Evaluation of atmospheric inputs as possible sources of antimony in pregnant women from urban areas

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ABSTRACT

Antimony and copper are common components of brake linings. The occurrence of these two metals in urban atmospheric aerosols has been related to vehicular use. Urine samples (n = 466) taken during the 32nd week of pregnancy were analyzed for Sb and Cu in pregnant women from an urban area (Sabadell, Catalonia, Spain). The geometric mean levels were 0.28 and 13 µg/g creatinine, respectively. Positive significant associations between urine concentrations of Sb and seasonality, intensity of physical exercise, working activities and traffic intensity at their home streets were observed. Cu showed the same trends but without statistical significance. In both cases, the estimated dietary ingestion of these two metals was larger than the inhalation inputs but the difference was much higher for Cu than for Sb. While Sb has no dietary role, Cu is an essential element which is also incorporated into humans through diet. The results suggest that inhalation of atmospheric particles may also constitute a source of Sb in pregnant women and general population of urban areas.

Key words: antimony, copper, pregnant women, atmospheric pollution, urine studies, urban areas, physical activity, seasonal changes.

1. INTRODUCTION

Atmospheric particles in urban areas have been linked to several health outcomes such as oxidative stress, inflammation, epoc and cardiovascular or cerebrovascular stroke (Perez et al., 2009; Pope and Dockery, 2006). Significant correlations between daily mortality and ambient air particulate matter (PM) have been identified in Barcelona (Ostro et al., 2011). However, urban particles constitute very complex mixtures. Insight on the origin of these deleterious health effects and possible remediation actions depend on the association of specific properties such as size, chemical composition and others to specific toxic outcomes.

Sb is a toxic metalloid that is present in the diet at low concentrations (Arnich et al., 2012). The intestinal absorption of this element in humans is 5-20% (Lauwers et al., 1990). Its high volatility involves high affinity for atmospheric PM which constitutes a potential pollution source of this compound by particle inhalation (Belzile et al., 2012). Few studies on concentrations of Sb in humans are available (Filella et al., 2013a; Filella et al., 2013b) and very limited information is found on prenatal and children exposure but its presence in amniotic fluid has been observed (Caserta et al., 2011).

Cu is an essential metal that is necessary for the function of some enzymes such as ceruloplasmine or cytocrom c oxydase. It is present in a wide variety of foods (Mason, 1979). Besides diet and the gastrointestinal system, it may also be incorporated through respiration (Wiseman and Zereini, 2014). To date, industrial activity is the main source of this metal to the environment but vehicular traffic has also become a potential source because of its current use in brake linings (Amato et al., 2009). This metal is also known for its toxicity at high concentrations, children are more susceptible to deleterious effects than adults (Mason, 1979).

Sb and Cu are present in urban particulate material (Amato et al., 2011). Inhalation of PM may be a source of these metals for human populations. Urine is an adequate matrix for heavy metals biomonitoring (Fort et al., 2014) and can be collected without invasive methods. The concentrations of Sb and Cu in women at any pregnancy stage provide representative results for the whole pregnancy period (Fort et al., 2014). In the present study urban atmospheric pollution is evaluated as potential source of prenatal exposure to these metals.

2. MATERIALS AND METHODS

2.1. Urine samples

A cohort of 657 pregnant women were recruited between 2004 and 2006 in their 12th week medical visit (Primary Care Center II Sant Fèlix of Sabadell, Catalonia) within the INMA research network (INfancia y Medio Ambiente - Childhood and environment) (Guxens et al., 2012). Recruitment conditions were: residence in Sabadell, age higher than 16 years, single pregnancy, volunteering for the program and scheduled birth at the Hospitals of Sabadell or Terrassa (a nearby city). Women suffering from chronic diseases, having communication impairment or pregnancy from assisted reproduction were excluded. After obtaining the consent from the admitted women, questionnaires were administered by trained interviewers in the 12th and 32th weeks of pregnancy. Dietary information was obtained by food frequency questionnaires obtained at both periods.

Pregnant women from this cohort provided a urine sample during the 32nd week of pregnancy (n = 466) which was collected in 100 mL polypropylene containers. The samples were stored in polyethylene tubes at -20ºC until analysis. This study was approved by the Research Ethics Committee of the CREAL. All information on participants was coded to maintain confidentiality.

