APPENDIX A6.1.3.8 TOLUENE - FACTSHEET

	HPHC	BoExp assessed by PMI
IUPAC name	Methylbenzene	S-benzylmercapturic acid
Usual abbreviation		S-BMA; SBMA
Other names	Toluene, toluol, methacide, methylbenzol, phenylmethane	N-Acetyl-S-benzyl-L-cysteine; S-benzyl-N-acetyl-L-cysteine (SBAC)
$CAS number	108-88-3	19542-77-9
Formula and molecular weight	C_7H_8 MW: 92.14	$C_{12}H_{15}NO_3S$ MW: 253.31

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Main characteristics

Chemical class	Monocyclic aromatic hydrocarbons
HPHC lists	FDA-93 [1]; FDA-18 [2]; HC [3, 4]; PMI-58; WHO-39 [5]
Source in cigarettes	Amount in MSS (3R4F): 73.6 µg (ISO); 208 µg (HCI) [6] Smoke phase: gas/vapor (majority) and particulate phases (<5 %) [7, 8] Presence in tobacco / pyrosynthesis (T°): can be found in uncombusted tobacco [9] / Main generation range 500–800°C [10]; 500–800°C [11]; precursors in tobacco are similar to those for isoprene and benzene, i.e . solanesol [12], organic acids (in particular stearic acid), terpenes, polyphenols [13-15], resinous and ether-extractable compounds [16]. In addition, proteins and sugars were found to form toluene upon pyrolysis [14].
Other sources [17, 18]	Toluene is released into the atmosphere principally from the volatilization of petroleum fuels and toluene-based solvents and thinners and from motor vehicle exhaust. Toluene's production and use as an intermediate in the production of benzoic acid, benzaldehyde, benzene, explosives, dyes and many other organic compounds may also result in its release to the environment through various waste streams. Toluene has been detected in emissions from volcanos, forest fires and crude oil.
Toxicity class (FDA) [1]	RT, RDT
Toxicity – CA [19]	IARC: group 3: not classifiable as to its carcinogenicity to humans [19] Considering the multiple exposure circumstances in eight studies mentioning exposure to toluene and the weak consistency of findings, the results are not strong enough to conclude that there is an association.
Toxicity – RT	In humans, respiratory tract irritation is experienced from exposure to toluene. Irritation of the upper airways and degeneration of the nasal epithelium have been observed in animal studies [17].
Toxicity – CT	-

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Toxicity – RDT	Reproductive toxicity has been observed in exposed humans (spontaneous abortion) and rats (high fetal mortality and retardation of embryonic growth) [19].
Toxicity – AD	-
Toxicity - others	Reversible neurological symptoms from acute exposure progressing from fatigue, headache, and decreased manual dexterity to narcosis with increasing exposure level, degenerative changes in white matter in chronic solvent abusers, and subtle changes in neurological functions including cognitive and neuromuscular performance, hearing, and color discrimination in chronically exposed workers [17]. All available toxicity data can be found on the Website Aggregated Computational Toxicology Resource (ACToR) [20].

ADME properties

Absorption and distribution [17] - Studies with volunteers and laboratory animals indicate that toluene is readily absorbed from the respiratory tract and, to a lesser extent, through the skin. Animals given toluene orally or by inhalation had high concentrations of toluene in their adipose tissue, brain, and bone marrow, and moderately high concentrations of toluene and its metabolites in liver and kidney. Autopsies of toluene-exposed humans indicate that absorbed toluene is distributed to lipid-rich and highly vascular tissues such as the brain.

Metabolism [17] - The liver is expected to be the prime site of toluene metabolism, based on the high concentration of cytochrome P450 (CYP) isozymes in the liver relative to other tissues. A scheme for toluene metabolism in humans and animals is presented in Figure 1. The primary initial steps in toluene metabolism in humans and laboratory animals is hydroxylation of the methyl side-chain (to form benzyl alcohol) catalyzed predominately by the CYP isozyme CYP2E1, followed by oxidation to benzoic acid. Most of the benzoic acid is then conjugated with glycine to form hippuric acid, but a small portion can be conjugated with UDP-glucuronate to form the acyl-glucuronide. Studies with volunteers and human liver microsomes indicate that a very small portion (<1–5%) of absorbed toluene can be metabolized by CYP1A2, CYP2B2, or CYP2E1 to ortho- or para-cresol (i.e. *o*- and *p*-cresol), which are excreted in the urine as sulfate or glucuronate conjugates.

