Waste Manag Assoc **61**(11): 1273-1280.
Lovreglio, P., M. N. D'Errico, S. Fustinoni, I. Drago, A. Barbieri, L. Sabatini, M. Carrieri, P. Apostoli and L. Soleo (2011). "Biomarkers of internal dose for the assessment of environmental exposure to benzene." Journal of Environmental Monitoring **13**(10): 2921-2928.
Mandin, C., M. Trantallidi, A. Cattaneo, N. Canha, V. G. Mihucz, T. Szigeti, R. Mabilia, E. Perreca, A. Spinazze, S. Fossati, Y. De Kluizenaar, E. Cornelissen, I. Sakellaris, D. Saraga, O. Hanninen, E. D. O. Fernandes, G. Ventura, P. Wolkoff, P. Carrer and J. Bartzis (2017). "Assessment of indoor air quality in office buildings across Europe - The OFFICAIR study." Science of the Total Environment **579**: 169-178.
Masiol, M., S. Squizzato, G. Formenton, R. M. Harrison and C. Agostinelli (2017). "Air quality across a European hotspot: Spatial gradients, seasonality, diurnal cycles and trends in the Veneto region, NE Italy." Science of the Total Environment **576**: 210-224.
McNabola, A., B. M. Broderick and L. W. Gill (2008). "Relative exposure to fine particulate matter and VOCs between transport microenvironments in Dublin: Personal exposure and uptake." Atmospheric Environment **42**(26): 6496-6512.
Misra, C., M. Singh, S. Shen, C. Sioutas and P. A. Hall (2002). "Development and evaluation of a personal cascade impactor sampler (PCIS)." Journal of Aerosol Science **33**(7): 1027-1047.
Monn, C. (2001). "Exposure assessment of air pollutants: a review on spatial heterogeneity and indoor/outdoor/personal exposure to suspended particulate matter, nitrogen dioxide and ozone." Atmos.Environ. **35**(1): 1-32.
Morawska, L., A. Afshari, G. N. Bae, G. Buonanno, C. Y. Chao, O. Hänninen, W. Hofmann, C. Isaxon, E. R. Jayaratne, P. Pasanen, T. Salthammer, M. Waring and A. Wierzbicka (2013). "Indoor aerosols: from personal exposure to risk assessment." Indoor Air **23**(6): 462-487.
Olson, D. A. and J. M. Burke (2006). "Distributions of PM2.5 source strengths for cooking from the Research Triangle Park particulate matter panel study." Environ Sci Technol **40**(1): 163-169.
Panis, L. I., B. de Geus, G. Vandenbulcke, H. Willems, B. Degraeuwe, N. Bleux, V. Mishra, I. Thomas and R. Meeusen (2010). "Exposure to particulate matter in traffic: A comparison of cyclists and car passengers." Atmospheric Environment **44**(19): 2263-2270.

Perbellini, L., F. Pasini, G. Faccini, B. Danzi, M. Gobbi, A. Zedde, P. Cirillo and F. Brugnone (1988). "Determinazione di solventi ad uso industriale nel sangue, nell'aria alveolare e nell'urina di un gruppo di donatori di sangue." Med Lav **79**: 460-467.
Pope, C. A., M. Ezzati and D. W. Dockery (2009). "Fine-particulate air pollution and life expectancy in the United States." N Engl J Med **360**(4): 376-386.
Raaschou-Nielsen, O., Z. J. Andersen, R. Beelen, E. Samoli, M. Stafoggia, G. Weinmayr, B. Hoffmann, P. Fischer, M. J. Nieuwenhuijsen, B. Brunekreef, W. W. Xun, K. Katsouyanni, K. Dimakopoulou, J. Sommar, B. Forsberg, L. Modig, A. Oudin, B. Oftedal, P. E. Schwarze, P. Nafstad, U. De Faire, N. L. Pedersen, C. G. Ostenson, L. Fratiglioni, J. Penell, M. Korek, G. Pershagen, K. T. Eriksen, M. Sørensen, A. Tjønneland, T. Ellermann, M. Eeftens, P. H. Peeters, K. Meliefste, M. Wang, B. Bueno-de-Mesquita, T. J. Key, K. de Hoogh, H. Concin, G. Nagel, A. Vilier, S. Grioni, V. Krogh, M. Y. Tsai, F. Ricceri, C. Sacerdote, C. Galassi, E. Migliore, A. Ranzi, G. Cesaroni, C. Badaloni, F. Forastiere, I. Tamayo, P. Amiano, M. Dorronsoro, A. Trichopoulou, C. Bamia, P. Vineis and G. Hoek (2013). "Air pollution and lung cancer incidence in 17 European cohorts: prospective analyses from the European Study of Cohorts for Air Pollution Effects (ESCAPE)." Lancet Oncol **14**(9): 813-822.
