

S-Benzylmercapturic Acid (S-BMA) Levels in Urine as an Indicator of Exposure to Toluene in the Kinshasa Population

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Abstract

Background and objectives: Toluene, one of the various volatile organic compounds frequently observed in ambient air, is an environmental toxicant associated with several adverse effects on the central nervous and reproductive systems. In DR Congo, data on environmental toluene exposure are scarce. The present study aims to assess the toluene exposure in the Kinshasa population through the measurement of S-BMA in urinary samples.

Methods: During January - December 2008, 220 subjects aged 6 to 70 years living in the urban area of Kinshasa (50.5% women) provided urine samples. 50 other participants from the sub-rural area were added as a control. S-BMA levels were measured by LC-MS/MS. Data were expressed as arithmetic means, geometric means, median levels, percentile 95th and range.

Results: The levels of S-BMA in Kinshasa [median (min - max) levels = 18.1 µg/L (0.3 - 97.1, 25.3)] were similar to those reported in the general population. Whereas the median S-BMA levels in this study were in accordance with those found in the literature, the urban area of Kinshasa had a high risk of the toluene exposure as compared to the sub-rural area of the region [median (min - max) levels were 4.3 µg/L (0.3 - 51.9) for sub-rural area and 10.5 µg/L (0.3 - 97.1) for urban area, $p < 0.01$].

Conclusions: The present study findings highlight the environmental toluene exposure in the Kinshasa population. The determination of toluene concentrations in airborne of Kinshasa and its limit values are needed for the protection of human health.

Keywords: S-benzylmercapturic acid; Toluene; Biomonitoring; Environmental Exposure; Kinshasa population

Introduction

Toluene is one of the various volatile organic compounds frequently observed in ambient air [1]. Toluene exposure by inhalation is associated with several adverse effects on the central nervous and reproductive systems [2,3,4]. The main sources of toluene exposure include the burning of fossil fuels, toluene-based solvents, thinners and motor vehicle exhaust [5,6]. Others identified sources include cigarette smoke, emissions from volcanoes, forest fires and crude oil [7, 8]. The vapor of toluene in ambient air has been identified as an important source of toluene exposure in the general population [9,10]. The biomarkers of toluene exposure highlighted in the literature include toluene in blood, urine and exhaled air, as well as its urinary metabolites: Hippuric acid, Orto-cresol, S-p-toluymercapturic acid and S-benzylmercapturic acid (S-BMA) [11]. Among these biomarkers, S-BMA has been proposed as a reliable biomarker of toluene exposure [12,13,14,15]. The present study aims to assess the toluene exposure in the Kinshasa population through the measurement of S-BMA in urinary samples.

Methods

Study Design

During January - December 2008, 220 subjects aged 6 to 70 years, not occupationally exposed to toluene, were selected using a two-stage systematic sampling approach according to [16,17]. After providing some information about this study, subjects were asked to complete a questionnaire, consent in the survey and provide a urine sample. This study was approved by the congolese committee of medical ethics.

Laboratory Method

Spot urine samples were collected in polystyrene containers and stored at -20°C until analysis in the Louvain Center for Toxicology and Applied Pharmacology (Brussels, Belgium). After thawing and mixing all frozen urine samples, 1 mL of urine aliquot was spiked with 25 μL of an internal standard solution (1 mg/L of D3-SBMA). The sample was diluted with nanopure water (1:1) and centrifuged using Isolute SAX 500 mg 3 mL SPE columns according to the previous procedure used by [18]. Samples were analyzed by using LC-MS/MS system, equipped with Waters Alliance 2795 LC Column: C18 Supersples 100 (125 mm x 4 mm). Solvent-A was 0.5% (v/v) aqueous acetic acid and solvent-B was methanol with 0.5% (v/v) acetic acid. The solvent elution program used a flow rate of 0.40 mL/min at 50°C . The mass spectrometer was operated in negative ion electrospray mode. Mass spectral data on ions were obtained in multiple reaction monitoring. The limit of detection (LOD) was 0.70 $\mu\text{g/L}$. The analytical methods used in this study are in accordance with the literature [19, 20, 21]. Additionally, urinary cotinine was determined by HPLC according to the methods previously described by Benowitz (1996).

The statistical data analysis was performed using NCSS version 2004 [22]. We expressed the results as AM (Arithmetic means), GM (Geometric means), median, P95th (percentile 95th) and range. Dealing with laboratory results below the LOD (limit of detection), we used LOD/2. All parametric tests and stepwise multiple linear regression analyses were used according to the statistical procedure described by [22].

Results and Discussion

In the present work, we assessed the environmental exposure to toluene in the Kinshasa population using a urinary S-BMA as a biomarker. The characteristics of the participants from urban area as well as rural area are reported in. The average of age was 31 years (min = 6 and max= 70), with 50.5% of female, 36% of current smokers and 77.3% of adults.

