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BENZENE

BASIC Guide: Biomonitoring and Surveillance of Chemical Exposure in Occupational Settings

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CHEMICAL NAME(S): **BENZENE**

EC No. 200-753-7 CAS No. 71-43-2

Biomarker(s)[1]	S-Phenyl mercapturic acid (SPMA) in urine	trans,trans-Muconic acid (t,t-MA) in urine	Urinary benzene (UB)
Molecular weight (g/mol)	239.29	142.11	78.11
Biological matrix	Urine	Urine	Urine
Range of use (Benzene air concentration ranges are intended for non-smokers)	From environmental to occupational exposure [1, 2] The linearity between SPMA and ambient concentration has been validated only down to 0.1 ppm.	Only when exposure is above 0.3 ppm.	From environmental to occupational exposure ranges with short-term exposure [3, 4].
Initial half-life (hours) i.e., apparent urinary elimination half-life	9-13 [5-7]	4-6 [8]	2 [7, 9]
Additional biomarker selection considerations	Active smoking elevates benzene biomarker levels, consider this when interpretating the data for smokers.	Sorbic acid intake and sorbate food preservatives, bioproducts from bacterial activity, cosmetics, pharmaceutical products, active smoking, metabolic enzyme polymorphism, and diet may significantly affect the background concentration of t,t-MA [10]. Smoking elevates benzene biomarker levels, consider this when interpretating the data for smoker but t,t-MA is less sensitive to active smoking than SPMA.	Active smoking elevates benzene biomarker levels, consider this when interpretating the data for smokers.
Type of sample[2]	Spot Urine	Spot Urine	Spot urine

1.Blood benzene and exhaled air can also be used as a biomarker but the fast kinetics require repeated samples (pre-shift, post shift and next morning post shift) and contextual information for useful interpretation [21,22]. Additionally, while breath analysis is more sensitive and specific than the urinary phenol test, however, owing to the short half-elimination time (minutes), UB appears to be a more appropriate biomarker. There are other exposure biomarkers for benzene including hydroquinone (HQ), catechol (CAT), and phenol, but they are unsuitable as biomarkers at low benzene doses because of their poor specificity and high background concentrations.
2. For cross-sectional studies, spot urine samples, preferably first morning voids could be used. First morning voids are preferable to other spot urine samples since they are collected at about the same time each day for all participants and the results may better correlate with those from 24 h samples (Kissel et al., 2005).

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Sampling time[3]	Workers: pre-shift (optional) and end-of-8hr shift (within 1 hour after cessation of work).	Workers: pre-shift (optional) and post-shift (within 1 hour after cessation of work).	Workers: pre-shift (optional) and end of exposure (task) or end of shift.
Sampling time after incidents[4]	Following an incident, collect a spot sample from every urine void at time intervals up to pre-shift next day (when possible). Label each sample with time of collection and time of incident.		
Sample collection at the community level	24 h urine, otherwise spot urine samples for sufficiently large population.		
Type of container for sample collection from the participants	Collect a urine sample in a urine container. Afterwards if necessary, aliquot into second 30 ml container suitable for freezing.	Collect a urine sample in a urine container. Afterwards if necessary, aliquot into second 30 ml container suitable for freezing.	Collect a urine sample in a urine container and Immediate transfer to head- space pre-sealed vial with a Teflon lined septum according to the requirements of the chemical analytical laboratory; after filling, keep it upside down to avoid loss of vapour.
Volume of samples	Recommended minimum of 10 ml for one analysis (duplicate aliquots plus some for creatinine).	Recommended minimum of 10 ml for one analysis (duplicate aliquots plus some for creatinine).	Depending on the volume of the vial according to the requirements of the chemical analytical laboratory.