Ragettli, M. S., E. Corradi, C. Braun-Fahrlander, C. Schindler, A. de Nazelle, M. Jerrett, R. E. Ducret-Stich, N. Kunzli and H. C. Phuleria (2013). "Commuter exposure to ultrafine particles in different urban locations, transportation modes and routes." Atmospheric Environment **77**: 376-384.
Rank, J., J. Folke and P. H. Jespersen (2001). "Differences in cyclists and car drivers exposure to air pollution from traffic in the city of Copenhagen." Sci Total Environ **279**(1-3): 131-136.
Rodes, C. and J. Thornburg (2005). Breathing Zone Exposure Assessment. Aerosols Handbook: Measurement, Dosimetry, and Health Effects. LS Ruzer, Harley, NH Boca Raton, CRC Press.
Schlink, U., A. Thiem, T. Kohajda, M. Richter and K. Strebel (2010). "Quantile regression of indoor air concentrations of volatile organic compounds (VOC)." Science of the Total Environment **408**(18): 3840-3851.
Schlitt, C., D. Cavallo, M. Taronna, A. Cattaneo, C. Peruzzo, G. Garramone, F. Vercelli, S. Pulvirenti and M. Maroni (2006). "Assessment of personal PM exposure in the PM-CARE project." Epidemiology **17**(6): S531-S531.
Son, B., P. Breysse and W. Yang (2003). "Volatile organic compounds concentrations in residential indoor and outdoor and its personal exposure in Korea." Environ Int **29**(1): 79-85.
Spinazze, A., A. Cattaneo, G. Garramone and D. M. Cavallo (2013). "Temporal Variation of Size-Fractionated Particulate Matter and Carbon Monoxide in Selected Microenvironments of the Milan Urban Area." J Occup Environ Hyg **10**(11): 652-662.
Spinazze, A., A. Cattaneo, D. R. Scocca, M. Bonzini and D. M. Cavallo (2015). "Multi-metric measurement of personal exposure to ultrafine particles in selected urban microenvironments." Atmospheric Environment **110**: 8-17.
Spinazze, A., G. Fanti, F. Borghi, L. Del Buono, D. Campagnolo, S. Rovelli, A. Cattaneo and D. M. Cavallo (2017). "Field comparison of instruments for exposure assessment of airborne ultrafine particles and particulate matter." Atmospheric Environment **154**: 274-284.
Spinazzè, A., D. Campagnolo, A. Cattaneo, P. Urso, I. A. Sakellaris, D. E. Saraga, C. Mandin, N. Canha, R. Mabilia, E. Perreca, V. G. Mihucz, T. Szigeti, G. Ventura, E. de Oliveira Fernandes, Y. de Kluizenaar, E. Cornelissen, O. Hänninen, P. Carrer, P. Wolkoff, D. M. Cavallo and J. G. Bartzis (2020). "Indoor gaseous air pollutants determinants in office buildings-The OFFICAIR project." Indoor Air **30**(1): 76-87.
Spinazzè, A., A. Cattaneo, C. Peruzzo and D. M. Cavallo (2014). "Modeling population exposure to ultrafine particles in a major Italian urban area." Int J Environ Res Public Health **11**(10): 10641-10662.
Tan, S. H., M. Roth and E. Velasco (2017). "Particle exposure and inhaled dose during commuting in Singapore." Atmospheric Environment **170**: 245-258.
Tsangari, X., X. D. Andrianou, A. Agapiou, P. Mochalski and K. C. Makris (2017). "Spatial characteristics of urinary BTEX concentrations in the general population." Chemosphere **173**: 261-266.
Urso, P., A. Cattaneo, G. Garramone, C. Peruzzo, D. M. Cavallo and P. Carrer (2015). "Identification of particulate matter determinants in residential homes." Building and Environment **86**: 61-69.

Weisel, C. P., J. Zhang, B. J. Turpin, M. T. Morandi, S. Colome, T. H. Stock, D. M. Spektor, L. Korn, A. M. Winer and J. Kwon (2005). "Relationships of Indoor, Outdoor, and Personal Air (RIOPA). Part I. Collection methods and descriptive analyses." Research Report (Health Effects Institute)(130 Pt 1): 1-107; discussion 109-127.
WHO (2006). WHO Air Quality Guidelines for Particulate Matter, Ozone, Nitrogen Dioxide and Sulfur Dioxide: Global Update 2005 (Summary of risk assessment), World Health Organization.
WHO (2010). WHO guidelines for indoor air quality: selected pollutants. Copenhagen, World Health Organization, Regional Office for Europe.