Benzyl alcohol is thought to be converted to benzoic acid in two steps by alcohol dehydrogenase and aldehyde dehydrogenase. Hippuric acid formation from benzoic acid (a common component of the diet) is catalyzed by acyl-CoA synthetase and acyl-CoA:amino acid N-acyltransferase. Conjugation of benzoic acid with glucuronic acid to form benzoyl glucuronide is catalyzed by UDP-glucuronyl transferase.

S-benzylmercapturic acid, a minor urinary metabolite identified in humans, is thought to be formed via conjugation of benzyl alcohol with glutathione (catalyzed by glutathione-S-transferases), followed by the concerted catalytic actions of γ-glutamyltranspeptidase,

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amino peptidase M, and N-acetyltransferase to release glutamic acid and glycine and add an acetyl group [21]. The formation of another minor human urinary metabolite, S-p-toluylmercapturic acid, is thought to proceed by a similar series of reactions from the proposed intermediate, 3,4-toluene epoxide [21].

The kinetics and metabolism of toluene in humans, rats and mice are very similar: the hippurate is in all cases by far the major metabolite, while in all species the ortho- and para-cresols are minor metabolites [22].

Excretion [17] - In both humans and rats, up to about 75–80% of inhaled toluene that is absorbed can be accounted for as hippuric acid in the urine [17, 21, 23]. Much of the remaining toluene is exhaled unchanged. In humans exposed by inhalation, rates of urinary excretion of ortho-cresol were about 1,000-fold lower than excretion rates for hippuric acid. The excretion of toluene and its metabolites is rapid, with the major portion occurring within 12 hours of exposure. No information is available regarding the in vivo conversion of toluene to BMA and whether it is slower than that to hippuric acid or o-cresol. By analogy to the half-life of 9h for S-phenylmercapturic acid excretion after occupational exposure to benzene, the half-life for S-BMA in men can, however, be estimated to be no more than 10h [24].

Analyses of kinetic data for toluene concentrations in blood, exhaled breath, and adipose tissue following inhalation exposure of humans and rats indicate that even though most absorbed toluene is rapidly eliminated from the body, a smaller portion (that which gets into adipose tissues) is slowly eliminated. Elimination half-lives ranged from about 12 to 65 hours in subcutaneous adipose tissue samples taken from 12 subjects at several times within 8 days of cessation of exposure to about 80 ppm toluene for four consecutive 30-minute periods. Increasing elimination half-lives were significantly correlated with increasing amounts of body fat [17].