3. For benzene, post-shift sampling is recommended to reflect exposure during the previous shift, though it may also capture elevated exposure from up to 48 hours earlier. Consistency in sampling (minimizing sampling variability) is crucial for long-term biomonitoring to ensure data comparability.
4. Given the likelihood of incidents occurring outside of office hours, it's advisable to develop an action plan and have sample containers ready. For post-incident sampling, it's crucial to record the time and date of collection and gather 3 to 4 spot samples within 24 hours. Not all samples need to be analyzed, but the most appropriate ones can be selected. SPMA samples remain stable for 2-3 days if kept cool, but for longer storage, freezing is recommended. Often, if there is an incident there will be various actions undertaken (clean-up, removing personnel involved from the scene etc.) and a urinary sample typically will be taken afterwards. So, timing is less critical, especially if multiple samples are collected. This is highly recommended as it will allow to estimate the actual concentrations people have been exposed to).

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Storage and Transportation (according to UN3373 for shipping)	Samples should be refrigerated and shipped to the laboratory as soon as possible (within 2 days). If longer storage required/anticipated, then store frozen in suitable containers and ship with ice or dry ice.		
Sample handling considerations (possibility of contamination and loses)	If vials are sealed and handled properly, contamination and losses are unlikely[5].	If vials are sealed and handled properly, contamination and losses are unlikely.	Samples must be transferred into the vials immediately after the micturition. Benzene is a volatile compound, so samples are prone to contamination from the environment and losses. Therefore, ensure clean handling and proper sealing.
Lab Storage	Up to 1 month at 4°C if acidified to pH 2 with 6M of hydrochloric acid (the conversion of SPMA precursor into SPMA will proceed) Frozen at -20°C sample is stable up to 1 year.	Up to 1 month at 4°C if acidified to pH 2 with 6M of hydrochloric acid. Frozen at -20°C sample is stable up to 1 year.	Frozen at -20°C a sample is stable up to several weeks.
Analytical method and laboratory[6]	HPLC-MS/MS, GC-MS [11-13]	HPLC-MS/MS [11], HPLC-UV (absorption at 259 nm)	HS-GC/MS, HS-SPME GC/MS
Method limit of quantification (LOQ)	Depends on the analytical method used. To separate occupational exposure from background exposure, the LOQ should be less than 0.3 µg/L.	Depends on the analytical method used. To separate occupational exposure from background exposure, the LOQ should be less than 150 µg/L.	Depends on the analytical method used. To separate occupational exposure from background exposure, the LOQ should be less than 0.3 µg/L.
QA/QC procedure at the laboratory	G-EQUAS http://www.g-equas.de/	G-EQUAS http://www.g-equas.de/	G-EQUAS http://www.g-equas.de/

5. In the laboratory acidification of the samples to a standardised pH of 2 should be considered. The amount of measured SPMA may change as a function both of pH and of storage conditions. An acidic pH leads to the conversion of its precursor, increasing the SPMA metabolite concentration. A study (Paci et al., 2007) showed that a previous hydrolysis procedure can increase SPMA urinary concentrations by factors of up to 5 compared to pH 2 condition and up to 100 when no acid treatment is performed. As urine pH is variable, the hydrolysis at a standardised pH (i.e. 2) is essential to be able to compare results. If the sample is stored at pH of 2 the hydrolysis will proceed, and the analyst must be aware of this.
6. An analytical method with appropriate performance, which is offered by at least one laboratory.

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Biomarker(s)	S-Phenyl mercapturic acid (SPMA) in urine	trans,trans-Muconic acid (t,t-MA) in urine	Urinary benzene (UB)
Recommended adjustments	Use creatinine for correction of density (spot urine sample) or Use Specific gravity.	Use creatinine for correction of density (spot urine sample) or Use Specific gravity.	None
Preferred units for expression of results	• µg SPMA/g creatinine when creatinine used for correction • µg SPMA/L when specific gravity is used for urinary dilution correction.	• µg t,t-MA/g creatinine when creatinine used for correction • µg t,t-MA/L when specific gravity is used for urinary dilution correction.	µg/L[7]
Conversion factor	1µg/g = 0.475 µmol/mol	1 µg/g = 0.800 µmol/mol	1 µg/L = 0.0128 µmol/L
Reproducibility of the method (overall coefficient of variation, CV)	The analytical methods should ideally achieve a reproducibility (CV of less than 15% or 20% at the LOQ as indicated by international guidelines). The lab report must clearly state the reproducibility of the method used.		

