Applied Research in Toxicology v.1 n.1 (2015) 25-31

Applied Research in Toxicology

journal homepage: www.appliedrestoxicol.com

ORIGINAL ARTICLE

Biological monitoring in Brazilian workers occupationally exposed to different xenobiotics

Natália Brucker[a,b], Angela Moro[a,b], Mariele Charão[a,b], Guilherme Bubols[a], Flávia V. Thiesen[c], Solange C. Garcia[a,b]*

[a]*Laboratory of Toxicology (LATOX), Department of Clinical Analysis, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil;* [b]*Post-graduate Program in Pharmaceutical Sciences, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil;* [c]*Toxicology Institute, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, RS, Brazil.*

ABSTRACT

Biological monitoring is required in order to prevent occupational diseases caused by chronic exposure to potentially toxic xenobiotics. Technological advances have enabled the exponential use of new agents with different physicochemical features and potential to cause toxic responses considering the lack of knowledge about biological effects. In Brazil, this issue is regulated by the NR 7 approved in 1994 which addresses the biological monitoring of 26 toxic agents and establishes their limits of biological exposure (LBE); however in relation to some agents, such as metals, the LBE values are obsolete and very lenient. Besides, there is lack of studies related to the biological monitoring of effect and susceptibility, especially considering lifestyle and exposure to mixture of xenobiotics. Comparative studies of exposure to different agents are therefore important, more specifically with biomarkers of exposure and laboratorial exams. In the present study, 80 subjects were evaluated and divided as occupationally-exposed: painters group (n = 20), gas station attendants group (n = 20), taxi drivers group (n = 20) and non-occupationally exposed controls (n = 20). Biomarkers of exposure, hematological, hepatic and renal parameters were investigated. Our results demonstrated that the occupationally-exposed workers were exposed to low levels of xenobiotics in their workplace. The routine biomarkers analyzed did not present important alterations, except to AST in painters. In addition, comparing gas station attendants with non-exposed group was observed decreased erythrocytes and hemoglobin levels ($p < 0.05$). On the other hand, scientific works have shown that low levels of exposure to xenobiotics may cause damage to DNA, proteins and lipids. Based on this, these alterations could be suggested as early biomarkers to prevent occupational diseases.

Keywords: occupational exposure; early biomarkers; xenobiotics.

1. Introduction

A great part of the population has been continuosly exposed to some kind of solvent or their vapours either at work or in the environment. Diseases possibly triggered or aggravated by exposure to environmental or occupational contaminants become common, especially the chronic exposure to these xenobiotics (1-3).

Biological and environmental monitoring determine the global exposure to which a subject is exposed and are intended to detect early and possibly reversible effects (4). Biomarkers used in biological monitoring indicate the interaction between xenobiotic and the exposed subjects and according to their toxicological importance are divided into biomarkers of exposure, effect and susceptibility (2).

Biomarkers of exposure are necessary to quantify xenobiotics present in the workplace and/or their metabolites in the blood or urine from exposed individuals (3) and are represented mostly by urinary metabolites, specifically regarding organic solvents and polycyclic aromatic hydrocarbons (PAH).

In addition, biomarkers of effect are widely used in biological monitoring due to their high correlation with the exposure level. Biomarkers of effect are defined as any measurable biochemical or physiological alteration that depending on its magnitude may be recognized as a potential health disturbance or a disease (2,3).

In Brazil, the Regulatory Norm Nr. 7 (NR-7) is a regulation that establishes some biological parameters to control the occupational exposure to different chemical agents, among them organic solvents. According to the NR-7, all institutions that employ workers are obligated to elaborate and implement the Program of Medical Control of the Occupational Health, whose goal is to promote and preserve the health of workers (5). Moreover, the Instrução Normativa Nr. 2 from December

*Corresponding author: Prof Dr Solange Cristina Garcia.
E-mail address: solange.garcia@ufrgs.br (S. C. Garcia).
Address: Avenida Ipiranga 2752, Santa Cecília, Porto Alegre, RS, Brazil. CEP.: 90610-000
Tel.: (+55) 51 3308-5297. Fax: (+55) 51 3308-543

20 1995, that regulates the prevention and health surveillance of workers occupationally exposed to benzene, also has the objective to promote occupational health and to detect as early as possible the negative effects caused by benzene (6).

