sciendo

interdisciplinary
toxicology

ORIGINAL ARTICLE

Toluene abuse markers in marginalized populations

Beáta HUBKOVÁ [1], **Oliver RÁCZ** [2], **Gabriel BÓDY** [3], **Eugen FRIŠMAN** [4], **Mária MAREKOVÁ** [1]

[1] Department of Medical and Clinical Biochemistry, Pavol Jozef Šafárik University in Košice, Faculty of Medicine, Košice, Slovakia
[2] Department of Pathological Physiology, Pavol Jozef Šafárik University in Košice, Faculty of Medicine, Košice, Slovakia
[3] Medirex a.s., Košice, Slovakia
[4] Clinic of Burns and Reconstructive Surgery, 1st Private Hospital Košice – Šaca, Slovakia

ABSTRACT

Toluene abuse is one of the most common addictions among marginalized Roma. The aim of the study was the comparison of urinary toluene metabolite levels in marginalized population of Eastern Slovakia as compared to the majority population, and to verify the validity of the answers, given in the questionnaires, regarding toluene abuse. The study was carried out as part of the HEPA-META project aiming to map the prevalence of health problems in marginalized Roma. The majority of people living outside the area of the segregated Roma communities comprised the control group. The total number of study participants was 770. Statistically significant differences in the levels of hippuric acid and *o*-cresol were found between Roma and the majority population. Variations in urinary hippuric acid levels in addition to toluene abuse can be caused also by dietary factors, medical treatment as well as alcohol consump-tion, which is frequent (not only) in marginalized communities.

KEY WORDS: toluene abuse; Roma; *o*-cresol; hippuric acid

Introduction

Toluene (methylbenzene) is a colorless, volatile liquid with a characteristic sweet odor. It occurs naturally in crude oil, and is produced industrially either from gasoline or coal. Toluene is mainly used as a precursor to benzene (over 50%), benzoic acid, *p*-xylene and is also used in the production of toluene di-isocyanate, precursor for poly-urethane foams. Furthermore, toluene is widely used as a solvent for various polymers, resins, and certain pharma-ceuticals. Its use as a solvent in adhesives, paints and inks is preferred over benzene due to its much lower toxicity.

The current Occupational Safety and Health Administration standard for toluene is 200 ppm for an 8-hour workday. Toluene levels of 500 ppm are considered dangerous to life and health (Public Health Statement for Toluene, 2015). Due to its lipophilic nature, toluene is absorbed relatively rapidly through the skin, the respi-ratory and gastrointestinal tract resulting in its rapid distribution throughout the body. In the brain, toluene selectively affects some ion channels. It increases the release of dopamine and the activity of dopaminergic neurons.

Acute intoxication is recognizable thanks to the symptoms of CNS disorders, including euphoria, hal-lucinations, delusions, tinnitus, dizziness, confusion, headache, vertigo, seizures, ataxia, stupor, and coma (Camara-Lemarroy *et al.*, 2015). Repeated chronic expo-sure can lead to white matter damage.

Toluene is detectable in the arterial blood after few seconds of inhalation. Approximately 80% of a dose is metabolized in the liver to hippuric acid and is excreted in urine. Ring hydroxylation to cresols is a minor metabolic pathway (Figure 1). On the other hand, hippuric acid is also a degradation product of different aromatic com-pounds in the digestive tract, while cresols are exclusive degradation products of toluene.

The highest prevalence of volatile solvent misuse worldwide is in marginalized populations [Beckley and Woodward, 2013]. Its background is associated with different conditions. Low educational level, indifferent attitude toward drug use, poor social status, poor health, and efforts to escape from the given problems. Toluene abuse has become widespread, especially among margin-alized young Roma in our region due to its availability and relatively low cost.