7. Creatinine-correction is not required for urinary benzene as the benzene accumulates in the bladder via osmosis due to a concentration gradient. It is widely accepted that unmetabolized solvents measured directly in urine do not require creatinine correction, and most biological monitoring guidance values adhere to this principle.

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Interpretation of results	
Reference Ranges[8]: Background Level (general population level)	International levels for instance: Biological Guidance Values (BGV) in Europe[9]: • 0.5 µg SPMA/g creatinine in nonsmokers (ECHA 2018) • 0.3 µg benzene/L urine in nonsmokers (ECHA 2018) National level of background exposure (national databases). Additionally, consider pre-shift sampling. If not available consult: https://www.intlexposurescience.org/i-hbm/ -OR BAuA - Biomonitoring Information System - Federal Institute for Occupational Safety and Health: https://www.baua.de/EN/Topics/Chemicals-biological-agents/Hazardous-substances/Biomonitoring. If not available consult: Other countries for instance: Canadian Biomonitoring Dashboard — Canada.ca Finland: reference value of 0.5 µg SPMA/g creatinine for nonsmokers based on the 95th percentile of occupationally non exposed population. For Smokers: Smoker levels are usually 10 times higher than non-smokers [14, 15].
Occupational Exposure Limits (OEL) ppm (mg/m³) 8-hour TWA	Check the international, national and/or company requirements (for instance, GESTIS - International limit values: https://ilv.ifa.dguv.de/limitvalues/4437 or SER Grenswaarde (currently not available in English): https://www.ser.nl/nl/thema/arbeidsomstandigheden/grenswaarden-gevaarlijke-stoffen/grenswaarden). EU directive 2022/431 0.2 ppm (0.66 mg/m³) as TWA 8h, the transitional measures state that Limit value is 0.5 ppm (1,65 mg/m³) from 5 April 2024 until 5 April 2026. • At EU level ECHA 2018 has suggested the level at 0.05 ppm (0.16 mg/m³) [16]. • At national level, the Netherlands has an OEL of 0.2 ppm (since 2017) [4].

8. Statistical ranges based on a reference population. Exceeding the reference range doesn't necessarily indicate a health risk but suggests higher exposure than the general population. Sensitivity can be assessed by comparing its LOQ with expected values in the general population and workers, avoid LOQ at the OBL level, aim for LOQ < 10% of OBL with < 50% variability.
9. The 95th percentiles for benzene and metabolites in the general population.

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Cancer risk threshold	• At EU level ECHA 2018 has suggested the level at 0.05 ppm (0.16 mg/m³) [16]. • At national level, the Netherlands has an OEL of 0.2 ppm (since 2017) [4].
Short Term Exposure Limit (STEL) in ppm (mg/m³)	Use of STEL values for Benzene is not recommended. Although some countries have proposed a STEL for benzene, this is fundamentally incorrect. STEL values are meant to protect workers from short-term peak exposures that could cause health damage, typically in compounds with steep dose-response curves. Benzene does not meet these criteria for setting a STEL.
Ratio of SPMA/t,t-MA	Ratio (R) can considerably reflect the effect of individual genetic differences. This ratio can be used when air benzene concentration is not less than 0.3 ppm (or concentrations above the currently proposed OELs). The excretion rates of these two metabolites are assumed to be constant, with ACGIH setting the theoretical threshold value of R at 20 [17, 18].
Biological limit values (BLV) corresponding to Inhalation Occupational Exposure Limits (OEL) ppm (mg/m³) 8-h TWA	Check for national requirements or international databases. Air level corresponding to different BLV is presented in **Table 1.**
Possible Co-Exposure	Co-exposure (for example with toluene) is not relevant at current exposure level of Benzene (1ppm). In the event of a higher exposure level to benzene further study of co-exposure is recommended.
Biomarkers of effect for Health surveillance[10]	
Peripheral blood changes (for exposure above 1 ppm [19])	Changes in peripheral blood counts, including red blood cell (RBC), haemoglobin (Hb), absolute neutrophil (ANC), white blood cell (WBC), and platelet counts (PLT), can assist in early disease diagnosis. Note: If exposure levels are below the modern standards (like binding OEL in Europe), those hematological parameters are not sensitive enough biomarkers for use in medical surveillance. Hematological effects are likely only at levels far higher than 1 ppm.