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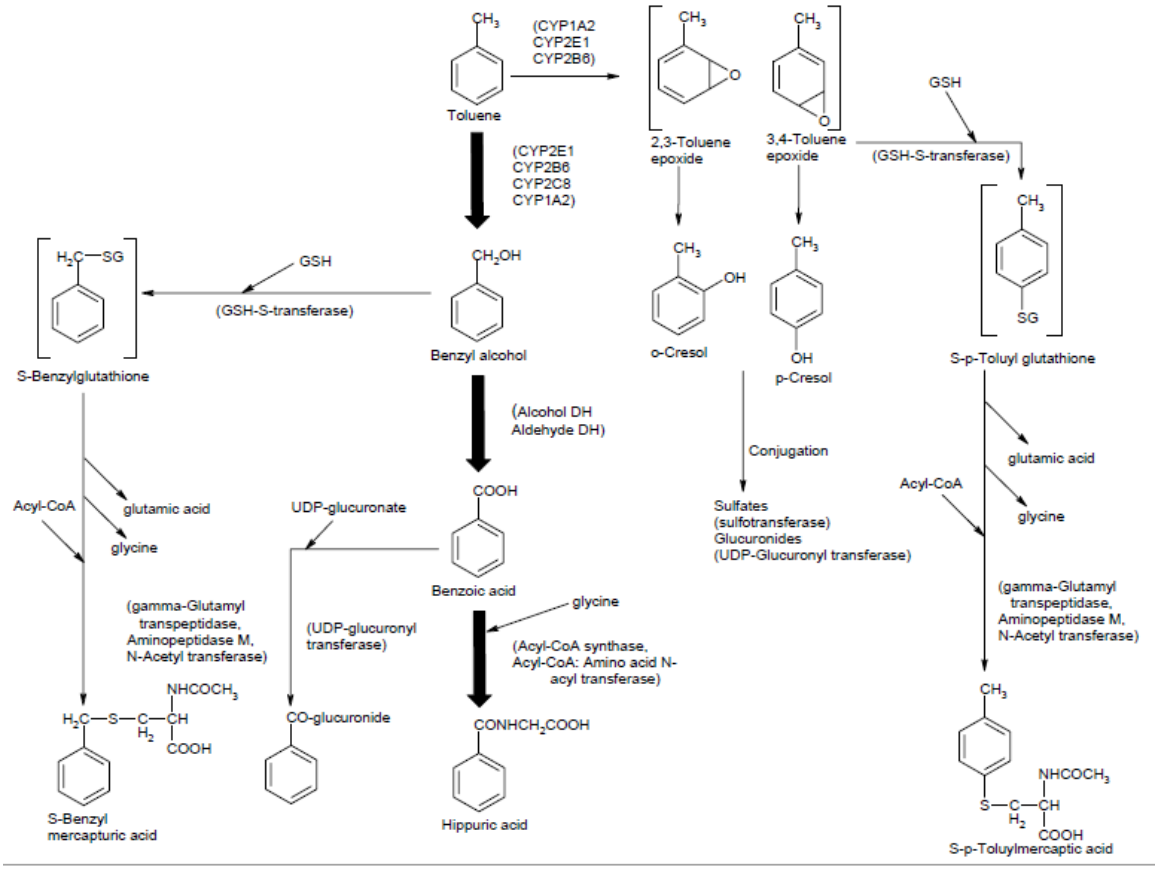


Figure 1 Toluene metabolism in humans and animal [17]

Figure source: Agency for Toxic Substances and Disease Registry

Exposure levels in smokers and non-smokers

Several studies have reported a correlation between occupational exposure to toluene and urinary excretion of hippuric acid, o-cresol, and *p*-cresol [17]. However, there are individual differences in the amounts of these excreted metabolites, and monitoring of urinary excretion of metabolites can only serve as a qualitative indication of exposure to toluene [17]. Hippuric acid excretion varied widely between subjects and the total amount of hippuric acid excreted was about 4 times greater than what would have been generated by toluene exposure alone. This indicates that there are a number of hippuric acid precursors present in the environment (e.g., benzoic acid in food) and exposure to these compounds varies, making hippuric acid excretion a poor indicator of toluene exposure. The consumption of green tea and/or coffee, for example, can result in an overestimation of urinary hippuric acid concentrations and cause false-positive results during the biological monitoring of workers exposed to low doses of toluene [25]. Both green tea and coffee can be converted to benzoic acid by bacterial flora in the colon, another precursor of hippuric acid [25]. In addition to the individual differences for urinary metabolites, possible ethnic differences in the excretion of hippuric acid and *o*-cresol have also been reported in Chinese, Turkish, and Japanese workers exposed to toluene (in printing, electric apparatus and shoe factories) [26]. Significant differences in

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o- and *p*-cresol/hippuric acid ratios in the urine were also seen in four different strains of rats exposed to toluene [27]. Measurement of *o*-cresol has shown high interindividual variability, and seems to lack sensitivity and specificity [28]. Unchanged toluene in urine was reported to be a suitable BoExp to toluene, with higheier specificity and sensitivity, and lower backgroung valudes than *o*-cresol, however, toluene levels were not influenced by cigarette smoking, only by airborne exposure [28]. S-BMA has been determined as a biomarker of exposure to toluene in environmental [29] and occupational studies [24, 30-34], although the sensitivity of analysis at low (occupational) exposure levels has been questioned [35, 36].