The Brazilian NR-7 contemplates the biological monitoring of most chemical substances, however there are xenobiotics not yet included in the health evaluation of workers, such as environmental pollutants, which are a wide variety of toxic agents that affect several groups of workers such as bus drivers, taxi drivers and traffic agents. Therefore, studies that aim to establish useful biomarkers in the health evaluation of occupationally exposed subjects are necessary.

Considering that the use of organic solvents (toluene and benzene) and that the constant presence of atmospheric pollutants in the workplace represent significant risk to the workers' health, the present study aims to determine biomarkers of exposure in different groups of exposed workers and investigate possible biomarkers of effect in order to evaluate the impact of the occupational exposure to toluene in painters, to benzene in gas station attendants and to atmospheric pollutants in taxi drivers in the end of the phrase and add a point after the word drivers.

2. MATERIALS AND METHODS

2.1. Study groups

The present study was approved by ethics committees from the Federal University of Rio Grande do Sul (No 20322/11 and No 21728/11) and the Federal University of Santa Maria (No 23081.015931/2006-59). All study participants signed informed consent. Groups of study consisted of 20 painters, 20 gas station attendants and 20 taxi drivers, occupationally exposed to low levels of toluene, benzene and atmospheric pollutants (mostly PAH), respectively. The control group was 20 subjects without occupational exposure to chemical and atmospheric agents. All subjects were non-smoker men resident in the state of Rio Grande do Sul, Brazil.

2.2. Collection of biological samples

At the end of the work shift after three consecutive days of exposure, urine and blood samples were collected. Non-occupationally exposed controls also provided urine and blood samples. Fifty milliliters of urine was collected for the determination of hippuric acid, trans,trans-muconic acid, 1-hydroxypyrene and creatinine levels. The blood venous samples from all the subjects were collected by venipuncture. The blood-EDTA sample was used for a hemogram and to measure carboxyhemoglobin levels. Another blood collection tube without anticoagulants was centrifuged at 1500 g for 10 min and the serum was used to determine aspartate aminotransferase, alanine aminotransferase, uric acid and urea levels. Besides, a questionnaire was applied to collect information such as lifestyle, smoking, alcohol consumption, history of chronic diseases and use of medications and vitamins.

2.3. Biomarkers of exposure

2.3.1. Urinary hippuric acid (toluene)

Quantification of urinary hippuric acid was performed by high performance liquid chromatographic (HPLC) with ultraviolet detection (Knauer®) according to Bulcão et al. (7).

2.3.2. Trans,trans-muconic acid (benzene)

Trans,trans-muconic acid (t,t-MA) levels were determined as described by Ducos et al. (8) with some modifications, utilizing solid phase extraction with 100 mg SAX cartridges and quantification by HPLC with ultraviolet detection.

2.3.3. 1-Hydroxypyrene (PAH)

1-Hydroxypyrene levels (1-OHP) were quantified by HPLC after enzymatic hydrolysis of the conjugated metabolite and solid phase extraction. Urine (2.5 mL) was diluted with 5 mL acetate buffer (pH 5). Then 10 µL of β-glucuronidase was added to samples and were incubated at 37ºC for 2 hours. Later, samples were cleaned up by solid phase extraction in 500 mg C18 cartridges (Chromsbond ®MN), previously activated with 2 mL methanol and 5 mL milli-Q water. Elution was performed with 2 mL isopropanol and samples were fully evaporated under stream of compressed air and reconstituted with 200 µL methanol. Samples were analyzed by HPLC with a fluorescence detector at 242/388 nm excitation/emission wavelengths, respectively. A LiChospher ® 100 RP-18 (150 x 4.6 mm x 5 µm) chromatographic column was used with a mobile phase composed of a mixture of milli-Q water:acetonitrile:methanol (30:35:35) with a 1mL/min flow. 1-OHP levels were adjusted by the urinary creatinine excretion.