One absorbed through the lungs, toluene is biotransformed and secreted rapidly in the urine (IARC, 1999; ATSDR, 2015). Several toluene exposure studies have been conducted among population in environmental and occupational settings using urinary metabolites as the indicator of toluene exposure [22, 23, 24, 25]. Some of these studies reported also that the urinary metabolites are GST-dependent [26, 27, 28]. Considering that GSTs are dimeric enzymes with two polymorphic genes in the general population [29], this information should be considered when interpreting urinary levels of S-BMA [27].

Median (range, P95th) urinary S-BMA was 18.1 µg/L (0.3 - 97.1, 25.3). Women had high levels of S-BMA as compared to men (0 for female, GM: 7.9 µg/L vs 1 for male, GM: 5.8 µg/L, $p < 0.01$). This can be explained by cooking activities mostly found in women in DR Congo (Tuakuila et al., 2013).

Comparing non-smokers and current smokers, higher levels of S-BMA were found in current smokers (0 for non-smokers, GM: 2.1 µg/L vs 1 for current smokers, GM: 11.8 µg/L, $p < 0.01$). This is in accordance with the results of other studies in the literature (IARC, 2004; Polzin et al., 2007; Schettgen et al., 2008; B'Humer, 2011; ATSDR, 2015). For example, Schettgen et al. (2008) reported median (min-max) levels for S-BMA in non-smokers and smokers of 8.2 µg/L (1.6 - 77.4) and 11.5 µg/L (0.9 - 51.2), respectively. B'Humer (2011) found median (min-max) levels for S-BMA in non-smokers and smokers of 6.9 µg/L (0.3 - 23.3) and 7.4 µg/L (1.3 - 28.3), respectively.

The Levels of S-BMA were lower in children as compared to adults (0 for 6-14 years, GM: 2.7 µg/L vs 1 for >14 years, GM: 11.2 µg/L, $p < 0.01$). This is because no child was a smoker.

Levels of S-BMA found in the present study were in accordance with those previously reported in the general population. The median (min - max, n) levels reported were 9.8 µg/L (0.9 - 77.4, n = 30) [28, 29].

The stepwise multivariable analyses were performed to compare urban area to sub-rural area (0 for sub-rural area/1 for urban area). The potential confounders were age (continuous variable), sex (qualitative variable) and smoking habits (urinary cotinine levels, continuous variable). The partial R² was 0.016 for age, 0.013 for sex, 0.126 for smoking habits and 0.019 for area. The variables used in this model explained approximately 17% (Total R² was 0.174) of the variance of the S-BMA levels : median (min - max) levels were 4.3 µg/L (0.3 - 51.9) for sub-rural area and 10.5 µg/L (0.3 - 97.1) for urban area. About 2-fold higher levels of S-BMA were found in the urban area of Kinshasa as compare to the sub-rural area of the same region.

This study had two major limitations. First, the participants were not randomly selected because of the absence population registers in Kinshasa. Second, passive smoking, an other source of toluene exposure in the general population, did not evaluate [30].

Despite these limitations, the levels of S-BMA in Kinshasa were similar to those reported in the general population. Whereas the median S-BMA levels in our study were in accordance with those found in the literature, the highest risk of exposure was observed in the urban area of Kinshasa. The present study findings highlight the environmental toluene exposure in the Kinshasa population. The determination of toluene concentrations in airborne of Kinshasa and its limit values are needed for the protection of human health [31].

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References

1. ACGIH (American Conference of Governmental Industrial Hygienists) (2014) TLVs and BEIs: Threshold limit values for chemical substances and physical agents & biological exposure indices. ACGIH; Cincinnati, Ohio.
2. ACGIH (American Conference of Governmental Industrial Hygienists) (2011) TLVs and BEIs for chemical substances and physical agents. Cincinnati, OH: ACGIH.
3. Ancelle T (2002) Statistique Epidémiologie. 1ère Ed. Maloine, Paris.
4. Angerer J, Schilbach M, Kramer A (1998) S-p-Toluymercapturic acid in the urine of workers exposed to toluene: A new biomarker of toluene exposure. Arch Toxicol. 72: 119-23.
5. ATSDR (Agency for Toxic Substances and Disease Registry) (2015) Toxicological Profile for Toluene (Draft for public comment). Atlanta, GA: US Department of Health and Human Services, Public Health Service.
6. Benowitz NL (1996) Cotinine as a biomarker of environmental tobacco smoke exposure. Epidemiol. Rev. 18: 188-204.
7. B'Hymer C (2011) Validation of an HPLC-MS-MS method for the determination of urinary S-benzylmercapturic acid and S-phenylmercapturic acid. J. Chromatogr. Sci. 49: 547-53.
8. Cosnier F, Cossec B, Burgart M, et al. (2013) Biomarkers of toluene exposure in rats: mercapturic acids versus traditional indicators (urinary hippuric acid and o-cresol and blood toluene). Xenobiotica 43: 651-60.
9. Fan R, Li J, Chen L, Xu Z, He D, et al. (2014) Biomass fuels and coke plants are important sources of human exposure to polycyclic aromatic hydrocarbons, benzene and toluene. Environ. Res 135: 1-8.
10. Fustinoni S, Mercadante R, Campo L, (2009) Self-collected urine sampling to study the kinetics of urinary toluene (and o-cresol) and define the best sampling time for biomonitoring. Int Arch. Occup. Environ. Health 82: 703-13.
11. Gericke C, Hanke B, Beckmann G, Baltes MM, Kühl KP, et al. (2001) Multicenter field trial on possible health effects of toluene: III. Evaluation of effects after long-term exposure. Toxicology 168: 185-209.
12. Goniewicz ML, Smith DM, Edwards KC, Blount BC, Caldwell KL, et al. (2018) Comparison of Nicotine and Toxicant Exposure in Users of Electronic Cigarettes and Combustible Cigarettes. JAMA Netw. Open 1: e185937.
14. Greenberg MM (1997) The Central Nervous System and Exposure to Toluene: A Risk Characterization. Environ. Res. 72: 1-7.
15. IARC (International Agency for Research on Cancer) (2004) IARC Monographs on the Evaluation of Carcinogenic Risk to Humans; Tobacco Smoke and Involuntary Smoking; IARC Scientific Publications: Lyon, France 83.
16. IARC (International Agency for Research on Cancer) (1999) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide. IARC - Lyon, 71. 830-855.
17. Hays SM, Aylward LL (2009) Using biomonitoring equivalents to interpret human biomonitoring data in a public health risk context. J Appl Toxicol 29: 275-88.