2.2. Chemical analysis

Aliquots (3 mL) of each urine sample (n = 466) were introduced in Teflon vessels together with 3 mL of Instra-Analysed 65% HNO3 (J.T. Baker, Germany) and 1.5 mL of Instra-Analysed 30% H2O2 (Baker). The vessels were closed and heated at 90ºC in an oven overnight. After cooling, the vessels were opened and placed on a plate heated at 250°C to evaporate the nitric acid. The resulting solid samples were dissolved with 3 mL of 4% HNO3 and stored in 7 mL plastic bottles which were subsequently kept in a refrigerator until instrumental analysis (Castillo et al., 2008; Krachler et al., 1998). This digestion protocol was validated by processing a Bio-Rad Level 1 urine reference sample (Lyphochek Urine Metals Control 1-69131; Marnes-la-Coquette, France) that contains metal concentrations close to those of the urine from our studied population. The resulting inter-assay relative standard deviation coefficients were 17% and 4% for Cu and Sb, respectively. Before analysis, an internal standard of 10 ppb of In was introduced and, depending on sample density, samples were diluted with MilliQ water to 30 mL or 60 mL to avoid non-spectral interference. Instrumental analysis was performed by a Q-ICP-MS X-SERIES II instrument (Thermo Fisher SCIENTIFIC). One MilliQ water blank was processed together with each batch of samples to control for possible contamination. Instrumental limit of detection was 0.2 ng/mL attending to the most reliable lowest calibration point. A concentration of 0.1 ng/mL was stablished for samples under limit of detection for statistical purposes.

All wet-lab material was thoroughly cleaned by soaking in 10% nitric acid for 24 h, which was followed by three rinses of Milli Q water. The Teflon vessels were cleaned after every use by rinsing with 10% nitric acid (three times), then heating in the oven at 90ºC overnight, and finally rinsing with a high amount of Milli Q water.

Creatinine was determined by the Jaffé method (kinetic with target measurement, compensated method) with Beckman Coulter© reactive in AU5400 (IZASA®).

2.3. Statistical analysis

Arithmetic and geometric means, standard deviations (SD), medians, percentiles, minimum and maximum values of Sb and Cu in the studied population groups were calculated for descriptive statistics. Normality was checked by the Kolmogorov-Smirnov test.

Pregnant women included in the study answered to questionnaires regarding lifestyle and environmental exposures as well as food frequency questionnaires, conducted by trained interviewers. Exposure to vehicular traffic in environmental questionnaires was classified in four groups of intensity at home street, namely rare, moderate, frequent and heavy.

Sampling season and physical activity were also considered for their potential influence on atmospheric pollution intake. The former was assigned attending to sampling date and annual distribution of seasons, while the second referred to self-reported total physical activity, classified in three categories, namely sedentary or little active, moderately active and quite or very active. Type of maternal and paternal occupation (manufacturers, non-manufacturers), height of the housing and working time during pregnancy were also considered. The Sb and Cu levels between different categories were assessed by univariate linear regression modeling of the log-transformed concentrations of each metal and the categorical variables. Maternal age, pre-conception BMI, parity (in three categories, namely primiparous, one previous children and two or more previous children), social class, cotinine in urine and weekly consumption of the main groups of dietary items included in the food frequency questionnaires were also tested, as these could be associated with metal concentrations. Finally, stepwise regression was performed for the selection of variables ($p < 0.20$) included in multivariate linear regression. Interactions between car traffic exposure and season, physical activity, working time during pregnancy and height of the housing were assessed.

All statistical analyses were performed using Stata 12.0 and R software packages (Team, 2014).

3. RESULTS

3.1. Characteristics of the population

Median age of the mothers at the time of their last menstrual period was 31 years, ranging between 18 and 42 years (Table 1). The mean BMI of these mothers before pregnancy was 23.6 kg/m^2 ranging between 14.9 and 53.8 kg/m^2. Among these, 18.4 and 7.6% of them were overweight and obese, respectively. Concerning parity, 53.3% of the mothers were primiparous, 39.1% had another infant and 7.5% had more than two infants. During the third trimester of pregnancy, 21.4% and 43.6% of women considered themselves quite-very active and sedentary-not active, respectively. Concerning vehicular traffic exposure, 40.9%, 25.4%, 24% and 9.5% of women reported living in streets with heavy, frequent, moderate and rare traffic, respectively. 88.7% of the women worked during all pregnancy and 24.2% of them worked in manufacturing or transport. Paternal occupation in manufacturing or transport was 46.3% of total. Height of the housing was grouped as between ground and 4th floor and above, encompassing 87.5% in the first case.