2.3.4. Carboxyhemoglobin carbon monoxyde

Carboxyhemoglobin (COHb) was quantified in whole blood-EDTA samples according to the spectrophotometric method described by Beutler and West (9).

2.4. Creatinine

Urinary creatinine levels were assessed with the spectrophotometric method using commercial kits (Doles, Goiânia, GO).

2.5. Hematological and biochemical analyses

Complete hemograms were performed with the automated method in Coulter T890. Biochemical parameters aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid and urea were analyzed using commercial kits.

2.6. Statistical analysis

Data were analyzed with the SPSS software. A normality test was applied to verify data distribution for each variable. Comparisons between groups were performed by the Mann-Whitney test and data are presented as mean ± standard error. The significance level was considered when $p<0.05$.

3. Results

Data from the lifestyle obtained after questionnaire application are presented in Table 1.

Figure 1 (A-D) presents results from biomarkers of exposure quantified in the study groups. No significant difference was observed in hippuric acid levels from painters compared to controls (Figure 1A; $p>0.05$), and all values obtained were within the limits established by the NR-7. Regarding t,t-MA, gas station attendants presented enhanced levels in relation to controls (Figure 1B; $p<0.05$). In addition, an increased urinary concentration of 1-OHP in taxi drivers was observed compared to controls (Figure 1C; $p<0.05$). Blood carboxyhemoglobin results showed no significant difference between taxi drivers and the control group (Figure 1D; $p>0.05$).

In relation to the hematological parameters (Table 2), no significant difference between painters and controls and also between taxi drivers and controls was found. However, gas station attendants showed significantly lower number of erythrocytes and hemoglobin levels compared to the control group ($p<0.05$). All hematological parameters though were within the reference values for adult men (10).

Enhanced AST and ALT values were found in painters and gas station attendants in comparison to controls ($p<0.05$), but only the group of painters presented AST values higher than the reference values. Taxi drivers did not show significant AST and ALT alterations compared to the control group ($p>0.05$) (Table 2).

None of the renal biomarkers analyzed showed significant differences among all groups of study ($p>0.05$).

4. Discussion

The long-term prevention of possible damages to the workers' health is an important task accomplished by the biological monitoring. In this context, the early detection of biomarkers that result from the exposure to potentially damaging substances in the work environment may significantly diminish the occurrence of health adverse effects. Data obtained from the biological monitoring enable the adoption of appropriate prevention and control measures along with a constant evaluation of possible risks associated to the exposure to chemical agents in the workplace (11).

Subjects occupationally exposed to paints are known to be in contact with a variety of xenobiotics once the formulation of paints includes a complex mixture of chemical substances (12), among which the organic solvent toluene stands out (13-15). According to Brazilian regulations, hippuric acid is the biomarker of exposure established for the biological monitoring of exposure to toluene and the present study showed that its levels in the group of painters were below the reference values (1.5 g/g creatinine) as well as the maximum values permitted for this biomarker (2.5 g/g creatinine). No significant difference in comparison to controls was observed, suggesting that the quantification of urinary hippuric acid is not specific to detect exposures to low levels of toluene, considering that hippuric acid is also found in urine samples from subjects not exposed to toluene, whose primary source is the dietary ingestion of benzoic acid and its precursors in foodstuffs (4). The low specificity of hippuric acid as biomarker of exposure to toluene has already been evidenced in studies from our group (16,17). Besides, the LBE values present in NR-7 for hippuric acid are very high in comparison to International Standards, for example those established by the ACGIH American Conference of Governamental Industrial Hygienists (18).

