Collective expert appraisal: summary of discussion with conclusions

Regarding the expert appraisal on recommending occupational exposure limits for chemical agents

Evaluation of biomarkers and recommendation for biological limit values and biological reference values for styrene [CAS no. 100-42-5]

This document summarises the work of the Expert Committee on expert appraisal for recommending occupational exposure limits for chemical agents (OEL Committee) and the Working Group on biomarkers (biomarkers WG).

Presentation of the issue

AFSSET which became ANSES in July 2010, received a solicited request on 12 June 2007 from the French Directorate General for Labour to conduct the scientific expert appraisal work required for setting occupational exposure limit values (OELVs) for styrene. Set by a 1985 Circular[1], France has an indicative 8h-OELV of 215 mg.m^{-3} (50 ppm) for styrene.

The Directorate General for Labour asked the Agency to reassess this value and, if necessary, to propose new occupational exposure limit values based on health considerations.

This request was entrusted to the Agency's OEL Committee which, in June 2010, issued a report recommending, for styrene:

- establishing an 8h-OEL of 100 mg.m^{-3};
- establishing a short-term limit value (STLV) of 200 mg.m^{-3};
- assigning the "skin notation";

ANSES decided to supplement its expert appraisal with an assessment of the biological monitoring data on styrene in an occupational environment, in order to establish the relevance of recommending monitoring of one or more biomarkers in addition to the atmospheric OEL and the establishment of biological limit values for the selected biomarker(s).

Scientific background

Biological monitoring of exposure in workplaces has emerged as a complementary method to atmospheric metrology for assessing exposure to chemical agents. Biological monitoring assesses a worker's exposure by including all the routes by which a chemical penetrates the

[1] Circular of 5 March 1985 amending and supplementing the Circular of 19 July 1982 as amended, on the acceptable values for concentrations of certain hazardous substances in workplace atmospheres

body (lung, skin, digestive tract). It is particularly worthwile when a substance has a systemic effect, and:

- when routes other than inhalation contribute significantly to absorption,
- and/or when the pollutant has a cumulative effect,
- and/or when the working conditions (wearing respiratory protection, inter-individual differences in respiratory ventilation, etc.) determine large differences in internal dose that are not taken into account by atmospheric metrology.

With regard to prevention of chemical risk in the workplace, the French Labour Code provides for the use of biological monitoring of exposure and biological limit values.

OEL Committee definitions

Biomarker of exposure: it is the parent substance, or one of its metabolites, determined in a biological matrix, whose variation is associated with exposure to the agent targeted. Biomarkers of early and reversible effects are included in this definition when they can be specifically correlated to occupational exposure.

Biological limit value (BLV): This is the limit value for the relevant biomarkers.

Depending on the available data, the recommended biological limit values do not all have the same scope:

- if the body of scientific evidence is sufficient to quantify a dose/response relationship with certainty, the biological limit values (BLVs) will be established on the basis of health data (critical dose for threshold effect substances or risk levels for non-threshold carcinogens);
- in the absence of such data for substances with threshold effects, the BLV will be calculated on the basis of the expected concentration of the biomarker of exposure when the worker is exposed to the 8h-OELV. For carcinogens, in the absence of sufficient quantitative data, the biological limit value is calculated on the basis of another type of effect (pragmatic BLV). These last values do not guarantee the absence of health effects, but aim to limit exposure to these substances in workplaces.

Whenever possible, the OEL Committee also recommends biological reference values (BRVs). These correspond to concentrations found in a general population whose characteristics are similar to those of the French population (preferentially for biomarkers of exposure) or in a control population not occupationally exposed to the substance under study (preferentially for biomarkers of effect).

These values cannot be considered to offer protection from the onset of health effects, but do allow a comparison with the concentrations of biomarkers assayed in exposed workers. These values are of particular interest in cases where it is not possible to establish a BLV.

Organisation of the expert appraisal

ANSES entrusted examination of this request to the Expert Committee on expert appraisal for recommending occupational exposure limits for chemical agents (OEL Committee). The Agency also mandated the Working Group on biomarkers for this expert appraisal.