3.2. Sb and Cu concentrations

The concentrations of Sb and Cu, in ng/mL and µg/g creatinine, are shown in Table 2. The distributions of concentrations of Sb and Cu were not parametric but skewed to the left. Descriptive statistics in ng/mL or µg/g creatinine were not significantly different (Table 2). Thus, description of results and discussions are referred to µg/g creatinine. The geometric mean Cu and Sb concentrations were 13 µg/g creatinine (interquartile range 9.8 µg/g creatinine; P90 27 µg/g creatinine) and 0.28 µg/g creatinine (interquartile range 0.35 µg/g creatinine; P90 0.85 µg/g creatinine), respectively. The concentrations of both metals were significantly correlated (Spearman’s correlation rho = 0.30, $p < 0.001$).

3.3. Seasonal differences

The geometric mean concentrations of both metals were highest in the urine samples collected in winter (17 µg/g and 0.31 µg/g creatinine of Cu and Sb, respectively; Table 2). The geometric mean concentrations of Sb were lowest in the urine collected in autumn (0.23 µg/g creatinine). Those of Cu were lowest in the urines collected in summer (9.7 µg/g creatinine; Table 2). In the univariate models the concentrations of Sb were significantly different between winter and spring or autumn ($p < 0.05$) while the levels of Cu were significantly lower in summer ($p < 0.001$) and spring and autumn ($p < 0.01$) than in winter (Fig. 1).

3.4. Physical activity

The geometric mean Sb urine concentrations were higher in quite or very active women than in those with more sedentary habits (0.35 and 0.28 µg/g creatinine vs 0.25 µg/g creatinine, respectively; Table 2). These differences were significant in the univariate models ($p < 0.01$; Fig. 2). On the other hand, the geometric mean Cu levels in sedentary women were higher than in moderately active women but lower than in quite or very active women. Only the difference between sedentary and moderately active women was significant in the univariate models ($p < 0.05$; Fig. 2).

3.5. Traffic pollution

The geometric means of the Sb urine concentrations were higher in women with homes in street categorized in the heavy or frequent traffic density groups than in those of streets with very low or rarely any traffic (0.29 and 0.30 µg/g creatinine vs 0.27 and 0.21 µg/g creatinine, respectively; Table 2). The geometric means of the Cu concentrations were slightly higher for women living in streets with heavy traffic than in streets with practically no traffic (14 µg/g creatinine vs 12 µg/g creatinine). In the univariate models for car traffic the Sb concentrations were significantly higher in the women group exposed to heavy or frequent vehicular traffic than in those rarely exposed ($p < 0.05$; fig. 3).

3.6. Other population characteristics

Women who worked during the whole pregnancy had lower concentrations of Cu and Sb than those who did not (13 µg/g and 0.28 µg/g and vs. 15 µg/g and 0.32 µg/g creatinine of Cu and Sb, respectively), but these differences were not statistically significant in the univariate linear regression models (Fig. 4). Mothers whose apartments were below the fifth floor had higher geometric mean Sb concentrations than those living above, between the 5th and the 12th floors (0.29 µg/g vs. 0.23 µg/g; Table 2). No difference in Cu geometric means was observed in relation to home altitude. Different Sb and Cu means were observed for maternal and paternal occupation but without statistically significance.

3.7. Multivariate analysis

Multivariate linear regression models were built considering all above mentioned variables. According to the backward stepwise selection, pre-conception BMI, social class, cotinine, and consumption of food items such as bread, cereal/pasta and candies were also included in final models for Sb. In the case of Cu, age, parity, height of the housing, paternal occupation, cotinine and consumption of fruits, nuts, potatoes, coffee or infusions and alcohol were the selected variables. The adjusted R^2 for final models was 0.10 and 0.14 for Sb and Cu, respectively.

Sb concentrations were again higher during winter than in spring and autumn ($p<0.05$ and 0.01, respectively). For Cu the multivariate models also showed higher significant concentration differences in winter than in the other seasons (Fig. 1).