Regarding the urinary t,t-MA analysis, our results were found to be within the biological limits established by ACGIH (500 µg/g creatinine), indicating that the gas station attendants in this study were exposed to low environmental levels of benzene, which could be a consequence of the efficacy of safety measures to control the amount of benzene in gasoline as well as its levels in the work environment (18-20). In Brazil, the maximum percentage of benzene allowed in gasoline is 1% (20). Based on the toxic effects that arise from the occupational exposure to benzene in humans, the presence of this solvent in gasoline has been decreasing exponentially over the years and substituted for less toxic solvents (21). The decreased levels however do not assure a complete health protection in exposed subjects, once there is no safe limit of exposure to carcinogenic substances such as benzene (22).

The present results showed an increase in the urinary biomarker of exposure to benzene in gas station attendants when compared to control group, thus demonstrating that t,t-MA can be considered a reliable biomarker in the biomonitoring of exposure even in exposures to low levels of benzene. On the other hand, the major disadvantage of t,t-MA as a biomarker of exposure is

Table 1. Data obtained using a questionnaire to each group (n=80).

	Controls (n = 20)	Painters (n = 20)	GSA (n = 20)	Taxi drivers (n = 20)
Age (years)	30.19 ± 1.60	27.80 ± 1.80	38.30 ± 1.80	40.30 ± 2.70
Time of occupational exposure (years)	NE	2.70 ± 0.71	12.50 ± 1.70	11.45 ± 2.15
Occasional alcohol drinking [n (%)]	16 (80)	16 (80)	13 (65)	12 (60)

The values are expressed as mean ± standard error of the mean (SEM).
(n) (%): Total number found per group and in parenthesis the perceptual.
Abbreviation: GSA: Gasoline station attendants. NE: non-occupationally exposed.

that its basal concentration is influenced by individual factors, such as tabagism and diet, considering that t,t-MA is also derived from sorbic acid metabolism, which is largely used as additive in different foodstuffs, hence the urinary presence of t,t-MA in subjects not occupationally exposed to benzene (23-25).

Urbanization, populational growth and a high vehicle fleet are associated with a progressive increase in the emission rates of atmospheric pollutants. Taxi drivers are a group of workers presenting long work shifts and continuously exposed to a heterogeneous mixture of toxic compounds (26-29). Taxi

Table 2. Hematological and biochemical parameters of the studied groups (n=80).

	Controls (n = 20)	Painters (n = 20)	GSA (n = 20)	Taxi drivers (n = 20)	[10]Reference Values
Erythrocytes (x10^6/mm^3)	5.19 ± 0.06	5.00 ± 0.08	4.94 ± 0.09[b]	5.15 ± 0.09	4.5-6.1
Hemoglobin (g/dL)	15.05 ± 0.14	14.76 ± 0.21	14.11 ± 0.23[b]	14.89 ± 0.18	12.8-17.8
Hematocrit (%)	43.81 ± 0.43	43.93 ± 0.62	44.29 ± 0.69	43.45 ± 0.60	39-53
Leukocytes (x10^3 mg/dL)	7.07 ± 0.33	6.92 ± 0.25	6.72 ± 0.24	6.34 ± 0.33	3.6-11
AST (U/L)	11.00 ± 0.67	53.80 ± 4.47[a]	29.55 ± 1.95[b]	10.15 ± 1.06	11-37
ALT (U/L)	13.71 ± 1.48	35.90 ± 3.54[a]	26.30 ± 3.53[b]	15.50 ± 3.85	11-37
Uric acid (mg/dL)	5.93 ± 0.17	4.34 ± 0.22	4.74 ± 0.28	5.78 ± 0.28	3.6- 7.7
Urea (mg/dL)	32.28 ± 1.78	35.70 ± 1.90	32.60 ± 1.77	31.15 ± 1.53	15-45

The values are expressed as mean ± standard error of the mean (SEM).
[a]p< 0.05 compared painters with controls
[b]p< 0.05 compared GSA with controls
Abbreviations: GSA: Gasoline station attendants; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