Correspondence address:
Dr. Beáta Hubková
Department of Medical and Clinical Biochemistry
Pavol Jozef Šafárik University in Košice, Faculty of Medicine
Trieda SNP 1, 040 11 Košice, Slovakia
E-MAIL: beata.hubkova@upjs.sk

Figure 1. Metabolism of Toluene. Adapted by Yacob & Zinalibdin (2010).
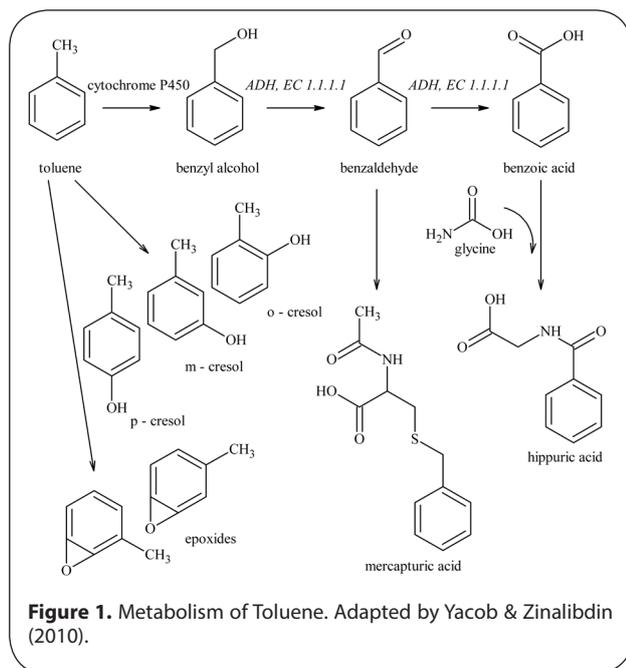
Materials and methods

Participants and data collection

The participants were Roma people living in the segregated settlements in Eastern Slovakia in the Košice County as well as representatives of the majority population from the same region aged 18–55 years, with an average age of 34±8 years (Madarasová Gecková *et al.*, 2014b). The participants recruited via local Roma community workers and general practitioners were divided into 3 categories based on the place of residence:

- Category 1: people from segregated Roma communities, identified as Roma, N=422 (155 men and 267 women)
- Category 2: representatives of the majority population living in the vicinity of the segregated Roma communities, N=134 (62 men and 72 women);
- Category 3: representatives of the majority population living outside the area of segregated Roma communities, N = 214 (107 men and 107 women).

All participants were asked to fill in a questionnaire which contained the following statements regarding toluene abuse: "never tried", "tried once or two times", "toluene user, frequency: less than once a month", "toluene user, frequency: several times a month", "toluene user, frequency: once a week", "toluene user, frequency: several times a week", "toluene user, frequency: daily".

On the day of urine analysis, the participants (after at least 8 hours fasting) obtained detailed instruction about first-morning urine sample collection. Compliance with this request was confirmed upon arrival.

Analysis

The analysis of urinary hippuric acid, *o*-cresol and creatinine was performed in first morning urine samples by reverse phase HPLC (Shimadzu, Japan) on a modified silicagel column with nonpolar octadecyl functional group (Nucleosil standard Expert Column; 100-3 C18, ID 4 mm, length 250 mm, Macherey – Nagel). Urine samples were injected manually in the volume of 40 μL at a flow rate of the mobile phase 0.8 mL/min at 30°C. Analytes were detected by UV/Vis detectors at 280 nm and at 220 nm as well as by fluorescent detector (excitation wavelength 280 nm, emission detected at 350 nm). The mobile phase composition during the isocratic mode of the elution was as follows: acetonitrile (ACN)/water 15/85 (v/v) with 0.05% addition of formic acid. The method performance was evaluated in terms of linearity, limit of detection and limit of quantification for every analyte. Urinary hippuric acid levels were quantified using UV/Vis detector at 220 nm, retention time 8.0 min. The quantification of *o*-cresol was performed based on the fluorescent detector signal at retention time 30 min. Correction according to the hydration was made by calculation of analyte concentration on urine creatinine concentration.

