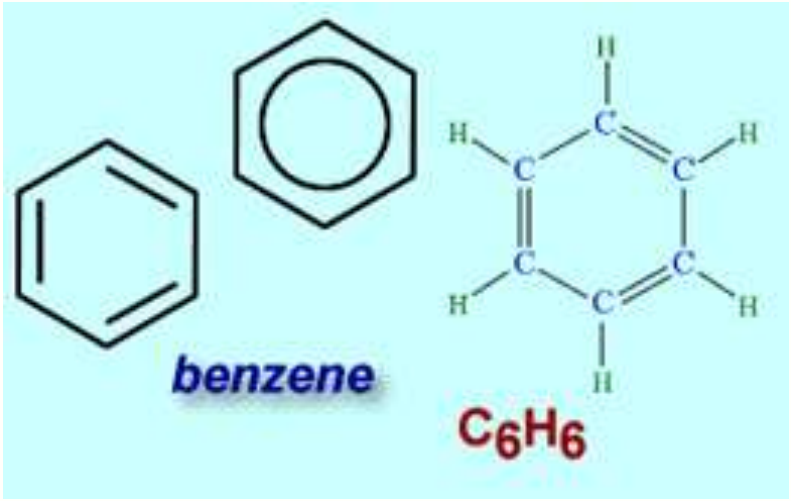
Department of Occupational Medicine - Formerly ISPESL
National Institute of Occupational Safety and Prevention

"Low levels of exposure to benzene: which biomarker is best for risk assessment ?

Research

Giovanna Tranfo
03/19/2012



Benzene is a known carcinogenic substance for humans (IARC group 1) and a widely used chemical. Being an ubiquitous pollutant, environmental exposure even to low doses is a common problem.

Occupational Exposure

Therefore, benzene is one of the main concerns for occupational medicine today: occupational exposure can occur in urban workers, chemical industry and specially in oil plants.






The benzene exposure biomarkers can be classified in three groups:

1. **dose biomarkers**

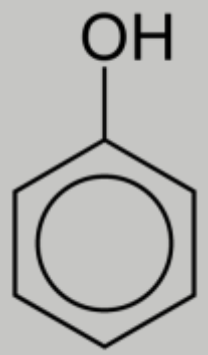
2. **effect biomarkers**

3. **susceptibility markers**

Risk assessment is based mainly on dose biomarkers, corroborated by information about susceptibility and effect biomarkers

A little of history….

The first dose biomarker adopted by ACGIH in 1987 for the assessment of benzene occupational exposure was urinary **phenol**:



for a benzene TLV TWA exposure of 10 ppm, the suggested BEI until 1996 was 50 mg/l in the urine at the end of the work shift.

Because of phenol low specificity the ACGIH recommended also to monitor **benzene**