The multivariate models for physical activity also showed significantly higher Sb concentrations for active and moderately active women than for those with sedentary habits ($p<0.01$; Fig.2). Cu showed the same trend but without statistical significance (Fig. 2).

Vehicular traffic density showed a statistically significant association with the urine Sb levels (Fig. 3). Women from streets with rare traffic had significantly lower urine concentrations than those living in streets with continuous and frequent traffic ($p < 0.01$) and those living in streets with moderate traffic ($p < 0.05$). The beta coefficients and significance levels were higher in the multivariate than in the univariate models.

Concerning Cu, the multivariate models also showed lower urine concentrations for women from streets with low traffic intensity than from moderate, frequent or heavy traffic but the differences were not significant.

According to the multivariate models, women that did not work during pregnancy had higher significant Sb concentrations than those who worked ($p<0.05$; Fig. 4). The same difference was observed for the Cu concentrations but the differences were not statistically significant. No significant differences were found for height of housing and type of maternal or paternal occupation.

Finally, Cu concentrations did not show any significant association with diet items, while Sb showed a positive association with the tertiles of intake of pasta/cereal. The beta coefficients of this association using the first tertile as the reference category were 0.32 (SD: 0.10; $p<0.01$) and 0.25 (SD: 0.10) for the second and third tertiles, respectively.

3.8. Variable interactions

Interactions between vehicular traffic exposure and working during pregnancy were also evaluated for both metals. No significant interactions were found for Cu but in the case of Sb they were significant. Accordingly, women who did not work had a more marked association between Sb content and vehicular traffic than working women (Fig. 5).

Calculation of the Spearman correlations for Sb and Cu concentrations over all samples showed a rho coefficient 0.3025 ($p<0.001$). However, calculation of these correlations per season separately only showed a significant correlation between Sb and Cu for the samples collected in winter (rho: 0.569; $p<0.001$).

4. DISCUSSION

4.1. Urinary Sb and Cu concentrations in other cohorts and environments

The urine concentrations of Sb in the Sabadell cohort were slightly higher than those reported in populations from Congo (Banza et al., 2009), Germany(Heitland and Köster, 2006) or USA (NHANES, 2009) but lower than those found in workers from a glass-producing plant (Lüdersdorf et al., 1987) as well as those in general population from Italy (Alimonti et al., 2000; Minoia et al., 1990) or USA between 1988 and 1994 (Paschal et al., 1998) (Table 3). The urine Cu levels were similar to those found in Japan (Ohashi et al., 2006), lower than those reported in Tarragona (Catalonia, Spain) (Schuhmacher et al., 1994), and higher than those in general population from Germany (Lüdersdorf et al., 1987; Seifert et al., 2000) or in pregnant women from Australia (Callan et al., 2013) (Table 3).

Sb and Cu have been previously analyzed in atmospheric $PM_{2.5}$ in Sabadell (Minguillón et al., 2012). In summer, concentrations of 2.5 and 5.5 ng/m^3 in a suburban background area were observed, respectively. These concentrations increased to 3 and 20 ng/m^3 in a dense traffic street, respectively. In comparison to other concentrations these results were higher than the annual means of regional forest environments (Montseny; Pey et al., 2010b) or in an urban background from Birmingham during spring (Taiwo et al., 2014) but they were lower than in an urban background from Barcelona (Pey et al., 2010b). Sb was markedly lower than those reported in atmospheric samples from Tijuana area (Minguillón et al., 2014).

4.2. Seasonality

The highest concentrations of Sb and Cu in Sabadell have been found in the atmospheric particles collected in winter (Minguillón et al., 2012) which reflects winter anti-cyclonic episodes and thermal inversion in the area (Pey et al., 2010a). The configuration of the geographic depression where Sabadell is located makes dispersion of the air pollutants particularly difficult when wind is not oriented along the depression axis (Minguillón et al., 2012). The higher Sb and Cu concentrations found in the maternal urine samples collected in winter are consistent with these observations.