S-BMA is considered to have a greater validity to evaluate exposure to toluene than hippuric acid or *o*- and *p*-cresol [24]. In an occupational study, urinary S-BMA concentration could distinguish the group with the highest exposure (tanker drivers) from the other two groups, and S-BMA concentration was significantly correlated with the concentrations of airborne toluene in the exposed groups and the entire sample [37]. The presence of background levels of S-BMA in the general population with no occupational exposure to toluene was observed in the controls. As to its origin, it did not seem to be attributable to cigarette smoking, as also reported by other authors [30, 38-40], and, according to the authors, could only be partly explained by the ubiquitous toluene pollution deriving from widely used consumer products.

In studies which have considered smokers and non-smokers (Table 1), determination of *S*-BMA using a high-performance liquid chromatography (HPLC) derivatization method with a limit of detection of 0.5 µg *S*-BMA/L urine could not differentiate between smokers and non-smokers, but was sufficient for determination of occupational toluene exposure [29]. Similarly, two other studies in which occupational exposure to toluene was assessed also failed to differentiate between smokers and non-smokers [30, 37]. A more recent study using HPLC and tandem mass spectrometry (HPLC-MS/MS) for the determination of *S*-BMA and S-PMA report a limit of determination of approximately 0.2 ng/mL for both compounds, but S-BMA could still not differentiate smokers and non-smokers, likely because of the high background exposure to toluene [41]. A German study using HPLC-MS/MS reports median levels of 8.2 (range: 1.6-77.4 µg/L) and 11.5 (range: 0.9-51.2 µg/L) in non-smokers and smokers, respectively, failing to confirm smoking status [38].

The reason for this difficulty to discriminate smokers from non-smokers based on the urinary levels of S-BMA has not been clarified, but the speculations drawn by Schettgen et al. [38] are either 1) that other environmental sources of toluene are contributing in such a way to the individual exposure that smoking is only a minor factor, or 2) that the specificity of S-BMA excretion as a biomarker of toluene exposure is insufficient for the determination of environmental exposures and possibly other metabolic routes might in part also lead to the formation (and excretion) of S-BMA. Benzyl alcohol, which is a widespread constituent of cosmetic products like shampoo, creams, after-shave lotions or fragrances, could largely influence the excretion of S-BMA. The presence of background levels of S-BMA in the general population with no occupational exposure to toluene has indeed been observed, even in the absence of cigarette smoking [25].

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Table 1 Representative urinary S-BMA levels in smokers and non-smokers

Author (year)	Levels in non-smokers (or ex-smokers)	Levels in smokers	Units; variability
Imbriani (1999) [30]	10. 2 ± 11.9 [10200 ± 11900]	9.1 ± 9.3 [9100 ± 9300]	µg/g creat; mean ± SD [pg/mg creat; mean ± SD]
Schettgen (2008) [38]	8.2 (1.6–77.4)	11.5 (0.9–51.2)	µg/L; ranges
Lovreglio (2010) [37]	8.58 ± 10.10 [8580 ± 10100]	10.27 ± 9.40 [10270 ± 9400]	µg/g creat; mean ± SD [pg/mg creat; mean ± SD]
Maestri (1997) [29]	<0.5 - 32.7 [<500-32700]	<0.5 - 29.9 [<500-29900]	µg/g creat; mean ± SD [pg/mg creat; mean ± SD]
B'Hym (2011) [41]	8.2 ± 7.8	10.5 ± 10.2	ng/mL; mean ± SD
Pluym (2015) [42]	3.1 (0.58-19.6) [3100 (580-19600)]	2.3 (1.36-4.39) [2300 (1360-4390)]	µg/g creat; median (min-max) [pg/mg creat; median (min-max)]
Alwis (2012) [43]	15 ± 32	16 ± 29	ng/mL; mean ± SD

List of abbreviations

3R4F	Kentucky 3R4F reference research cigarette [44]
ACToR	Aggregated Computational Toxicology Resource
AD	Addictive
ADME	Absorption and Distribution, Metabolism, and Excretion
CA	Carcinogen
CAS number	Chemical Abstracts Service number
Creat	Creatinine
CT	Cardiovascular toxicant
CYP	Cytochrome P450
FDA	Food and Drug Administration
HC	Health Canada
HCI	Health Canada Intense regime [45]
HPHC	Harmful and Potentially Harmful Constituents
HPLC	High-performance liquid chromatography
HPLC-MS/MS	High performance liquid chromatography-tandem mass spectrometry
IARC	International Agency for Research on Cancer
ISO	International Organization for Standardization smoking regime [46]
IUPAC	International Union of Pure and Applied Chemistry
MSS	Main stream smoke
MW	Molecular weight
PMI	Philip Morris International
RDT	Reproductive or developmental toxicant