in exhaled breath prior to the next shift

with an acceptable maximum concentration of

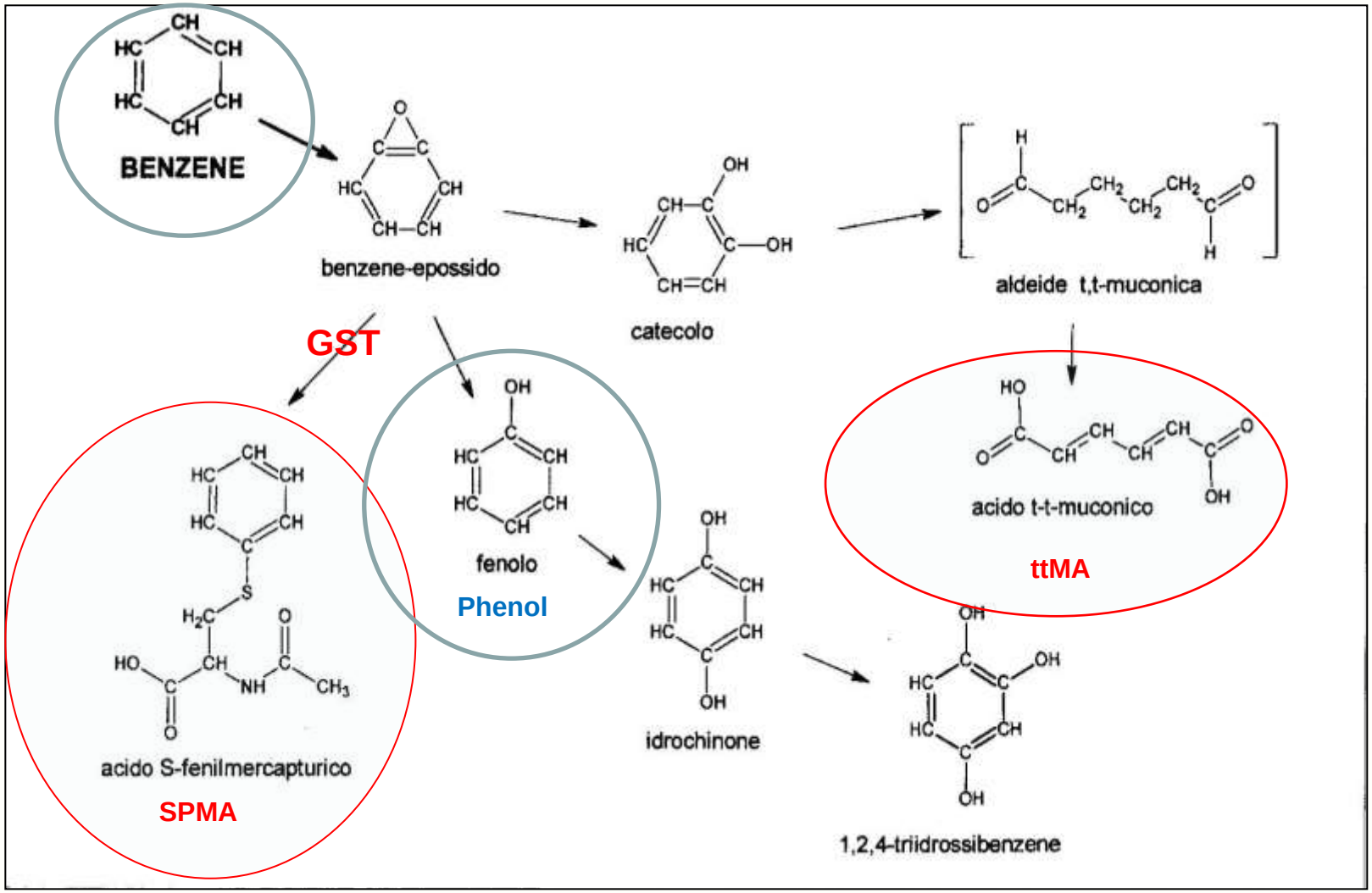
0.08 ppm for mixed-exhaled breath

and 0.12 ppm for end-exhaled breath.

SPMA and t,t-MA

- In 1997 S-phenylmercapturic acid (SPMA) was adopted and in 2000 trans,trans-muconic acid (t,t-MA) was added.
- The ACGIH BEI measured in the end-shift urine of workers are the following:
- **SPMA 25 µg/g of creatinine**
- **t,t-MA 500 µg/g of creatinine**

Benzene metabolic pathway



Glutathione-S-Transferase (GST) enzymes conjugate glutathione to toxic molecules (*benzene*) forming metabolites easier to be excreted (*SPMA*)

Confounding factors

Cigarette smoking causes the inhalation of significant amounts of benzene, besides many other toxic substances. Therefore smoking is an important confounding factor in the benzene exposure assessment, and the smoking habit must be investigated by means of interviews with the workers.

If very low levels of occupational benzene exposure are reached nowadays thanks to the growing culture of prevention and to the reinforcement of the laws on safety and health at work, occupational exposure to benzene appears to be negligible in comparison with that deriving from cigarette smoking.

Journal of Chromatography B, 878 (2010) 2502–2505

Contents lists available at ScienceDirect

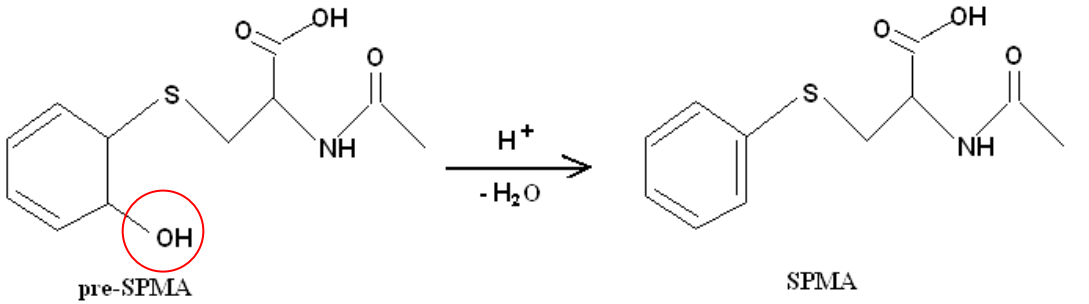
Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Enrichment and properties of urinary pre-S-phenylmercapturic acid (pre-SPMA)☆

Katharina Sterz[a], Dominique Köhler[a], Thomas Schettgen[b], Gerhard Scherer[a,*]

In urine samples of benzene exposed subjects, a precursor of SPMA is present : N-acetyl-S (1,2-dihydro-2-hydroxyphenyl)-L-cysteine (pre-SPMA). It can be transformed into SPMA by means of acid hydrolysis. The amount of SPMA measured therefore depends on the degree of hydrolysis, that is dependent on the urine pH, on the sample storage conditions and on the analytical pretreatment.



pre-SPMA SPMA

Therefore acidic hydrolysis before analysis is mandatory!

t,t-MA and SPMA urinary concentrations can be determined quantitatively using HPLC-MS/MS a very sensitive and specific technique, using isotopic dilution in order to compensate for the matrix effect

Biomarkers, March–April 2007; 12(2): 111–122

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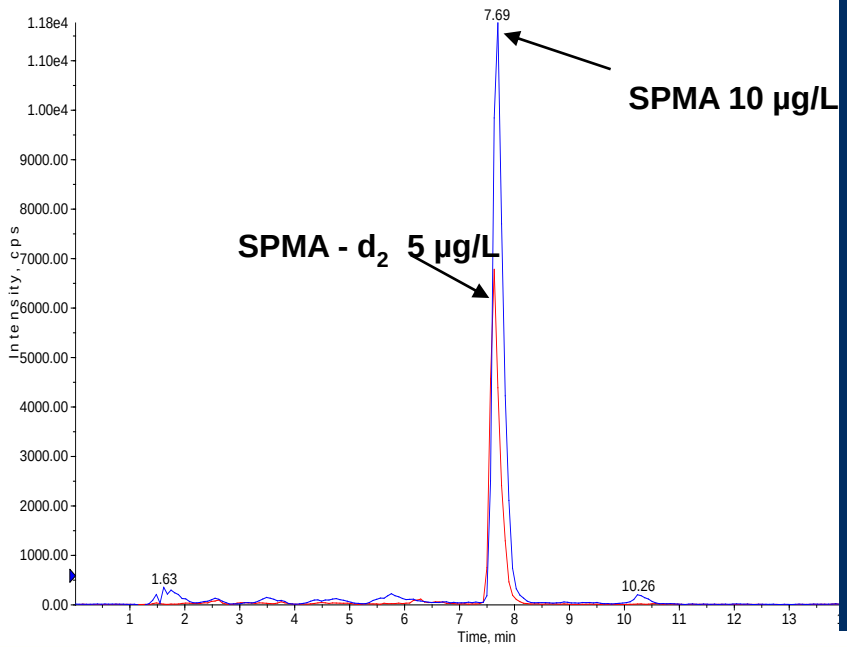
INAIL

Determination of free and total *S*-phenylmercapturic acid by HPLC/MS/MS in the biological monitoring of benzene exposure

E. PACI, D. PIGINI, A. M. CIALDELLA, P. FARANDA, & G. TRANFO

Italian Institute for Occupational Safety and Prevention (ISPESL), Occupational Hygiene Department, Monte Porzio Catone, Italy



Quantitative analysis has been performed using a commercial deuterium isotope of SPMA as internal standard.