10. Oxidative DNA damage is strongly associated with benzene-induced cancer. Urine 8-hydroxy-2-deoxyguanosine (8-OHdG), a biomarker associated with oxidative stress, represents as a non-invasive biomarker of early genotoxic damage in workers exposed to low-dose benzene. However, as polycyclic aromatic hydrocarbons (PAHs) and butadiene can also induce oxidative DNA damage, 8-OHdG is a nonspecific indicator of benzene exposure, suggesting that 8-OHdG can be used for mixture risk assessment.

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Table 1. *Biological limit values (BLV) corresponding to Inhalation Occupational Exposure Limits (OEL)*

Biomarker	ECHA/RAC [14]		AGS- the German Technical Guideline (TRGS) 910 (tolerance concentration at additional lifetime cancer risk of 4:1,000)[11]		AGS-TRGS 910 (acceptable concentration at additional lifetime cancer risk of 4:10,000)[11]		BAT-C1A/C1B-Suva[12]		Biological reference values (BAR)-DFG[13]		Exposure equivalents for carcinogenic substances (EKA)[14]		SCOEL[15]		ACGIH	
	BLV-ECHA/RAC	corr. OEL[a]	BLV-AGS-TRGS 910	corr. OEL	BLV-AGS-TRGS 910	corr. OEL	BAT	corr. OEL	BAR-DFG:	corr. OEL	EKA	corr. OEL	BLV-SCOEL	corr. OEL	BEI	corr. OEL
Urine Benzene	0.7 µg benzene/L urine	0.05 ppm (0.16 mg/m³)	5 µg benzene/L	0.6 ppm (1,9 mg/m³)	0.8 µg benzene/L	0.06 ppm (0.2 mg/m³)	n/a	0.2 ppm (0.7 mg/m³)	0.3 µg benzene/L	n/a	0.5 µg benzene/L urine	0.03 ppm (0.1 mg/m³)	n/a	< 1.0 ppm (< 3.25 mg/m³)		0.02 ppm (0.065 mg/m³)
SPMA	2 µg SPMA/g creatinine (sampling time: end of exposure or end of working shift)		25 µg SPMA/g creatinine		3 µg SPMA/g creatinine		8 µg SPMA/g creatinine		0.3 µg SPMA/g creatinine		1.5 µg SPMA/g creatinine (sampling time: end of exposure or end of working shift)[b]		46 µg SPMA/g creatinine		25 µg/g creatinine[c]	
t,t-MA	n/a	n/a	500 µg t,t-MA/g creatinine (sampling time: end of exposure or end of shift		No equivalent value for 0.06 ppm benzene in workplace air, due to background level		n/a		150 µg t,t-MA/g creatinine		300 µg t,t-MA/g creatinine (sampling time: end of exposure or end of shift				500 µg/g creatinine	

a corresponding OEL 8-hour TWA for non-smokers; b for long-term exposures after several previous shifts [20] c the TLV has been lowered from 0.5 ppm to 0.02 ppm, but the BEIs for SPMA and ttMA have not yet been adapted. Therefore, there is an update gap which will be filled in one of the ACGIH Guideline updates.

11. https://www.baua.de/DE/Themen/Chemikalien-Biostoffe/Gefahrstoffe/Biomonitoring/Biomonitoring-Auskunftssystem/Gefahrstoffinfo?biomonitoringQuery=benzene&xcas=71-43-2
12. https://www.suva.ch/de-ch/services/grenzwerte#gnw-location=%2F
13. https://series.publisso.de/pgseries/overview/mak
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Revision History

Revision Number	*Date of Change*	*Description of changes*
Version 1.0	2025-02	Version 1.0 is created

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