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RT	Respiratory toxicant
SD	Standard deviation
WHO	World Health Organization

References

[1] U.S. Department of Health and Human Services, FDA (Food and Drug Administration). [Docket No. FDA-2012-N-0143]: harmful and potentially harmful constituents in tobacco products and tobacco smoke; established list. Fed Regist. 2012; 77(64):20034-7.

[2] FDA (Food and Drug Administration). Guidance for industry - Reporting harmful and potentially harmful constituents in tobacco products and tobacco smoke under Section 904(a)(3) of the Federal Food, Drug, and Cosmetic Act - Draft guidance. 2012.

[3] Health Canada. Tobacco reporting regulations. Modified 2011; Available from: http://www.hc-sc.gc.ca/hc-ps/tobac-tabac/legislation/reg/indust/method/index-eng.php#main (Accessed on 21 May 2014).

[4] Health Canada. Consolidation - Tobacco reporting regulations. DORS/2000-273. 2013.

[5] WHO Study Group, Ashley DL, Ayo-Yusuf OA, Boobis AR, da Costa e Silva VL, Djordjevic MV, Gray N, Gupta P, Hammond SK, Hatsukami D, Henningfield J, Opperhuizen A, Zaatari G. WHO study group on tobacco product regulation - Report on the scientific basis of tobacco product regulations: fifth report of a WHO study group. 2015.

[6] Roemer E, Schramke H, Weiler H, Buettner A, Kausche S, Weber S, Berges A, Stueber M, Muench M, Trelles-Sticken E, Pype J, Kohlgrueber K, Voelkel H, Wittke S. Mainstream smoke chemistry and in vitro and in vivo toxicity of the reference cigarettes 3R4F and 2R4F. Beitrage zur Tabakforschung International/ Contributions to Tobacco Research. 2012; 25(1):316-35.

[7] Baker RR. Smoke generation inside a burning cigarette: modifying combustion to develop cigarettes that may be less hazardous to health. Progress in Energy and Combustion Science. 2006; 32(4):373-85.

[8] Moldoveanu S, Coleman Iii W, Wilkins J. Determination of benzene and toluene in exhaled cigarette smoke. Beitrage zur Tabakforschung International/ Contributions to Tobacco Research. 2008; 23(2):107-14.

[9] Rodgman A, Perfetti TA. The chemical components of tobacco and tobacco smoke 2nd ed: CRC Press, Taylor & Francis Inc (United States); 2013.

[10] Torikai K, Yoshida S, Takahashi H. Effects of temperature, atmosphere and pH on the generation of smoke compounds during tobacco pyrolysis. Food Chem Toxicol. 2004; 42(9):1409-17.

[11] Burton HR, Childs GJ. The thermal degradation of tobacco. VI. Influence of extraction on the formation of some major gas phase constituents. Beiträge zur Tabakforschung. 1975; 8(4):174-80.

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[12] Scholtzhauer WS, Severson RF, Chortyk OT, Arrendale RF, Higman HC. Pyrolytic formation of polynuclear aromatic hydrocarbons from petroleum ether extractable constituents of flue-cured tobacco leaf. J Agric Food Chem. 1976; 24(5):992-7.

[13] Piade JJ, Wajrock S, Jaccard G, Janeke G. Formation of mainstream cigarette smoke constituents prioritized by the World Health Organization - Yield patterns observed in market surveys, clustering and inverse correlations. Food Chem Toxicol. 2013; 55:329-47.

[14] Torikai K, Uwano Y, Nakamori T, Tarora W, Takahashi H. Study on tobacco components involved in the pyrolytic generation of selected smoke constituents. Food Chem Toxicol. 2005; 43(4):559-68.