Transitions:

-238.1 → -109.1 for S-PMA

-240.1 → -109.1 for the deuterium-labeled internal standard.

LOD 0.05 µg/L, and LLOQ 0.1 µg/L

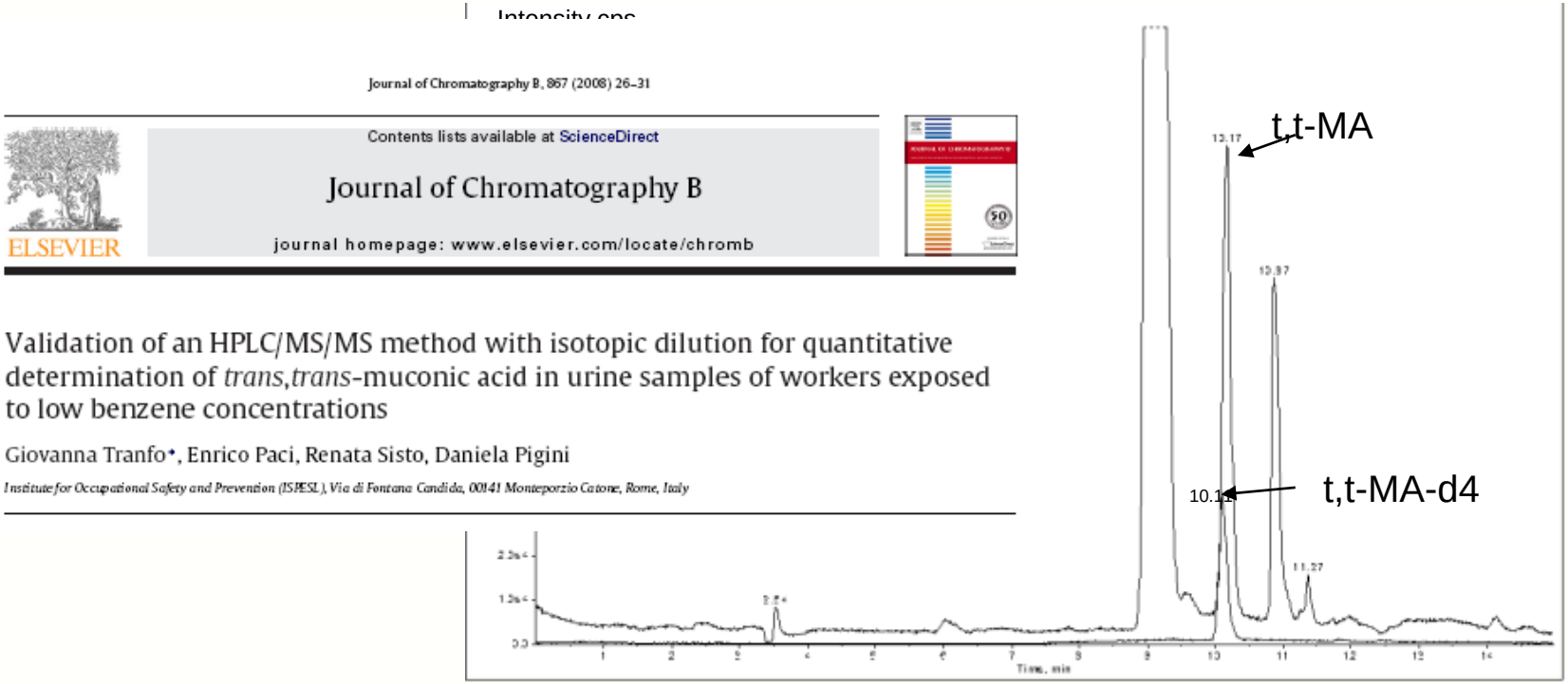
t,t – MA analysis

The following m/z ion combinations (precursor → product) were monitored and in the negative mode:

m/z 141 → m/z 97 for t,t-MA

m/z 145 → m/z 100 for the deuterium labeled internal standard.

LOD and LLOQ were respectively 0.5 µg/L and 1.5 µg/L



Journal of Chromatography B, 867 (2008) 26–31

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Validation of an HPLC/MS/MS method with isotopic dilution for quantitative determination of *trans,trans*-muconic acid in urine samples of workers exposed to low benzene concentrations

Giovanna Tranfo*, Enrico Paci, Renata Sisto, Daniela Pigini

Institute for Occupational Safety and Prevention (ISPESL), Via di Fontana Candida, 00141 Monteporzio Catone, Rome, Italy

t,t-MA is a metabolite of sorbic acid

Sorbic acid (SA), a common food preservative, could be an important confounding factor when t,t - MA is used as a biomarker for low doses exposures to Benzene.
Negri et al. suggest a possible use of urinary SA determination as a tool to correct t,t-MA levels measured in subjects exposed to low benzene concentrations.
However, because of its rapid elimination urine, if sampling for occupational benzene biomonitoring is done a few hours after meals, it can be considered negligible (DFG 2006).