Data were analyzed using IBM SPSS Statistics 22 data analysis software. Statistical analysis was performed via the following statistical tests: Kolmogorov-Smirnov test of normality; Shapiro-Wilk test of normality, non-parametric Wilcoxon test of statistical significance to compare the urine parameters in Roma and in the control groups.

Participation in the study was voluntary and anonymous. Detailed information about our study and its procedures was given to all respondents, and informed consent was obtained prior to medical examination.

Ethic committee approval

The Ethics Committee of the P. J. Šafárik University in Košice, Faculty of Medicine, approved the study (No. 104/2011).

Results

Levels of hippuric acid showed a log normal distribution of values. The median values were in the majority population lower in men compared to women in all investigated populations. The 75th percentile of the values of urinary hippuric acid in Roma men as well as in Roma women was above 570 mg/g creatinine and 690 mg/g creatinine, respectively; while in the majority population the value of the 75th percentile was lower. Non-parametric Wilcoxon test indicated a statistically significant difference in urinary hippuric acid levels in Roma men (Category 1) when compared to men at group 3 (*p*=0.003). Statistically significant difference was observed in Roma women and majority women in Category 2 (*p*=0.0001) as well as in Category 3 (*p*=0.019, Table 1).

Similarly, to the hippuric acid, levels of *o*-cresol showed a log normal distribution of values (both Kolmogorov-Smirnov test of normality as well as the Shapiro-Wilk test of normality had a *p*-value <0.01). The median of the values in the categories did not differ and has a value close to zero μg/g of creatinine. We detected differences in the inter-quartile ranges (75th percentile – 25th percentile)

Table 1. Urinary hippuric acid level of the study respondents.

Category		N	Median [mg/g creatinine]	percentile [mg/g creatinine]		Test of Normality K – S[a] / S – W[b]	Non-parametric Wilcoxon test
				25th	75th		
Men	1	155	338	227.0	573.3	1.09×10^{-19}/ 4.60×10^{-14}	
	2	62	302	180.9	449.5	3.24×10^{-4}/ 5.25×10^{-7}	$p=0.227$
	3	107	250	159.8	416.6	1.17×10^{-2}/ 2.27×10^{-5}	$p=0.0295^{**}$
Women	1	267	506	345.7	698.5	0.200/ 0.702	
	2	72	331	225.1	512.4	4.83×10^{-4}/ 6.22×10^{-9}	$p=0.000140^{***}$
	3	107	436	275.1	596.2	4.46×10^{-5}/ 9.96×10^{-5}	$p=0.0186^{*}$

a) K – S: Kolmogorov-Smirnov test of normality; b) S – W: Shapiro-Wilk test of normality; *p*-values are rounded to three significant digits

Table 2. Urinary o-cresol level of the study respondents.

Category		N	percentile [μg/g creatinine]		Test of normality K – S[a] / S – W[b]	Non-parametric Wilcoxon test
			25th	75th		
Men	1	155	0.000	0.331	6.25×10^{-23}/ 5.58×10^{-14}	
	2	62	0.000	0.315	4.16×10^{-27}/ 6.38×10^{-16}	$p=0.471^{ns}$
	3	107	0.000	0.000	8.05×10^{-35}/ 4.07×10^{-15}	$p=0.0138^{**}$
Women	1	267	0.000	0.316	1.28×10^{-20}/ 2.20×10^{-12}	
	2	72	0.000	0.000	2.76×10^{-38}/ 2.98×10^{-14}	$p=0.0636^{ns}$
	3	107	0.000	0.000	8.09×10^{-28}/ 2.68×10^{-14}	$p=0.813^{ns}$