The significant correlation of the urine concentrations of Sb and Cu in the samples collected in winter is consistent with the finding of highest concentrations of these metals in the atmospheric particles. The Sb and Cu urine concentrations from the samples collected in the other seasons shows no correlation. This difference is consistent with the above reported inputs and metabolic role of these two metals. Both metals have been demonstrated to be highly soluble in pulmonary fluid (Wiseman and Zereini, 2014). Sb has been reported to be more absorbed through the respiratory than the gastrointestinal track (Iavicoli et al., 2002) and can therefore be incorporated from air pollution. Cu may be incorporated from this source and also from diet and is retained by metabolic needs. The excretion behavior of this last metal may only reflects environmental exposure in conditions of high atmospheric pollution such as winter thermal inversion.

4.3. Physical activity

Associations between physical activity and increased metal excretion have been reported (Campbell and Anderson, 1987; Kovacs et al., 2012), but some of these studies showed that the most important way of excretion of trace metals during physical exercise was sweat (Genuis et al., 2011). During pregnancy women have higher nasofaringeal and faringeal capillarity which increases the absorption capacity of air pollutants (Plaat and Arrandale, 2012). Higher intake of metal pollution should be reflected in higher urine excretion of these elements as observed in the present study for Sb (Fig. 2). The more intense respiration during physical activity may lead to higher inhalation of particles and its components.

Conversely, urine Cu excretion did not show significant associations with physical exercise. As mentioned above, diet is a more important source of this metal into humans than Sb. The lack of association of Cu excretion with physical activity suggests that the statistically significant cases may reflect higher intake by inhalation of atmospheric pollution and not general mobilization processes of all stored metals at higher metabolic activity.

4.4. Influence of traffic pollution

Since the late 90s, antimony (III) sulphide, Sb_2S_3, is used in brake linings after elimination of asbestos which led to an increase of Sb in the atmospheric PM (Garg et al., 2000; Wåhlin et al., 2006). Braking at traffic lights and stop signs enhance brake lining wear (Apeagyei et al., 2011). The high temperatures reached in this action enhance the oxidation of Sb_2S_3 into antimony oxide that is much more soluble in water than the original sulfide. Sb_2O_3 is classified as possible carcinogenic to humans (Group 2B) by the International Agency for Research on Cancer (Sundar and Chakravarty, 2010). As mentioned above, previous atmospheric pollution studies have shown significant contributions from road traffic to the Sb and Cu content of PM (Minguillón et al., 2012) and the atmospheric occurrence of Cu and Sb has been attributed to brake lining metal emissions (Adachi and Tainosho, 2004; Amato et al., 2011; Amato et al., 2009; Hjortenkrans et al., 2007). Brake lining wear has been considered to be responsible for 90 and 99% of airborne Cu and Sb, respectively (Thorpe and Harrison, 2008).

The observed dependence of urine Sb from traffic activity (Fig. 3) is consistent with these observations on the metal composition of urban particles. Accordingly, the women living in homes with higher vehicular traffic nearby showed higher concentrations of Sb in urine (Fig. 3). Cu showed the same trend but the differences were not statistically significant (Fig. 3).

In the case of Cu, traffic pollution may influence much less than diet in the intake of this metal in pregnant woman. Thus, although pulmonary solubility of Cu in PM was reported to be above 80% (Wiseman and Zereini, 2014), an occupational study on electrolytic department workers presumably exposed to this metal as consequence of emissions to the air did not show high concentrations of Cu in urine (Nieboer et al., 2007).

In the present study, the exposure to car traffic was specifically considered in the area where the homes of the pregnant women were located (Fig. 3). Accordingly, the association was stronger for the women who did not work during the pregnancy period as they remained more time near their homes than the working women. This difference was observed for both Sb and Cu.

4.5. Dietary and atmospheric apportion of Sb and Cu.

The average concentrations of Sb and Cu in the supply waters of the city are 0.86 µg/l and 3.6 µg/l, respectively (Casas et al., 2001). These values are far below the public health goals of 6 µg/l and 1300 µg/l, for Sb and Cu, respectively, of the National Primary Drinking Water Regulations from the US EPA (EPA, 2009).

Calculation of the total dietary ingestion of Sb for mean weight women of 76 kg using reported data from UK (Rose et al., 2010) assuming an intestinal absorption of 5-20% (Lauwers et al. 1990) results into estimated of 0.15-0.61 µg/day. The equivalent Cu intake assuming a mean intestinal absorption of 30-40% (Wapnir, 1998) is 390-520 µg/day.