The methodological and scientific aspects of this group's work were regularly submitted to the OEL Committee. The report produced by the working group takes account of the observations and additional information provided by the Committee members.

This expert appraisal was therefore conducted by a group of experts with complementary skills. It was carried out in accordance with the French Standard NF X 50-110 "Quality in Expertise Activities".

Preventing risks of conflicts of interest

ANSES analyses interests declared by the experts before they are appointed and throughout their work in order to prevent potential conflicts of interest in relation to the points addressed in expert appraisals.
The experts' declarations of interests are made public on ANSES's website (www.anses.fr).

Description of the method

A rapporteur in the biomarkers WG was mandated by the Agency to produce a summary report on biomarkers of exposure and the recommendation of biological limit values (BLVs) and biological reference values for the biomarker(s) considered as relevant. An ANSES officer also contributed to this report.

The summary report on the biomarkers for styrene results from bibliographical information taking into account the scientific literature published on this substance until 2011. The bibliographical research was conducted in the following databases: Medline, Toxline, HSDB, ToxNet (CCRIS, GENE-TOX, IRIS), ScienceDirect. The rapporteur reassessed the original articles or reports cited as references whenever he considered it necessary, or whenever the Committee requested it.

The collective expert appraisal work and its conclusions and recommendations were adopted on 13 March 2012 by the OEL Committee (term of office 2010-2013)

The collective expert appraisal work and the summary report were submitted to public consultation from 10 December 2012 to 10 February 2014. The persons or organizations who contributed to the public consultation are listed in appendix 2. The comments received were reviewed by the OEL Committee (term of office 2014-2016) who adopted this version on 18 March 2014.

Result of the collective expert appraisal

Introduction

About a hundred scientific papers or reports were selected for the evaluation of biological monitoring data on styrene identified in the *Medline* database or on specific websites, using the following keywords:

- styrene, biomarker, biomonitoring, mandelic acid, phenylglyoxylic acid, urine, blood, analysis, method, GC, HPLC

Toxicokinetics data

Percutaneous absorption of styrene vapour is low with a proportion ranging from 0.1 to 5% of estimated respiratory absorption (Riihimaki and Pfaffli, 1978; Wieczorek, 1985). In liquid form, styrene's absorption rate on the hands is around 1 ± 0.5 $\mu g.cm^{-2}.min^{-1}$ (Berode and Droz, 1985).

Experimental studies in humans and animals have shown that pulmonary absorption is rapid and its rate varies between 66 and 93% (Engstrom et al., 1978; Cohen et al., 2002). Physical activity should be taken into account when interpreting results, since the increase in alveolar ventilation leads to increased pulmonary uptake of styrene (Bergert and Nestler, 1991; Wigaeus et al., 1983; Löf et al., 1986a; Truchon et al., 2009).

There are no data in humans on the absorption of styrene by ingestion.

Inhaled styrene is widely distributed in all tissues and organs (muscles, heart, liver, kidneys, lungs, spleen, brain). The highest concentrations are usually found in adipose tissue, and some

of the styrene can accumulate there temporarily (Vyskocil et al., 1997a; IARC 2002; INRS 2006; ATSDR 2007; INERIS 2008; Engstrom et al., 1978). Clearance of styrene in the blood is biphasic, with an initial rapid phase (half-life of 0.58 hours) followed by a slower phase (half-life of 13 hours), indicating two-compartment toxicokinetics.

The primary metabolic pathway is cytochrome P-450-dependent oxidation to styrene 7,8-oxide, a compound with known genotoxic potential (Vodicka et al., 2006). Styrene 7,8-oxide is mainly hydrolysed by microsomal epoxide hydrolase (mEH) to form styrene glycol, which is unstable and rapidly converted through the action of alcohol dehydrogenase and aldehyde dehydrogenase to mandelic acid, which can itself give rise to phenylglyoxylic acid. Mandelic acid is the major metabolite of styrene and accounts for 85% of urinary metabolites eliminated after an eight-hour exposure, while mandelic and phenylglyoxylic acids account for 95% of styrene metabolites (Ong et al., 1994). It should be noted that alcohol consumption inhibits (smaller area under the curve for the excreted mass of metabolites) and slows down the metabolism of styrene into its mandelic and phenylglyoxylic acids (Berode et al., 1986; Cerny et al., 1990).