a) K – S: Kolmogorov-Smirnov test of normality; b) S – W: Shapiro-Wilk test of normality; *p*-values are rounded to three significant digits
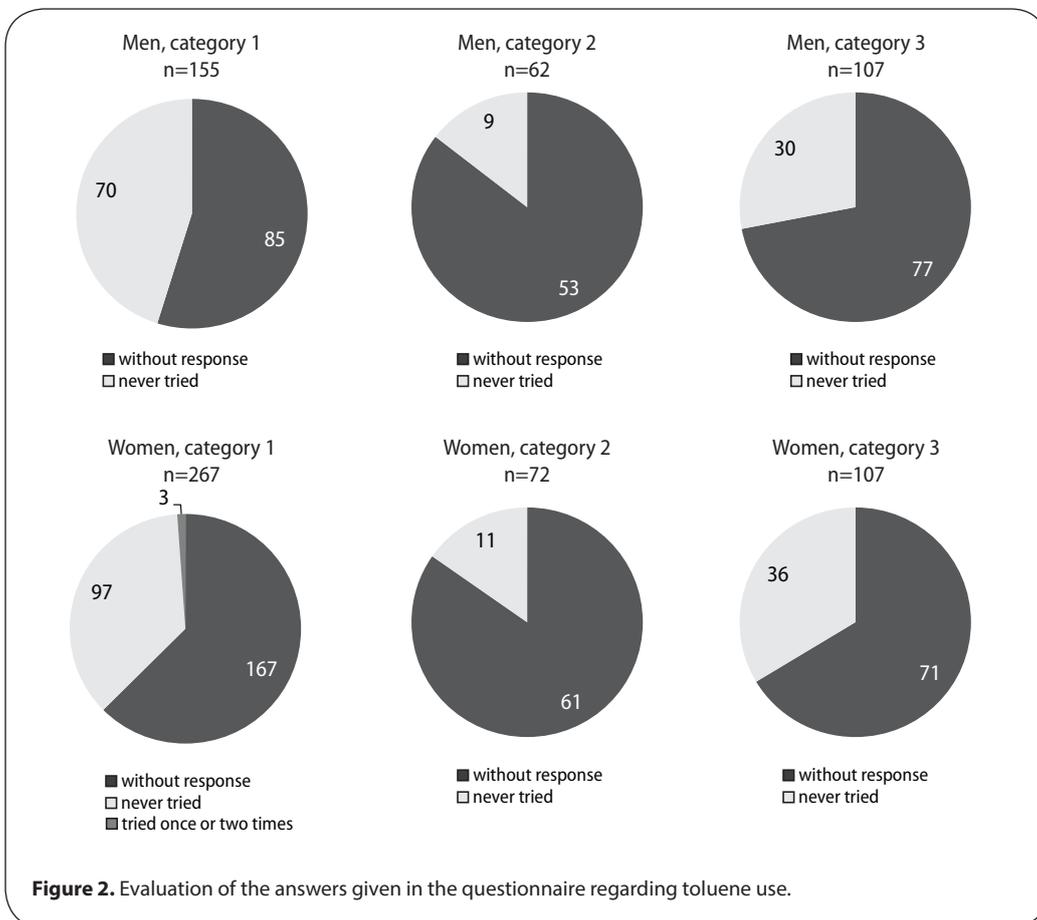

Figure 2. Evaluation of the answers given in the questionnaire regarding toluene use.

of the values among the categories. Non-parametric Wilcoxon test indicated a statistically significant differences in *o*-cresol in Roma men (Category 1) compared to men in Category 3 (*p*=0.014; Tabdle 2).

Results of the questionnaire monitoring toluene use among study participants is shown in Figure 2. The majority of participants did not respond to this question (66.7% of total participants, 66.3% of men and 67.0% of women) at all, or answered "never" (32.9% of total participants, 33.6% of men and of women 32.3%). Answer " tried once or two times " was responded only by three Roma women of Category 1.

From this perspective, it was interesting to compare the urinary toluene metabolite levels with the denied or negative answers given in the questionnaire. The statistical comparison of respondents who admitted toluene use with the other participants was hampered with extremely low number of admitters. The three values of urinary hippuric acid in subjects who admitted toluene use were 191.44, 346.1, and 1049.1 mg/g creatinine. The same was true for the urinary levels of *o*-cresol. The values of urinary *o*-cresol levels of probands who reported experiences with toluene were 0.426, 0.112, and 2.343 μg/g creatinine, respectively.