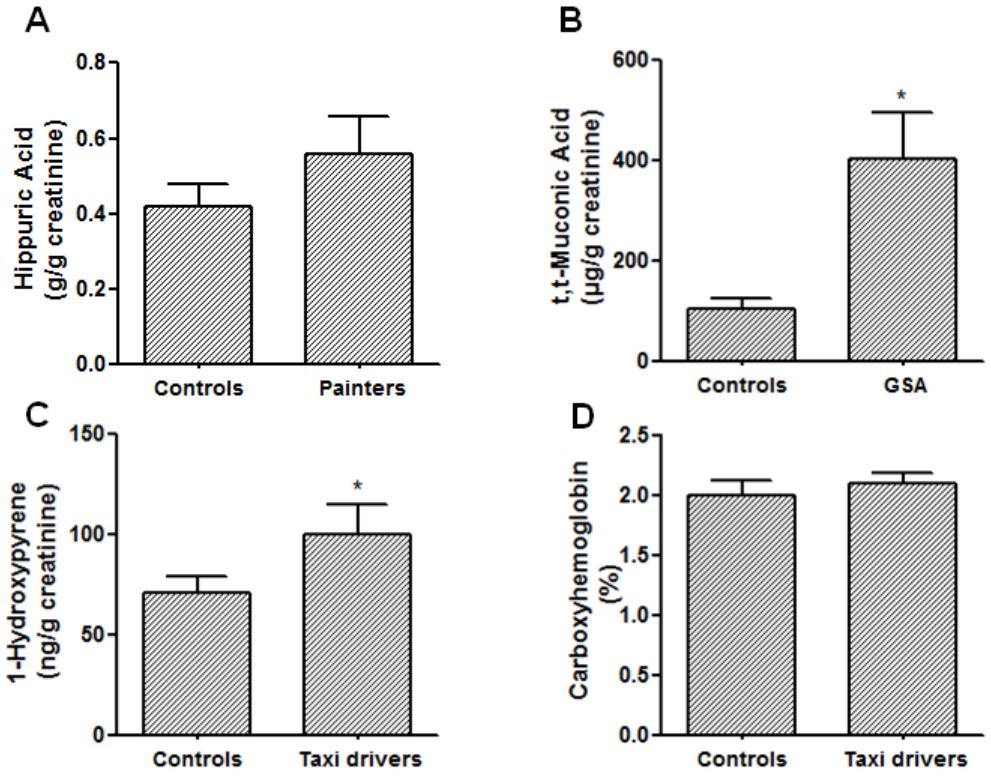

Figure 1. Biomarkers of exposure between occupationally-exposed and non-exposed subjects: (A) urinary hippuric acid levels from controls (n=20) and painters (n=20). (B) Trans, trans-muconic acid levels in urine from controls (n=20) and gasoline station attendants (GSA) (n=20). (C) Urinary 1-hydroxypyrene levels from controls (n=20) and taxi drivers (n=20). (D) Carboxyhemoglobin levels in blood from controls (n=20) and taxi drivers (n=20). Data are expressed as mean ± SEM. *p<0.05.

drivers are usually self-employed and Brazilian law lacks specific rules for health evaluation in this professional category. Carboxyhemoglobin is utilized as a biomarker of exposure to carbon monoxyde and its quantification in this study did not indicate differences between taxi drivers and the control group. Besides, 1-OHP urinary levels were significantly increased in taxi drivers compared to controls. These data indicate the need to investigate biomarkers of exposure that are useful in the evaluation of occupational exposure to pollutants. Quantification of the metabolite formed from PAH biotransformation, 1-hydroxypyrene, is suggested by some authors as a biomarker to estimate exposure to environmental pollutants related to vehicular traffic (30-33).

In reference to hematological and biochemical parameters in this study, painters presented neither hematological nor renal alterations; however a significant increase of hepatic enzymes in these subjects was found in comparison to controls. This finding may be a consequence of the recognized hepatotoxicity elicited by toluene. In chronic exposures, toluene induces liver damages which have been reported by increased liver transaminases and development of hepatitis in occupationally exposed subjects (34,35).