Other minority metabolites can be produced by binding styrene 7,8-oxide with glutathione to form phenylhydroxyethylmercapturic acids. (Sumner et al., 1994; Lauwerys et al., 2007). Mercapturic acids may only account for 1% of the total amount of styrene absorbed (De Rooij et al., 1998). Several authors have examined the possibility of using urinary mercapturic acids as biological indicators of exposure. These specific styrene biomarkers are well correlated to the intensity of exposure (Ghittori et al., 1997; Maestri et al., 1997a; Maestri et al., 1997b). However, urinary measurement of specific phenylhydroxyethylmercapturic acids is not recognised as a routinely useable method.

Several studies have shown that a proportion of between 0.7 and 4.4% of the absorbed dose is excreted unchanged in exhaled air (IARC 1994; Vyskocil et al., 1997b). Respective fractions of unmetabolised styrene excreted in the urine and sweat are probably less than 1% (INRS, 2006).

The kinetic parameters of the elimination of styrene and its metabolites are summarised in the following table.

Summary of toxicokinetic data on elimination of styrene and its metabolites

	Route of elimination	% of elimination by this route	Elimination ½ life	References
Parent substance				
Styrene	Expired air	0.7 - 4.4% 0.7 - 2.2% 2.6%	Biphasic: 13 - 52 minutes and 4 - 20 hours An apparent half-life extended to 3 days may occur in relation to body fat	INRS, 2006 INERIS, 2008 ATSDR, 2007 ACGIH, 2007
	Urine	< 1%		INRS, 2006
	Sweat	< 1%		INRS, 2006
Metabolites				
Mandelic acid	Urine	85% of the absorbed dose	Biphasic: 4 - 9 h and 17 - 26 h	INRS, 2006 ACGIH, 2007 ATSDR, 2007
Phenylglyoxylic acid	Urine	10% of the absorbed dose	Biphasic: 8 - 10 hours (+ slower elimination phase)	INRS, 2006 ACGIH, 2007
Total phenylhydroxyethyl-mercapturic acids	Urine	< 1%		INRS, 2006 ATSDR, 2007
2-Vinylphenol	Urine	< 1%		INRS, 2006 ATSDR, 2007
4-Vinylphenol	Urine	< 1%		INRS, 2006 ATSDR, 2007
Hippuric acid	Urine	< 1%		INRS, 2006

Selection of biological indicators of exposure

Five biomarkers for styrene are available and well documented. They are: styrene in urine, styrene in blood, styrene in expired air, mandelic acid in urine and phenylglyoxylic acid in urine. The sum of both mandelic acid and phenylglyoxylic acid is also recognised as a very useful biological indicator of exposure.

Due to their specificity and close correlation with atmospheric exposure, the three biomarkers that involve measuring styrene unchanged in exhaled air, blood or urine are theoretically the biomarkers of choice and provide a quite comparable level of information on exposure.

However, because of the disadvantages associated with transporting and storing samples (reproducibility of assays), measurement of styrene in exhaled air cannot reasonably be proposed for the biological monitoring of workers exposed to this solvent. Similarly, pre-analytical difficulties (transfer of blood in vials for analysis and blood clotting during heating of the sample required for headspace injection) and the invasive nature of sampling mean that measurement of styrene in blood has not been adopted as a relevant biomarker. In keeping with common practice for biological monitoring of occupational exposure, when there is the choice of an equally valid biomarker, insofar as the measurement of styrene in blood is no more advantageous (specificity, sensitivity) than the measurement of urinary styrene, the use of blood sampling for routine biological monitoring is not recommended, due to the invasive nature of the sampling. Among the proposals for measuring styrene in a biological medium, it is therefore the **determination of urinary styrene that has been deemed relevant**.

Urinary mandelic and phenylglyoxylic acids are also relevant, not only because they alone account for more than 90% of the mass of solvent absorbed, but also because of their close correlation with exposure and their half-life of several hours, which enables sampling at the end

of the work shift. The fact that these indicators are well documented and widely used in the biological monitoring of exposure to styrene is also a major advantage.