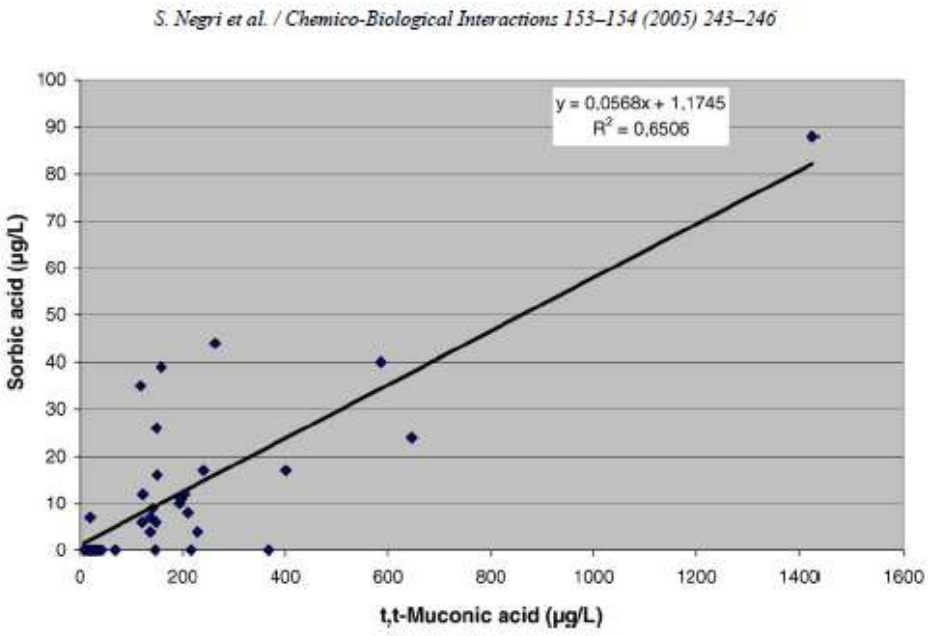
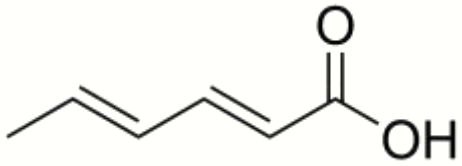
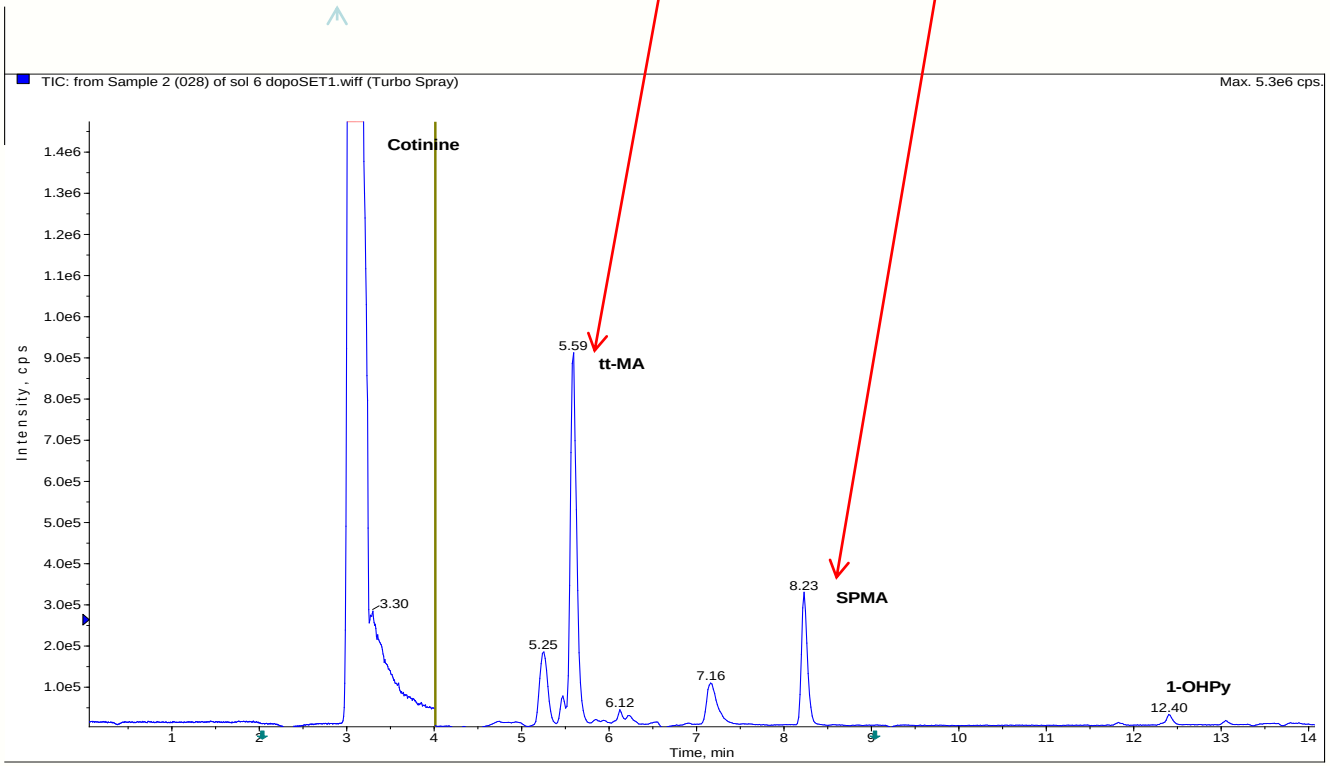


Fig. 2. Correlation between urinary *t,t*-muconic acid and sorbic acid in 36 subjects not exposed to benzene.