On the other hand, taking into account the reported concentrations of Sb and Cu in $PM_{2.5}$ in the suburban background area of Sabadell (Minguillón et al., 2012) and a daily inhalation of 22 m^3 of air in pregnant women (Brochu et al., 2006) estimates of Sb inhalations of 0.1 and 0.055 µg/day during winter and summer, respectively, are obtained. The estimates for Cu are 0.12 and 0.44 µg/day during these two seasons, respectively.

According to these values, the dietary contribution of both metals is higher than the theoretical atmospheric input. Nevertheless, this difference is much broader for Cu than for Sb, which is consistent with the previously reported lack of statistical significance of the Cu atmospheric inputs when comparing urine concentrations of this metal and determinants of atmospheric pollution intake. In the multivariate analysis, no dietary item was associated with Cu and consumption. In the case of Sb there was an association of urine concentrations and consumption of cereal/pasta involving 5% of total daily intake, but the beta coefficients of the tertiles of consumption were lower than those of traffic exposure, physical exercise and seasonality.

4.6. Strengths and limitations of the study

Although the associations between Sb and atmospheric inputs are found to be statistically significant, the current study has some limitations. Vehicular traffic exposure was only evaluated at home and through questionnaire variables. Deployment of a network of aerosol samplers for monitoring specific exposures to traffic particles in the different home areas would increase the robustness of the associations but this approach was beyond the technical and economic possibilities of this study. In addition, 35% of the samples had non-detectable levels, which may be a cause for bias. Nevertheless, final models included different variables that modulate exposure to atmospheric pollutants and the results for all of them were consistent. Further studies considering more markers of traffic exposure, including not only those registering exposure at home but also a complete picture of daily exposure to vehicular traffic should be performed for a better assessment of these findings.

5. CONCLUSIONS

Atmospheric inputs are possibly responsible for the observed differences in urine Sb concentrations from pregnant women living in urban areas. The occurrence of this metal in the atmosphere has been attributed to traffic activity as consequence of its use in brake linings. The associations of Sb content in urine of pregnant women with seasonality, physical activity and traffic intensity near their homes is consistent with some dependence of the intake of this metal from atmospheric sources. These associations suggest that despite the estimated dietary inputs of this metal are somewhat higher than the estimated inhalation intake, the atmospheric inputs of Sb may be significant for the overall incorporation of this metal in populations of modern urban areas, e.g. in pregnant women.

Cu is also used in brake linings but the high predominance of inputs of this essential metal from dietary components make unlikely the significance of the atmospheric urban inputs in the overall human intake. This is consistent with the lack of statistical significance of the observed differences in Cu urine concentrations when grouped according to atmospheric pollution indicators.

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TABLES

Table 1. Main characteristics of the participating pregnant women

Characteristics	N	%
Age (years)	[a] 30.9 (17-42)	
<25	36	7.7
25-29	149	32.0
30-35	199	42.7
>35	82	17.6
Pre-pregnancy BMI (kg/m^2)	[a] 23.6 (14.9-53.8)	
<20	74	16.0
20-25	267	57.9
25-30	85	18.4
≥30	35	7.6
Parity		
0	248	53.3
1	182	39.1
≥2	35	7.5
Social class		
Non manual	239	51.2
Manual	228	48.8
Origin		
Spanish	419	90.3
Latin American	33	7.1
Rest of Europe	10	2.2
Others	2	0.43
Physical activity		
Sedentary / little active	202	43.6
Moderately active	162	35.0
Quite / very active	99	21.4
Smoking		
Never	231	50.2
Smoking at the beginning of	132	28.4
Smoking throughout pregnancy	71	15.3
Cotinine		
< 4 ng/mL	199	42.8
≥ 4 ng/mL	265	57.1
Traffic intensity near the		
Heavy	190	40.9
Frequent	118	25.4
Moderate	112	24.1
Rare	44	9.5
Worked all pregnancy		
Yes	409	88.7
No	52	11.3
Maternal occupation		
Manufacturers - transporters	113	24.3
Rest	352	75.7
Paternal occupation		
Manufacturers - transporters	251	46.3
Rest	216	53.7
Height of housing		
0-4th	407	87.5
5th-12th	58	12.5
Season		
Winter	125	27.0
Spring	114	24.6
Summer	125	26.0
Autumn	99	21.4

[a] Arithmetic mean (Range)

Table 2. Statistics of the concentrations of Sb and Cu in general population and in the groups defined by influence of traffic pollution, season and physical activity.