However, given the instability of phenylglyoxylic acid in urine, assaying this metabolite alone is not considered relevant. On the other hand, because the instability of phenylglyoxylic acid leads to the reformation of mandelic acid, determining the sum of these two metabolites overcomes this problem and is therefore a more robust biomarker of exposure than measurement of mandelic acid alone.

Therefore, urinary styrene and urinary mandelic and phenylglyoxylic acids were selected as relevant for the biological monitoring of exposure to styrene.

The determination of mandelic acid alone, although affected by the instability of phenylglyoxylic acid, is still an interesting biological indicator for monitoring exposure to styrene. Information relating to mandelic and phenylglyoxylic acids considered separately is provided in the annex to the expert report.

Information on biological indicators of exposure identified as relevant for the biological monitoring of exposed workers

Name	**URINARY STYRENE**	
Other substances giving rise to this biomarker	none	
Concentrations found in exposed workers or volunteers	- <u>Field studies:</u> Ong et al., 1994 - exposure ≈ 40 $mg.m^{-3}$ (n = 34) Urinary styrene: 26 $\mu g.L^{-1}$ (geometric mean, sampling time NS) Prieto et al., 2002 - exposure ≈ 70 $mg.m^{-3}$ (n = 34) Urinary styrene: 5 $\mu g.L^{-1}$ (geometric mean, end of shift) Gobba et al., 1993b - exposure ≈ 90 $mg.m^{-3}$ (n = 198) Urinary styrene: 58 $\mu g.L^{-1}$ (arithmetic mean, end of shift) Maestri et al., 1999 - exposure ≈ 100 $mg.m^{-3}$ (n = 22) Urinary styrene: 26 $\mu g.L^{-1}$ (arithmetic mean, end of shift) Imbriani et al., 1986 - exposure ≈ 110 $mg.m^{-3}$ (n = 69) Urinary styrene: 48 $\mu g.L^{-1}$ (arithmetic mean, end of shift) - <u>Studies in volunteers:</u> NS	
Conversion factor	MW: 104.15 1 $\mu g.L^{-1}$ = 0.0096 $\mu mol.L^{-1}$ 1 $\mu mol.L^{-1}$ = 104.15 $\mu g.L^{-1}$	
Concentrations in the general population	NS	
Recommended limit values for exposed workers	USA - ACGIH (BEI)	NS
	Germany - DFG (BAT)	
	Quebec - IRSST (IBE)	
	Finland - FIOH (BAL)	