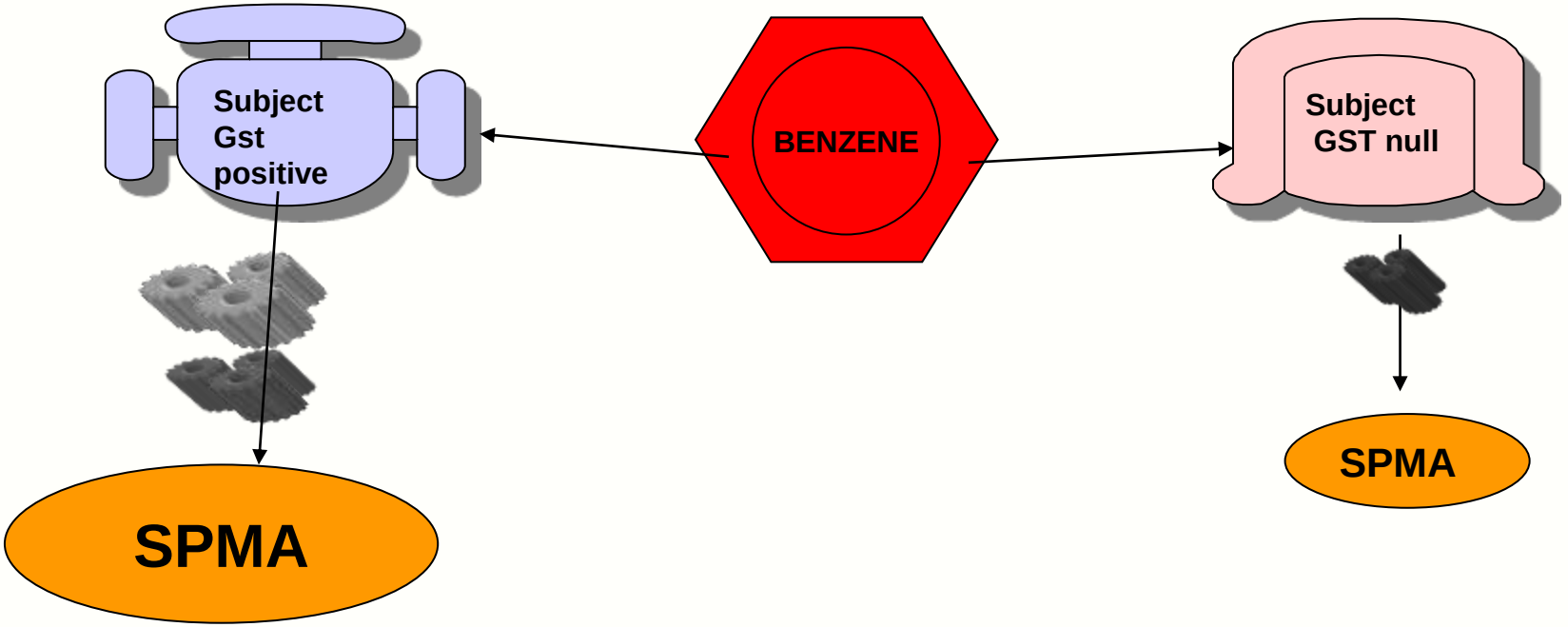
Simultaneous determination can be performed for the benzene metabolites t,t-MA and SPMA by HPLC tandem mass spectrometry.



Individual susceptibility

The genetic polymorphisms of the genes coding **GSTT1 and GSTM1** enzymes involved in benzene detoxification can influence the levels of SPMA.

A lower excretion of SPMA, but not of t,t-MA was found in GSTT1 and GSTM1 null subjects compared to the positive ones



= **Glutathione – S – Transferases**

t,t-MA/SPMA ratio (R)

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Low occupational exposure to benzene in a petrochemical plant: Modulating effect of genetic polymorphisms and smoking habit on the urinary t,t-MA/SPMA ratio

Antonella Mansi[a], Roberta Bruni[a], Pasquale Capone[a], Enrico Paci[b], Daniela Pigini[a], Carla Simeoni[c], Rossella Gnerre[c], Maddalena Papacchini[c], Giovanna Tranfo[b,*]

The theoretical t,t-MA/SPMA ratio (R) calculated from the ratio between the ACGIH BEIs is 20 (500/25 = 20) But measured R is not a constant number.

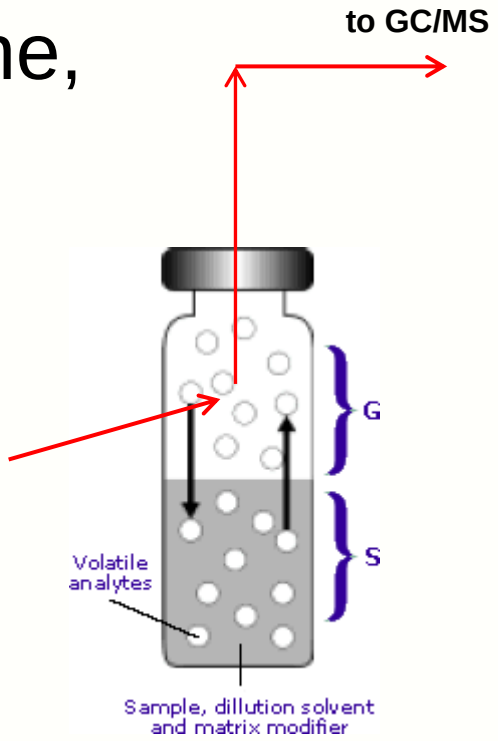
R shows the effect of the individual genetic differences independently from the benzene exposure:

R values are significantly higher for GSTT1 or GSTM1 null subjects than for positive ones.

Benzene in urine

Urinary unchanged benzene has been proposed as a new biomarker for exposure to very low concentrations of benzene, similar to those observed in the general environment.