	Sb	**Cu**
General cohort		
% detection	65	100
Concentration (ng/mL)		
Arithmetic mean (SD)	0.45 (1.2)	14 (9.6)
Geometric mean (IQR)	0.25 (0.31)	11 (11)
P_{90}	0.68	26
Concentration (µg/g creatinine)		
Arithmetic mean (SD)	0.56 (2.0)	16 (11)
Geometric mean (IQR)	0.28 (0.35)	13 (9.8)
P_{90}	0.85	27
Traffic intensity near the homeplace (µg/g creatinine)		
Rare	0.21[a] (0.35)[a]	12[a] (9.5)[a]
Moderate	0.27 (0.33)	12 (10.4)
Frequent	0.30 (0.37)	14 (10.8)
Heavy	0.29 (0.29)	14 (9.3)
Season (µg/g creatinine)		
Winter	0.31 (0.43)[a]	17 (12)[a]
Spring	0.26 (0.28)	14 (7.2)
Summer	0.30 (0.40)	9.7 (7.6)
Autumn	0.23 (0.23)	13 (9.8)
Physical activity (µg/g creatinine)		
Sedentary / little active	0.25 (0.33)[a]	14 (9.3)[a]
Moderately active	0.28 (0.27)	12 (8.4)
Quite / very active	0.35 (0.44)	14 (15)
Worked during all pregnancy (µg/g creatinine)		
Yes	0.28 (0.32)	16 (9.6)
No	0.32 (0.46)	13 (13)
Height of housing (µg/g creatinine)		
Ground to 4th	0.29 (0.37)	13 (10)
5th-12th	0.23 (0.24)	13 (9.4)
Maternal occupation (µg/g creatinine)		
Manufacturer – transporter	0.24 (0.25)	14 (10)
Rest	0.30 (0.39)	13 (7.8)
Paternal occupation (µg/g creatinine)		
Manufacturer – transporter	0.28 (0.32)	14 (9.0)
Rest	0.29 (0.36)	12 (11)

[a] geometric mean (interquartile range)

Table 3. Comparison of the urine Sb and Cu concentrations in this cohort with previous studies (µg/g creatinine)

Reference	Sampling years	Location	N	Sb	Cu
Present work[b]	2004-06	Sabadell	461	0.28	13
NHANES report, 2009[a]	2001-02	USA	2500	0.13	…
Ohashi et al., 2006[be]	2000-05	Japan	1000	…	13
Banza et al., 2009[b]	2006-07	DR Congo	179	0.07	17
Paschal et al., 1998[b]	1988-94	USA	496	0.67	…
Schuhmacher et al., 1994[b]	nr	Tarragona	434	…	27
Seifert et al., 2005[b]	1990-92	Germany	4000	…	9.5
Alimonti et al., 2005[a]	nr	Italy	50	0.68	…
Heitland et al., 2006[b]	2005	Germany	87	0.037	5
Minoia et al., 1990[b]	nr	Italy	306/507	0.79[d]	23
Callan et al., 2013 [af]	2008-11	Australia	173	…	10.4
Lüdersdolf et al., 1987 [ag]	nr	Germany	109	1.9	…

[a]Median. [b]Geometric mean. [c]Arithmetic mean. [d]µg/L. [e]Women only . [f] Pregnant women [g] Men from a glass-producing plant

FIGURE CAPTIONS

Figure 1 Results of the univariate and multivariate models for the influence of sampling season in the concentrations of Cu and Sb in the urine of pregnant women. The reference category is indicated.

Figure 2 Results of the univariate and multivariate models for the influence of maternal physical activity in the concentrations of Cu and Sb in the urine of pregnant women. The reference category is indicated.

Figure 3 Results of the univariate and multivariate models for the influence of traffic in the concentrations of Cu and Sb in the urine of pregnant women. The reference category is indicated.

Figure 4. Results of the univariate and multivariate models for the influence of working during pregnancy in the concentrations of Cu and Sb in the urine of pregnant women. The reference category is indicated.

Figure 5. Predicted values of Sb (interval: 95% CI) from the multivariate linear regression models representing the interaction between working during pregnancy and car traffic exposure.