Discussion

Survey conducted by Važan *et al.* (2011) in seven Roma settlements in Eastern Slovakia showed a 2% incidence of chronic toluene users. In our study, during the evaluation of the questionnaire we detected only three women of the Roma ethnic minority admitting toluene use (0.39% of total study participants and 1.12% of Roma women). Elevated urinary toluene metabolites were observed in 21 cases (2.73% of total study participants), of which 14 were Roma women and 1 Roma man. This comprises 5.24% and 0.65% of Roma women and men, altogether 3.55% of the Roma participants. The reason for the controversy between the results of questionnaire and the metabolite analysis could be as follows:

- Weak response rate – only one participant from three responded to this question at all, although the overall response rate to other questions regarding the health status was much higher (Madarasová Gecková *et al.*, 2014a).
- Feeling of shame.
- Fear of possible consequences after admitting to toluene abuse.
- Participants were aware of the fact that their urine will be analyzed and stopped toluene use some days before the investigation.
- Differences in toluene metabolism between men and women and other factors affecting toluene degradation.
- Gouva *et al.* (2015) devoted a study to shame and anxiety feelings of Roma living in Greece. According to their findings, Roma women are subject to shame more likely than Roma men are. In our study, we find the opposite, although the numbers are low. If anxiety

and shame is associated with increased outrightness when responding a question on drug abuse or not, is an open question

The study of Huang *et al.* (1994) is devoted to metabolization of toluene in 233 toluene-exposed workers. They found that at the same level of toluene exposure, the concentration of urinary toluene metabolites is higher in women compared to men. They also described a lower urinary excretion of hippuric and methyl-hippuric acid in smokers and alcohol consumers after toluene exposition as compared to nonsmokers and abstainers.

Unfortunately, there are no data documenting the relationship between smoking, alcohol consumption and conversion of toluene to cresol. So far, no study was addressed to gender based cresol formation. Our results indicate that the *o*-cresol formation following toluene exposure is similar to the formation of hippuric acid, and is increased in women compared to men.

The poor socio-economic status has undoubtedly considerable influence on the quality of life and is the most common cause of marginalization. From a medical point of view, the marginalized populations represent the most threatened group of the society. The isolated Roma communities constitute the largest group of marginalized people in Eastern Slovakia. Loss of employment, disability, volatile compound – or alcohol addiction, or lack of adaptability to the customs of the majority are the causes but also the consequences of a vcious circle of the lifestyle of marginalized Roma. Their worse health status in comparison with the majority is also confirmed (Hubková *et al.*, 2014). They more likely to suffer from chronic hepatitis B and metabolic syndrome (Janicko *et al.*, 2014), have a higher risk of cardiovascular diseases (Babinska *et al.*, 2013), Roma women have a higher incidence of nephropathy compared to non-Roma females (Rosenberger *et al.*, 2014).

There is a considerable effort to improve the social and health status of marginalized Roma. Some of the projects emerging from the surveys conducted in Roma settlements, such as integration of Roma children in the education process, are already under implementation. However, most of them require long time to achieve perceptible result. Some former ineffective attempt to tackle inhalant abuse ought to be replaced by specifically targeted educational projects focusing on parental and early childhood education.

In contrast to the answers given in the questionnaires about toluene abuse, our work confirmed the suitability of HPLC detection of urinary *o*-cresol as a marker of toluene abuse. Our results indicate that the formation of *o*-cresol after exposure to toluene is more pronounced among women than among men. Furthermore, the results clearly point to the need of using objective laboratory tests and the establishment of cut-off values for the metabolites of toluene as markers of exposition and abuse. From point of view of the public health, the objective HPLC method is a valuable tool for the evaluation of the health status of marginalized population groups in order to address their health problems arising from toluene abuse effectively.