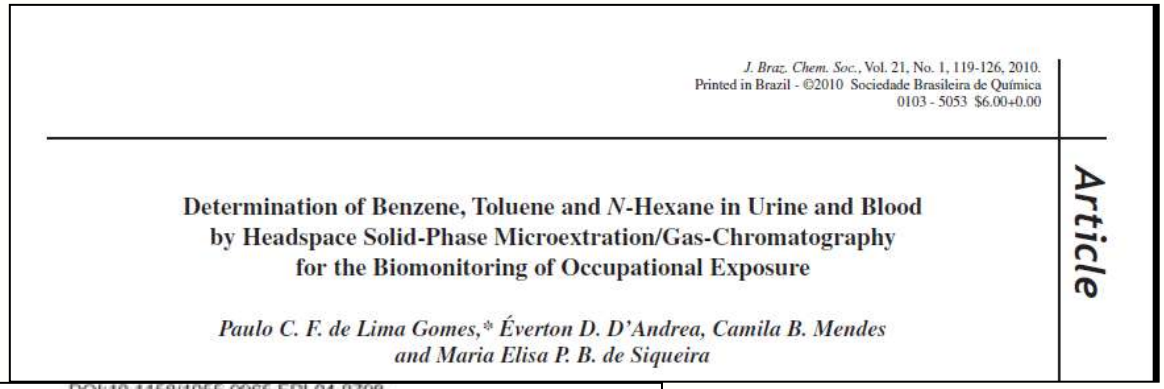
It can be determined in the head space of urine samples by means of GC/MS.



Toxicology and Industrial Health 2008; 24: 155–160
http://tih.sagepub.com

Possible sources of urinary benzene among nonoccupationally exposed Japanese subjects

Shigeru Suna[1], Tomohiro Hirao[2], Fumiyuki Asakawa[3], Takeshi Suzue[1], Toshifumi Mannami[4] and Famihiko Jitsunari[1]



J. Braz. Chem. Soc., Vol. 21, No. 1, 119-126, 2010.
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0103 - 5053 $6.00+0.00

Article

Determination of Benzene, Toluene and *N*-Hexane in Urine and Blood by Headspace Solid-Phase Microextration/Gas-Chromatography for the Biomonitoring of Occupational Exposure

Paulo C. F. de Lima Gomes, Éverton D. D'Andrea, Camila B. Mendes and Maria Elisa P. B. de Siqueira*

DOI:10.1158/1055-9965.EPI-04-0798

Monitoring Low Benzene Exposure: Comparative Evaluation of Urinary Biomarkers, Influence of Cigarette Smoking, and Genetic Polymorphisms

Silvia Fustinoni,[1] Dario Consonni,[1] Laura Campo,[1] Marina Buratti,[1] Antonio Colombi,[1] Angela C. Pesatori,[1] Matteo Bonzini,[1] Pier A. Bertazzi,[1] Vito Foà,[1] Seymour Garte,[2] Peter B. Farmer,[3] Leonard S. Levy,[4] Mauro Pala,[5] Federico Valerio,[5] Vincenzo Fontana,[5] Arianna Desideri,[5] and Domenico F. Merlo[5]

Unchanged benzene in the urine should be not affected by genetic polymorphism or other confounding factors; however, no consensus on its use has yet been reached.

Urinary benzene was found a valid biomarker for occupational exposure to low benzene concentrations, but it is strongly affected by smoking habit and does not show any real advantage over SPMA.

The analytical determination of this parameter still presents some difficulties that have hindered its adoption in routine practice mainly in the pre-analytical phases of collection and preservation of samples.

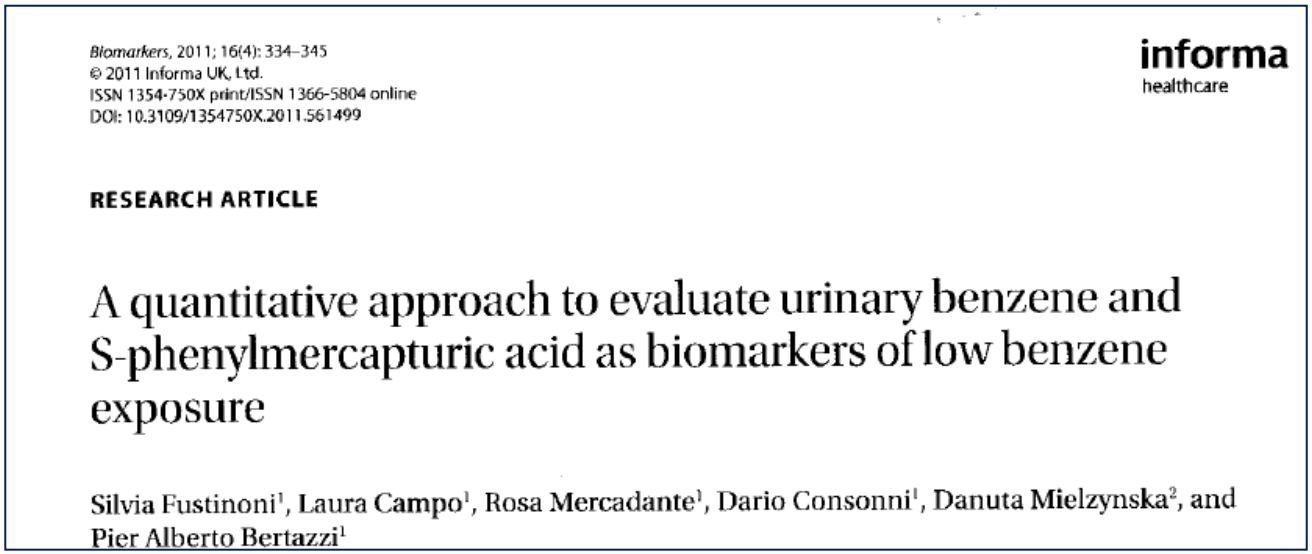
Int Arch Occup Environ Health (2010) 83:341–356
DOI 10.1007/s00420-009-0469-7

ORIGINAL ARTICLE

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

Piero Lovreglio · Anna Barbieri · Mariella Carrieri · Laura Sabatini · Maria Enrica Fracasso · Denise Doria · Ignazio Drago · Antonella Basso · Maria Nicolà D'Errico · Giovanni Battista Bartolucci · Francesco Saverio Violante · Leonardo Soleo