Gas station attendants have also exhibited no biochemical alterations in renal parameters, but hematological parameters were found to be significantly impaired in relation to controls, namely erythrocytes and hemoglobin levels. These findings though do not present clinical relevance in the biological monitoring of benzene exposure once all parameters ranged inside reference values. In addition, liver transaminases were increased in gas station attendants versus controls. Although the increased hepatic enzymes did not exceed reference values, these alterations may be associated to the benzene-induced toxic effects after occupational exposures, considering the extensive biotransformation suffered by benzene in the liver through phase I and II reactions which produce highly toxic metabolites (22).

The adoption of hematological and biochemical biomarkers may be useful in the process of biological monitoring in occupationally exposed subjects to toluene and benzene. However an alternative that has become reliable for the early detection of biological alterations induced by these xenobiotics is the evaluation of oxidative damage biomarkers, as previously demonstrated by our group (16,17,36).

Taxi drivers in this study did not present alterations in hematological, hepatic or renal biomarkers compared to the control group. The evaluated parameters did not show clinical relevance in the biological monitoring of taxi drivers. Recent studies suggest that inhalation of environmental pollutants such as PAH adhered to particulate matter are involved with the activation of inflammatory mediators (37-39). In this case, for a better evaluation of taxi drivers' health conditions and detection of alterations related to the toxic effects of continuous exposure to environmental pollutants, an evaluation of biomarkers of early effect would be necessary, e.g. quantification of serum inflammatory mediators that may contribute to the atherosclerotic process, as we have previously reported (40).

5. Conclusion

Taking together the findings reported from our group, we conclude that the groups of workers in this study are exposed to low levels of xenobiotics in their work environments as shown by analysis of biomarkers of exposure. In parallel, some biomarkers of effect studied were not effective in determining alterations in biological systems. Once Brazil has numerous workers chronically exposed to xenobiotics, these toxicity evidences indicate the need to select biomarkers that present suitable sensibility to evaluate low levels of exposure and to detect early signs of damages caused by occupational exposures along with the development of effective strategies to promote lifestyle changes in these workers.

Acknowledgments

The authors wish to thank all the workers who volunteered to participate in this study. This work was supported by FAPERGS (No. 1017503/2010), CNPq/Universal (No. 479613/2009-5 and 484096/2011-7) and CNPq/MCT (No. 479613/2009-5). CAPES provided a PhD research fellowships to N. Brucker, A. Moro and M. Charão. S.C. Garcia is recipient of CNPq research fellowship.

References

1. Angerer J, Ewers U, Wilhelm M. Human biomonitoring: state of the art. Int J Hyg Environ Health 2007; 210:201-28.
2. Manini P, De Palma G, Mutti A. Exposure assessment at the workplace: implications of biological variability. Toxicol Lett 2007; 168:210-8.
3. Manno M, Viau C, Cocker J, Colosio C, Lowry L, Mutti A, Nordberg M, Wang S. Biomonitoring for occupational health risk assessment (BOHRA). Toxicol Lett 2010; 192:3-16.
4. Oga S, Carvalho M, Batistuzzo J. Fundamentos de Toxicologia. 3th ed São Paulo: Atheneu; 2008.
5. BRASIL. Ministério do Trabalho e Emprego. Nr-7. In: Segurança e Medicina do Trabalho. 50 ed. 1994.
6. BRASIL. Ministério do Trabalho e Emprego. Nr-15. In: Segurança e Medicina do Trabalho. 50 ed. 1995.
7. Bulcão R, Santa Maria L, Charão M, Moro A, Roehrs M, Garcia SC, Limberger R. Quantificação Simultânea de Indicadores Biológicos de Exposição a Solventes. Quim Nova 2008; 31:1343-8.
8. Ducos P, Gaudin R, Robert A, Francin JM, Maire C. Improvement in HPLC analysis of urinary trans,trans-muconic acid, a promising substitute for phenol in the assessment of benzene exposure. Int Arch Occup Environ Health 1990; 62:529-34.
9. Beutler E, West C. Simplified determination of carboxyhemoglobin. Clin Chem 1984; 30:871-4.
10. Burtis CA, Ashwood ER, Bruns DE. Tietz-Fundamentos de Clínica Química. 6th ed. São Paulo: Elsevier; 2008.