[15] Torikai K, Uwano Y, Nakamori T, Tarora W, Takahashi H. Erratum to "Study on tobacco components involved in the pyrolytic generation of selected smoke constituents" [Food and Chemical Toxicology 43 (2005) 559–568] Food Chem Toxicol. 2005; 43(9):1449. Erratum in Food Chem Toxicol. 2005 Sep;43(9):1449. Torikaiu, K [corrected to Torikai, K]. .

[16] Obi Y, Muramatsu M, Shimada Y. Evaluation of tobacco quality from pyrolytic aspects. Agric Biol Chem. 1970; 34(8):1220-3.

[17] U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. Toxicological profile for toluene. 2000; Available from: http://www.atsdr.cdc.gov/toxprofiles/tp56.pdf (Accessed on 09 December 2014).

[18] National Center for Biotechnology Information (NCBI). Compound Summary for CID 1140: toluene. Available from: http://pubchem.ncbi.nlm.nih.gov/compound/1140?from=summary (Accessed on 15 January 2015).

[19] IARC (International Agency for Research on Cancer). Monographs on the evaluation of carcinogenic risks to humans: re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide: re-evaluation of some organic chemicals, hydrazine and hydrogen peroxide. 2-nitropropane. 1999; Vol. 71:1079-94.

[20] Aggregated Computational Toxicology Resource (ACToR). Chemical summary: toluene (108-88-3). Available from: http://actor.epa.gov/actor/GenericChemical?casrn=108-88-3 (Accessed on 15 September 2014).

[21] Angerer J, Schildbach M, Kramer A. S-p-toluylmercapturic acid in the urine of workers exposed to toluene: a new biomarker for toluene exposure. Arch Toxicol. 1998; 72(2):119-23.

[22] IARC (International Agency for Research on Cancer). Monographs on the evaluation of carcinogenic risks to humans; re-evaluation of some organic chemicals hydrazine and hydrogen peroxide: toluene. 1989; Vol. 71.

[23] Wang RS, Nakajima T. Effects of ethanol and phenobarbital treatments on the pharmacokinetics of toluene in rats. Br J Ind Med. 1992; 49(2):104-12.

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[24] Inoue O, Kanno E, Kasai K, Ukai H, Okamoto S, Ikeda M. Benzylmercapturic acid is superior to hippuric acid and o-cresol as a urinary marker of occupational exposure to toluene. Toxicol Lett. 2004; 147(2):177-86.

[25] Munaka M, Katoh T, Kohshi K, Sasaki S. Influence of tea and coffee on biomonitoring of toluene exposure. Occup Med (Lond). 2009; 59(6):397-401.

[26] Inoue O, Seiji K, Watanabe T, Kasahara M, Nakatsuka H, Yin SN, Li GL, Cai SX, Jin C, Ikeda M. Possible ethnic difference in toluene metabolism: a comparative study among Chinese, Turkish and Japanese solvent workers. Toxicol Lett. 1986; 34(2-3):167-74.

[27] Inoue O, Seiji K, Ishihara N, Kumai M, Ikeda M. Increased o- and p-cresol/hippuric acid ratios in the urine of four strains of rat exposed to toluene at thousands-ppm levels. Toxicol Lett. 1984; 23(2):249-57.

[28] Fustinoni S, Mercadante R, Campo L, Scibetta L, Valla C, Consonni D, Foa V. Comparison between urinary o-cresol and toluene as biomarkers of toluene exposure. J Occup Environ Hyg. 2007; 4(1):1-9.

[29] Maestri L, Ghittori S, Imbriani M. Determination of specific mercapturic acids as an index of exposure to environmental benzene, toluene, and styrene. Ind Health. 1997; 35(4):489-501.

[30] Imbriani M, Ghittori S, Cavalleri A. [Significance of urinary concentrations of S-benzyl-N-acetylcysteine (S-BMA) in subjects exposed to toluene] Italian. G Ital Med Lav Ergon. 1999; 21(4):329-33.

[31] Buratti M, Brambilla G, Fustinoni S, Pellegrino O, Pulviremti S, Colombi A. Determination of monobromobimane derivatives of phenylmercapturic and benzylmercapturic acids in urine by high-performance liquid chromatography and fluorimetry. J Chromatogr B Biomed Sci Appl. 2001; 751(2):305-13.