A comparison between Urinary benzene and SPMA presented in 2011 for sampling conditions and analytical techniques concludes that: ***although urinary benzene presents critical sampling conditions, due to the sophisticated analytical requirements SPMA determination is less suitable for investigations on the general population, while both markers are suggested for occupational exposure assessment.***



Biomarkers, 2011; 16(4): 334–345

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DOI: 10.3109/1354750X.2011.561499

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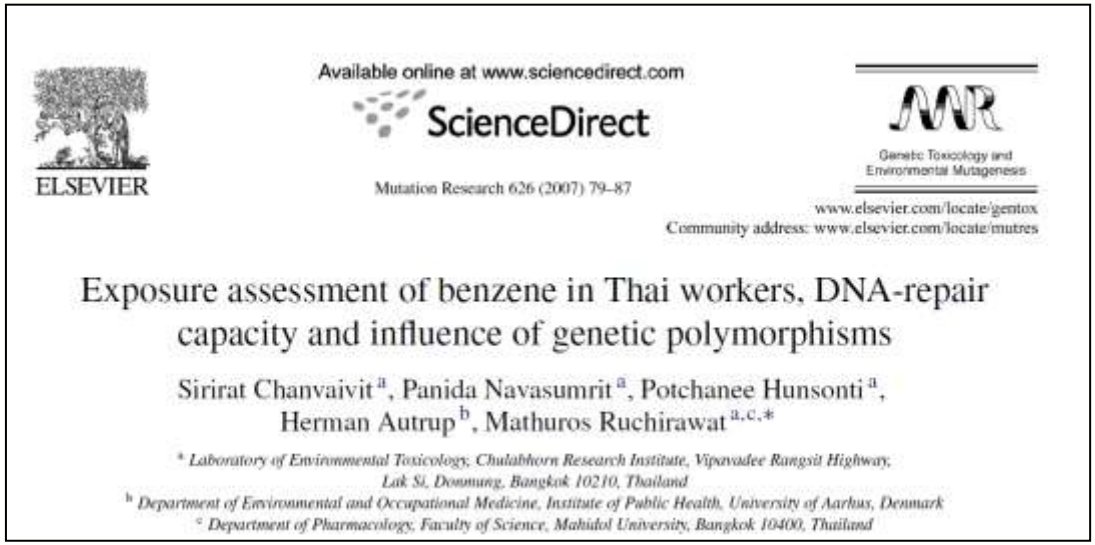
RESEARCH ARTICLE

A quantitative approach to evaluate urinary benzene and S-phenylmercapturic acid as biomarkers of low benzene exposure

Silvia Fustinoni[1], Laura Campo[1], Rosa Mercadante[1], Dario Consonni[1], Danuta Mielzynska[2], and Pier Alberto Bertazzi[1]

Benzene in blood

In some cases benzene is measured in blood by means of GC/MS often with the SPME (Solid phase micro extraction) technique. Blood is quite an invasive sample, but sometimes it is already available because it is the matrix for different kind of analysis, like genetic polymorphisms, DNA damage or repair capacity tests, or DNA adducts.



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Mutation Research 626 (2007) 79–87

Genetic Toxicology and Environmental Mutagenesis

www.elsevier.com/locate/gentox

Community address: www.elsevier.com/locate/mutres

Exposure assessment of benzene in Thai workers, DNA-repair capacity and influence of genetic polymorphisms

Sirirat Chanvaivit [a], Panida Navasumrit [a], Potchanee Hunsonti [a], Herman Autrup [b], Mathuros Ruchirawat [a,c,*]

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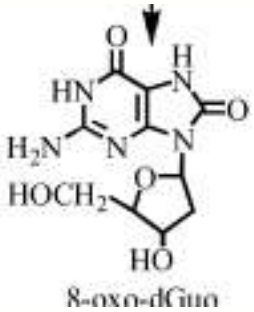
[b] Department of Environmental and Occupational Medicine, Institute of Public Health, University of Aarhus, Denmark

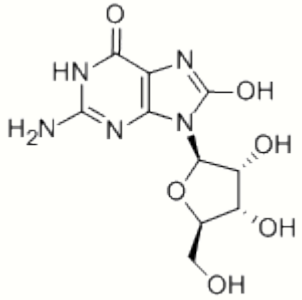
[c] Department of Pharmacology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

Benzene levels in blood of laboratory workers were 4-fold and in gasoline service attendants were 10-fold higher than those of control workers. Individual benzene exposure correlated well with the levels of benzene in blood ($R^2 = 0.6509$, $p < 0.01$).

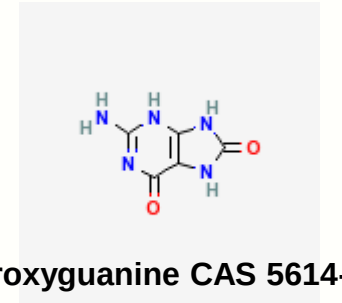
Nucleic Acids Oxidation Biomarkers

The advent of liquid chromatography–tandem mass spectrometry (LC-MS/MS) has enabled the determination of **extracellular oxidized guanine derivatives in urine**, such as 8-oxodGuo, 8-oxo-7,8-dihydroguanosine (8-oxoGuo), and 8-oxo-7,8-dihydroguanine (8-oxoGua); the non-invasive determination of biomarkers of nucleic acid oxidation in the same urinary sample used to assess exposure biomarkers could be useful to assess RNA oxidation and, in the case of DNA, also the effectiveness of repair pathways. In addition, owing to the antioxidant properties of urine these biomarkers are stable for several years when samples are properly stored.