11. Bernard A, Lauwerys R. Assessment of human exposure to chemicals through biological monitoring. In: Kopfler FC; Craun GR. Environmental Epidemiology. Chelsea, 1986
12. IARC. (International Agency for Research on Cancer). Some organic solvents, resin monomers and related compounds, pigments and occupational exposure in the paint manufacture and painting. Monograph on the evaluation of carcinogenic risk to humans. Lyon: IARC, 1989.
13. WHO. (World Health Organization). Biological Monitoring of Chemical Exposure in the Workplace. Geneva: WHO, 1996.
14. Moon CS, Lee JT, Chun JH, Ikeda M. Use of solvents in industries in Korea; experience in Sinpyeong-Jangrim industrial complex. Int Arch Occup Environ Health 2001; 74:148-52.
15. Samoto H, Fukui Y, Ukai H, Okamoto S, Takada S, Ohashi F, Moriguchi J, Ezaki T, Ikeda M. Field survey on types of organic solvents used in enterprises of various sizes. Int Arch Occup Environ Health 2006; 79: 558-67.
16. Moro AM, Brucker N, Charao M, Bulcao R, Freitas F, Baierle M, Nascimento S, Valentini J, Cassini C, Salvador M, Linden R, Thiesen F, Buffon A, Moresco R, Garcia SC. Evaluation of genotoxicity and oxidative damage in painters exposed to low levels of toluene. Mutat Res 2012;746:42-8.
17. Moro AM, Charao M, Brucker N, Bulcao R, Freitas F, Guerreiro G, Baierle M, Nascimento S, Waechter F, Hirakata V, Linden R, Thiesen FV, Garcia SC. Effects of low-level exposure to xenobiotics present in paints on oxidative stress in workers Sci Total Environ 2010;408:4461-7.
18. ACGIH. (American Conference of Governamental Industrial Hygienists). Documentation of the Threshold Limit Values for Chemical Substances and Physical Agents and Biological Indices. Cincinnati: ACGIH, 2011.
19. EPA. (Environmental Protection Agency). Control of hazardous air pollutants from mobile sources. EPA, 2011.
20. Fundacentro. Acordo e Legislação sobre o Benzeno - 10 Anos. Fundacentro, São Paulo, 2005.
21. Chanvaivit S, Navasumrit P, Hunsonti P, Autrup H, Ruchirawat M. Exposure assessment of benzene in Thai workers, DNA-repair capacity and influence of genetic polymorphisms. Mutat Res 2007; 626: 79-87.
22. Weisel CP. Benzene exposure: An overview of monitoring methods and their findings. Chem Biol Interact 2010; 184:58-66.
23. Pezzagno G, Maestri L, Fiorentino ML. Trans, trans muconic acid, a biological indicator to low levels of environmental benzene: some aspects of its specificity. Am J Ind Med 1999; 35:511-8.
24. Negri S, Bono R, Maestri L, Ghittori S, Imbriani M. High-pressure liquid chromatographic-mass spectrometric determination of sorbic acid in urine: verification of formation of trans, trans muconic acid. Chem Biol Interact 2005; 153-154:243-6.
25. Hoet P, De Smedt E, Ferrari M, Imbriani M, Maestri L, Negri S, De Wilde P, Lison D, Haufroid V. Evaluation of urinary biomarkers of exposure to benzene: correlation with blood benzene and influence of confounding factors. Inter Arch Occup Environl Health 2009; 82:985-95.
26. Miller-Schulze JP, Paulsen M, Toriba A, Tang N, Hayakawa K, Tamura K, Dong L, Zhang X, Simpson CD. Exposures to particulate air pollution and nitro-polycyclic aromatic hydrocarbons among taxi drivers in Shenyang, China. Environ Sci Technol 2010; 44:216-21.