- 18.Hoffmann K, Krause C, Seifert B, Ullrich D (2000) The German Environmental Survey 1990/92 (GerES II): Sources of personal exposure to volatile organic compounds. *J. Expo. Ann. Environ. Epidemiol.* 10: 115-25.
- 19.Inoue O, Seiji K, Nakatsuka H, Kasahara M, Watanabe T, et al. (1988) Relationship between exposure to toluene and excretion of urinary metabolites in Korean female solvent workers. *Ind. Health* 26: 147-52.
- 20.Inoue Q, Kanno E, Yusa T, Kalizaki M, Ukai H, Okamoto, S., et al., 2002. Urinary benzylmercapturic acid as a marker of occupational exposure to toluene. *Int. Arch. Occup. Environ.* 75: 341-7.
- 21.Lovreglio P, Barbieri A, Carrieri M, Sbatatini L, Fracasso ME, et al.(2010) Validity of new biomarkers of internal dose for use in the biological monitoring of occupational and environmental exposure to low concentrations of benzene and toluene. *Int. Arch. Occup. Environ. Health* 3: 341-56.
- 22.Mathias PI, B'Hymer C (2016) Mercapturic acids: recent advances in their determination by liquid chromatography/mass spectrometry and their use in toxicant metabolism studies and in occupational and environmental exposure studies. *Biomarkers* 21: 293-315.
- 23.Pierce CH, Chen Y, Dills RL, Kalman DA, Morgan MS, 2002. Toluene metabolites as biological indicators of exposure. *Toxicol. Letters* 129: 65-76.
- 24.Polzin GM., Kosa-Maines, R.E., Ashley, D.L., Watson, C.H., 2007. Analysis of volatile organic compounds in mainstream cigarette smoke. *Environ. Sci. Technol.* 41: 1297-302.
- 25.Rosting C, Olsen R (2020) Biomonitoring of the benzene metabolite s-phenylmercapturic acid and the toluene metabolite S-benzylmercapturic acid in urine from firefighters. *Toxicol. Let* 329; 20-5
- 26.Sabatini L, Barbieri A, Indiveri P, Mattioli S, Violante FS (2008) Validation of an HPLC–MS/MS method for the simultaneous determination of phenylmercapturic acid, benzylmercapturic acid and o-methylmercapturic acid in urine as biomarkers of exposure to benzene, toluene and xylenes. *J. Chromatogr. B* 863: 115-22.
- 27.Schettgen T, Musiol A, Alt A, Kraus T (2008) Fast determination of urinary S-phenylmercapturic acid (S-PMA) and S-benzylmercapturic acid (S-BMA) by column-switching liquid chromatography–tandem mass spectrometry. *J. Chromatogr.B: Anal. Technol. Biomed. Life Sci.* 863: 283-92.
- 28.Tuakuila J (2013) S-phenylmercapturic (S-PMA) levels in urine as an indicator of exposure to benzene in the Kinshasa population. *Int. J. Hyg. Environ. Health* 216: 494-8.
- 29.Tuakuila J, Lison D, Lantin AC, Mbuyi M, Deumer G, et al. (2012) Worrying exposure to trace elements in the population of Kinshasa, Democratic Republic of Congo. *Int. Arch. Occup. Environ. Health* 85: 925-39.
- 30.Tuakuila J, Mbuyi F, Kabamba M, Lantin AC, Lison D, et al. (2010) Blood lead levels in the Kinshasa population: a pilot study. *Arch. Public Health.* 68: 30-41.
- 31.Ukai H, Kawai T, Inoue O, Maejima Y, Fukui Y, et al. (2007) Comparative evaluation of biomarkers of occupational exposure to toluene. *Int. Arch. Occup. Environ. Health* 81: 81-93.