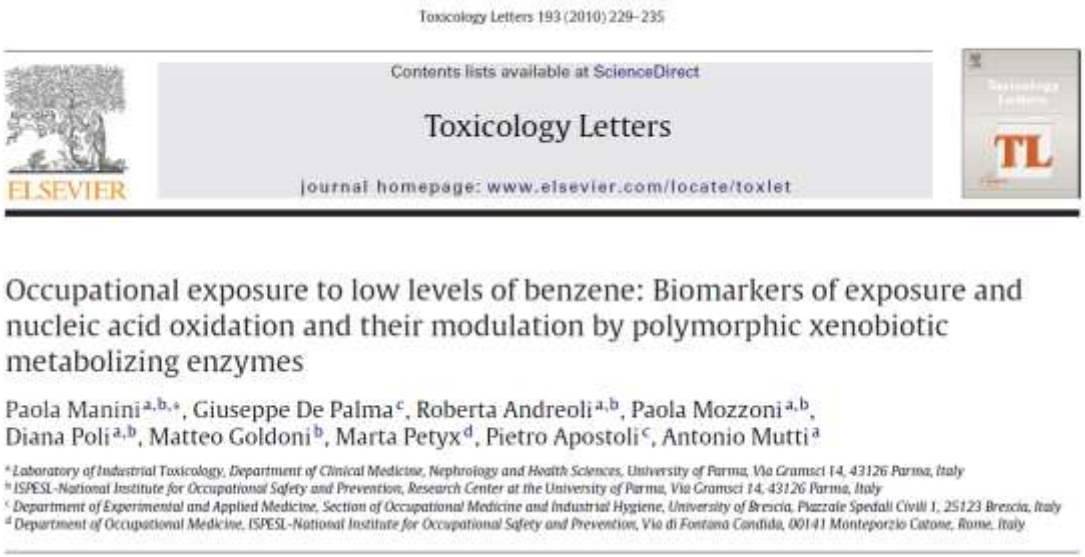


CAS: 3868-31-3
8-HYDROXYGUANOSINE



8-Hydroxyguanine CAS 5614-64

Higher concentrations of 8-oxoGua were detected in smoking traffic policemen compared to non-smoking subjects ($p<0.0001$), whereas higher concentrations of 8-oxodGuo were observed in smoking gasoline pump attendants compared to the corresponding non-smoking group ($p < 0.05$). In taxi drivers, the levels of 8-oxoGuo and 8-oxoGua were higher in smokers than in non-smokers ($p<0.05$).



Toxicology Letters 193 (2010) 229–235

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Occupational exposure to low levels of benzene: Biomarkers of exposure and nucleic acid oxidation and their modulation by polymorphic xenobiotic metabolizing enzymes

Paola Manini[a,b,*], Giuseppe De Palma[c], Roberta Andreoli[a,b], Paola Mozzoni[a,b], Diana Poli[a,b], Matteo Goldoni[b], Marta Petyx[d], Pietro Apostoli[c], Antonio Mutti[a]

[a] Laboratory of Industrial Toxicology, Department of Clinical Medicine, Nephrology and Health Sciences, University of Parma, Via Gramsci 14, 43126 Parma, Italy
[b] ISPESL-National Institute for Occupational Safety and Prevention, Research Center at the University of Parma, Via Gramsci 14, 43126 Parma, Italy
[c] Department of Experimental and Applied Medicine, Section of Occupational Medicine and Industrial Hygiene, University of Brescia, Piazzale Spedali Civili 1, 25123 Brescia, Italy
[d] Department of Occupational Medicine, ISPESL-National Institute for Occupational Safety and Prevention, Via di Fontana Candida, 00141 Monteporzio Catone, Rome, Italy

Albumin adducts

Research

Albumin Adducts of Electrophilic Benzene Metabolites in Benzene-Exposed and Control Workers

Yu-Sheng Lin,[1] Roel Vermeulen,[2] Chin H. Tsai,[1] Suramya Waidyanatha,[1] Qing Lan,[2] Nathaniel Rothman,[2] Martyn T. Smith,[3] Luoping Zhang,[3] Min Shen,[2] Guilan Li,[4] Songnian Yin,[4] Sungkyoon Kim,[1] and Stephen M. Rappaport[1]

[1]Department of Environmental Sciences and Engineering, School of Public Health, University of North Carolina, Chapel Hill, North Carolina, USA; [2]National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, Maryland, USA; [3]School of Public Health, University of California, Berkeley, California, USA; [4]Chinese Center for Disease Control and Prevention, Beijing, China

Metabolism of benzene produces reactive electrophiles, including benzene oxide (BO), 1,4-benzoquinone (1,4-BQ), and 1,2-benzoquinone (1,2-BQ), that are capable of reacting with blood proteins to produce adducts. Levels of BO-Alb, 1,4-BQ-Alb, and 1,2-BQ-Alb in 250 benzene-exposed workers and 140 control workers in Tianjin, China are reported. Albumin was isolated from serum or plasma, dried to constant weight, and analyzed by derivatization and gas chromatography mass spectrometry;

at benzene concentrations < 1 ppm there are rather small contributions of benzene-derived adducts to adducts arising from unknown dietary and endogenous sources. Above 1 ppm, the contributions of benzene exposure to albumin adducts become apparent. Benzene exposures were associated with increased production of albumin adducts of BO, 1,4-BQ and 1,2-BQ.

Results also indicate that none of the three albumin adducts would be useful biomarkers of benzene exposure in ambient populations, where air concentrations rarely exceed 0.1 ppm, or in working populations where exposures are consistently maintained at < 1 ppm.

Conclusions

For the occupational assessment to benzene exposure we suggest that:

1. both **t,t-Ma and SPMA** are determined to identify possible interferences: simultaneous analysis is possible
2. **their ratio R** is regarded as a marker of individual susceptibility: infact at the same exposure levels, higher R values are associated with GSTT1 or GSTM1 null genotypes, considered a risk factor for cancer disease. Further studies are needed to identify a R “warning” threshold value