27. Lewne M, Nise G, Lind ML, Gustavsson P. Exposure to particles and nitrogen dioxide among taxi, bus and lorry drivers. Int Arch Occup Environ Health 2006; 79: 220-6.
28. Burgaz S, Demircigil GC, Karahalil B, Karakaya AE. Chromosomal damage in peripheral blood lymphocytes of traffic policemen and taxi drivers exposed to urban air pollution. Chemosphere 2002; 47:57-64.
29. Manini P, De Palma G, Andreoli R, Poli D, Mozzoni P, Folesani G, et al. Environmental and biological monitoring of benzene exposure in a cohort of Italian taxi drivers. Toxicol Lett 2006; 167:142-51.
30. Freire C, Abril A, Fernandez MF, Ramos R, Estarlich M, Manrique A, Aquirre A, Olea N. Urinary 1-hydroxypyrene and PAH exposure in 4-year-old Spanish children. Sci Total Environ 2009; 407:1562–9.
31. Hansen AM, Wallin H, Binderup ML, Dybdahl M, Autrup H, Loft S, Knudsen LE. Urinary 1-hydroxypyrene and mutagenicity in bus drivers and mail carriers exposed to urban air pollution in Denmark. Mutat Res 2004; 557:7-17.
32. Rossbach B, Preuss R, Letzel S, Drexler H, Angerer J. Biological monitoring of occupational exposure to polycyclic aromatic hydrocarbons (PAH) by determination of monohydroxylated metabolites of phenanthrene and pyrene in urine. Int Arch Occup Environ Health 2007; 81:221-9.
33. Merlo F, Andreassen A, Weston A, Pan CF, Haugen A, Valerio F, Reggiardo G, Fontana V, Garte S, Puntoni R, Abbondandolo A. Urinary excretion of 1-hydroxypyrene as a marker for exposure to urban air levels of polycyclic aromatic hydrocarbons. Cancer Epidemiol Biomarkers Prev 1998; 7:147-155.
34. Svensson BG, Nice G, Erfuth EM, Olsson H. Neuroendocrine effects in printing workers exposed to toluene. Br J Ind Med 1992; 49:402-8.
35. Barberino JL, Carvalho FM, Silvany-Neto AM, Cotrim HP, Goes RC, Rosa H, Gidi JF, Valladares CM, Guedes F. Liver changes in workers at an oil refinery and in a reference population in the state of Bahia, Brazil. Rev Panam Salud Publica 2005; 17:30-7.
36. Moro AM, Charão MF, Brucker N, Durgante J, Baierle M, Bubols G, Goethel G, Fracasso R, Nascimento S, Bulcão R, Gauer B, Barth A, Bochi G, Moresco R, Gioda A, Salvador M, Farsky S, Garcia SC. Genotoxicity and oxidative stress in gasoline station attendants. Mutat Res 2013; 754:63-70.

37. Delfino RJ, Staimer N, Tjoa T, Gillen DL, Polidori A, Arhami M, et al. Air pollution exposures and circulating biomarkers of effect in a susceptible population: clues to potential causal component mixtures and mechanisms. Environ Health Perspect 2009; 117:1232-8.
38. Brook RD, Urch B, Dvonch JT, Bard RL, Speck M, Keeler G, et al. Insights into the mechanisms and mediators of the effects of air pollution exposure on blood pressure and vascular function in healthy humans. Hypertension 2009; 54:659-67.
39. Huttunen K, Siponen T, Salonen I, Yli-Tuomi T, Aurela M, Dufva H, et al. Low-level exposure to ambient particulate matter is associated with systemic inflammation in ischemic heart disease patients. Environ Res 2012;116:44-51.
40. Brucker N, Moro AM, Charão MF, Durgante J, Freitas F, Baierle M, Nascimento S, Gauer B, Bulcão RP, Bubols GB, Ferrari PD, Thiesen FV, Gioda A, Duarte MM, de Castro I, Saldiva PH, Garcia SC. Biomarkers of occupational exposure to air pollution, inflammation and oxidative damage in taxi drivers. Sci Total Environ 2013; 463-464C:884-93.