[32] Inoue O, Kanno E, Yusa T, Kakizaki M, Ukai H, Okamoto S, Higashikawa K, Ikeda M. Urinary benzylmercapturic acid as a marker of occupational exposure to toluene. Int Arch Occup Environ Health. 2002; 75(5):341-7.

[33] Barbieri A, Sabatini L, Accorsi A, Roda A, Violante FS. Simultaneous determination of t,t-muconic, S-phenylmercapturic and S-benzylmercapturic acids in urine by a rapid and sensitive liquid chromatography/electrospray tandem mass spectrometry method. Rapid Commun Mass Spectrom. 2004; 18(17):1983-8.

[34] Chou JS, Lin YC, Ma YC, Sheen JF, Shih TS. Measurement of benzylmercapturic acid in human urine by liquid chromatography-electrospray ionization-tandem quadrupole mass spectrometry. J Anal Toxicol. 2006; 30(5):306-12.

[35] Aylward LL, Barton HA, Hays SM. Biomonitoring Equivalents (BE) dossier for toluene (CAS No. 108-88-3). Regul Toxicol Pharmacol. 2008; 51(3 Suppl):S27-36.

[36] Inoue O, Kawai T, Ukai H, Maejima Y, Fukui Y, Ohashi F, Okamoto S, Takada S, Sakurai H, Ikeda M. Limited validity of o-cresol and benzylmercapturic acid in urine as biomarkers of occupational exposure to toluene at low levels. Ind Health. 2008; 46(4):318-25.

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[37] Lovreglio P, Barbieri A, Carrieri M, Sabatini L, Fracasso ME, Doria D, Drago I, Basso A, D'Errico MN, Bartolucci GB, Violante FS, Soleo L. 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. 2010; 83(3):341-56.

[38] Schettgen T, Musiol A, Alt A, Kraus T. 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 Analyt Technol Biomed Life Sci. 2008; 863(2):283-92.

[39] Sabatini L, Barbieri A, Indiveri P, Mattioli S, Violante FS. Validation of an HPLC-MS/MS method for the simultaneous determination of phenylmercapturic acid, benzylmercapturic acid and o-methylbenzyl mercapturic acid in urine as biomarkers of exposure to benzene, toluene and xylenes. J Chromatogr B Analyt Technol Biomed Life Sci. 2008; 863(1):115-22.

[40] Takahashi S, Kagawa M, Shiwaku K, Matsubara K. Determination of S-benzyl-N-acetyl-L-cysteine by gas chromatography/mass spectrometry as a new marker of toluene exposure. J Anal Toxicol. 1994; 18(2):78-80.

[41] B'Hymer C. Validation of an HPLC-MS-MS method for the determination of urinary S-benzylmercapturic acid and S-phenylmercapturic acid. J Chromatogr Sci. 2011; 49(7):547-53.

[42] Pluym N, Gilch G, Scherer G, Scherer M. Analysis of 18 urinary mercapturic acids by two high-throughput multiplex-LC-MS/MS methods. Anal Bioanal Chem. 2015; 407(18):5463-76.

[43] Alwis KU, Blount BC, Britt AS, Patel D, Ashley DL. Simultaneous analysis of 28 urinary VOC metabolites using ultra high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-ESI/MSMS). Anal Chim Acta. 2012; 750:152-60.

[44] Chepiga TA, Morton MJ, Murphy PA, Avalos JT, Bombick BR, Doolittle DJ, Borgerding MF, Swauger JE. A comparison of the mainstream smoke chemistry and mutagenicity of a representative sample of the US cigarette market with two Kentucky reference cigarettes (K1R4F and K1R5F). Food Chem Toxicol. 2000; 38(10):949-62.

[45] Health Canada, Tobacco Control Programme. Determination of “tar”, nicotine and carbon monoxide in mainstream tobacco smoke. T-115. 1999; Available from: http://edge.rit.edu/edge/P10056/public/Health%20Canada%20Nicotine (Accessed on 06 January 2016).

[46] International Organization for Standardization (ISO). Routine analytical cigarette smoking machine - Definitions and standard conditions (ISO 3308:2012). Out of print. 2000; Revised version (2012) available from: http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=60404.

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