Acknowledgements

REFERENCES

Agency for Toxic Substances and Disease Registry Division of Toxicology and Human Health Sciences. [webpage on the Internet]. Public Health Statement for Toluene. Published on September 2015. [updated 2015 January 21; cited 2017 June 29]. Available from: http:// www.atsdr.cdc.gov/phs/phs.asp?id=159&tid=29

Babinska I, Veselska ZD, Bobakova D, Pella D, Panico S, Reijneveld SA, Jarcuska P, Jarcuska P, Zezula I, Geckova AM, HEPA-META team. (2013). Is the cardiovascular risk profile of people living in Roma Settlemets Worse in Comparison with the Majority Population in Slovakia? *Int J Public Health* **58**: 417–425.

Beckley JT, Woodward JJ. (2013). Volatile Solvents as Drugs of Abuse: Focus on the Cortico-Mesolimbic Circuity. *Neuropsychopharmacol* **38**: 2555–2567.

Camara-Lemarroy CR, Rodríguez-Gutiérrez R, Monreal-Robles R, González-González JG. (2015). Acute toluene intoxication – clinical presentation, management and prognosis: a prospective observational study. *BMC Emerg Med* **15**: 19.

Gouva M, Mentis M, Kotrotsiou S, ParalikasT, Kotrotsiou E. (2015). Shame and Anxiety Feelings of a Roma Population in Greece. *J Immigr Minor Health* **17**: 1765–1770.

Huang MY, Jin C, Liu YT, Li BH, Qu QS, Uchida Y, Inoue O, Nakatsuka H, Watanabe T, Ikeda M. (1994). Exposure of workers to a mixture of toluene and xylenes. I. Metabolism. *Occup Environ Med* **51**: 42–46.

Hubková B, Maslanková J, Stupak M, Guzy J, Kovácová A, Pella D, Jarcuska P, Mareková M, HepaMeta Team. (2014). Assessment of Clinical Biochemical Parameters in Roma Minority Residing in Eastern Slovakia Compared with the Majority Population. *Cent Eur J Public Health* **S2014**: S12–S17.

Janicko M, Senajová G, Drazilová S, Veselíny E, Fedacko J, Siegfried L, Kristian P, Virág L, Pella D, Mareková M, Gecková AM, Kalanin P, Jarcuska P, Halánová M, HepaMeta Team. (2014). Association between metabolic syndrome and hepatitis B Virus Infection in the Roma Population in Eastern Slovakia: A population-based Study. *Cent Eur J Public Health* **S2014**: S37–S42.

Madarasová Gecková A, Babinská D, Veselská ZD, Bosáková L, Kolarcik P, Jarcuska P, Pella D, Halánová M, HepaMeta Team. (2014a). Socioeconomic Characteristics of the Population Living in Roma Settlements and Their Association with Health and Health-related Behaviour. *Cent Eur J Public Health* **S2014**: S57–S64.

Madarasová Gecková A, Jarcuska P, Mareková M, Pella D, Siegfried L, Jarcuska P, Halánová M, HepaMeta Team. (2014b). HepaMeta – Prevalence of Hepatitis B/C and Metabolic Syndrome in Population Living in Separated and Segregated Roma Settlements: a Methodology for a Cross-sectional Population-Based Study Using Community-Based Approach. *Cent Eur J Public Health* **S2014**: S6–S11.

Rosenberger J, Majrníková M, Jarcuska P, Pella D, Mareková M, Gecková AM, Halánová M, HepaMeta Team. (2014). Higher Prevalence of Nephropathy in Young Roma Females Compared with Non-Roma Females. *Cent Eur J Public Health.* **S2014**: S28–S31.

Važan P, Khan MR, Poduška O, Stastná L, Miovský M. (2011). Chronic toluene misuse among Roma youth in Eastern Slovakia. *Subst Use Misuse* **46**: 57–61.

Yacob AR, Zinalibdin MR. (2010). High Performance Liquid Chromatography Determination of Urinary Hippuric Acid and Benzoic Acid as Indices for Glue Sniffer urine. *World Academy of Science Engineering and Technology International Journal of Chemical, Molecular, Nuclear, Materials and Metallurgical Engineering* **4**: 237–242.