NS: not specified

Name	**URINARY MANDELIC AND PHENYLGLYOXYLIC ACIDS**	
Other substances giving rise to these biomarkers	Ethylbenzene, alpha phenylaminoacetic acid, phenylglycine, phenylglycol, styrene glycol	
Concentrations found in exposed workers or volunteers	- <u>Field studies:</u> Ong et al., 1994 - exposure ≈ 40 $mg.m^{-3}$ (n = 34) Mandelic acid + phenylglyoxylic acid (geometric mean): 190 $mg.g^{-1}$ creatinine (end of shift) 35 $mg.g^{-1}$ creatinine (start of shift, next day) Maestri et al., 1999 - exposure ≈ 100 $mg.m^{-3}$ (n = 22) Mandelic acid + phenylglyoxylic acid (arithmetic mean): 775 $mg.g^{-1}$ creatinine (end of shift) Gobba et al., 1993b - exposure ≈ 115 $mg.m^{-3}$ (n = 65) Mandelic acid + phenylglyoxylic acid (arithmetic mean): 1980 $mg.L^{-1}$ (end of shift) Calabrese et al., 1996 - exposure ≈ 150 $mg.m^{-3}$ (n = 20) Mandelic acid + phenylglyoxylic acid (arithmetic mean): 360 $mg.g^{-1}$ creatinine (end of shift) - <u>Studies in volunteers:</u> Guillemin et al., 1976 - exposure ≈ 430 $mg.m^{-3}$ (n= 9) Mandelic acid + phenylglyoxylic acid (arithmetic mean): 1500 $mg.g^{-1}$ creatinine (end of shift) 350 $mg.g^{-1}$ creatinine (start of shift, next day)	
Conversion factor	- For mandelic acid MW: 152 1 $mg.L^{-1}$ = 0.0066 $mmol.L^{-1}$ 1 $mmol.L^{-1}$= 152 $mg.L^{-1}$ 1 $mg.g^{-1}$ creatinine = 0.74 $mmol.mol^{-1}$ creatinine 1 $mmol.mol^{-1}$ creatinine = 1.35 $mg.g^{-1}$ creatinine - For phenylglyoxylic acid MW: 150 1 $mg.L^{-1}$ = 0.0067 $mmol.l^{-1}$ 1 $mmol.L^{-1}$= 150 $mg.L^{-1}$ 1 $mg.g^{-1}$ creatinine = 0.75 $mmol.mol^{-1}$ creatinine 1 $mmol.mol^{-1}$ creatinine = 1.33 $mg.g^{-1}$ creatinine	
Concentrations in the general population	Manini et al., 2004, general population in Italy (n = 129) - 97^{th} percentile: 3.03 $mg.g^{-1}$ creatinine - Median: NS - Geometric mean: 0.61 $mg.g^{-1}$ creatinine	
Recommended limit values for exposed workers	USA - ACGIH (BEI)	400 $mg.g^{-1}$ creatinine (end of shift) – 2003[a]
	Germany - DFG (BAT)	600 $mg.g^{-1}$ creatinine (end of shift - end of week) – 2002[a]
	Quebec - IRSST (IBE)	NS
	Finland - FIOH (BAL)	150 $mg.L^{-1}$ (start of shift – end of week) – 2009[b]
	Switzerland - SUVA (VBT)	500 $mg.g^{-1}$ creatinine (end of shift) – 2012[b]

[a] *Latest modification*
[b] *Latest update*

Study of the relationship between concentrations of biomarkers of exposure and health effects

Effects observed in humans following chronic exposure to styrene concern widely varying target organs. The most frequently described effects are neurotoxic effects, but respiratory, cardiovascular, gastrointestinal, haematological, hepatic, renal, endocrine and immunological effects have also been reported (AFSSET Report "OELV: health and measurement of styrene, 2009).

Several tests indicate neurotoxicity, and concern dyschromatopsia, slower reaction times, poorer performance in memory and dexterity tests, headaches, mood changes and impaired hearing.

Urinary styrene

A study by Gobba et al. (1993b) on 73 workers in the plastics industry (fibreglass-reinforced plastic) exposed on average to an atmospheric concentration of 64 $mg.m^{-3}$ (urinary concentrations of styrene of up to 69 $\mu g.L^{-1}$) indicates a significant increase in the prevalence of dyschromatopsia in the group of exposed workers compared to the control group (53 non-occupationally exposed controls).

Sum of urinary mandelic acid + phenylglyoxylic acid

A study by Mutti on a group of 50 workers exposed to styrene showed impaired word learning in workers with urinary levels of the sum of mandelic and phenylglyoxylic acids greater than 200 $mg.g^{-1}$ of creatinine (150 $mmol.mol^{-1}$ of creatinine) and impaired logical memory and visual perception disorders in workers with urinary levels of mandelic and phenylglyoxylic acids greater than 400 $mg.g^{-1}$ of creatinine (300 $mmol.mol^{-1}$ of creatinine) (Mutti et al., 1984).

A study by Toppila et al. (2006) focused on 252 workers in the reinforced plastics industry. Average concentrations of mandelic acid and phenylglyoxylic acid (sum) were equal to 1.4 $mmol.L^{-1}$. The authors report that exposure to these low concentrations of styrene affects postural stability, indicating a balance disorder. It should be noted that the exposures and concentrations are poorly documented in this study, meaning that the dose-response relationship cannot be analysed.

In a study by Calabrese et al. (1996), average concentrations of mandelic acid and phenylglyoxylic acid (sum) at the end of the shift were equal to 348.1 $mg.g^{-1}$ of creatinine (± 196.7) with no reported decrease in hearing at these exposure levels.

A study by Triebig et al. (2001) on 22 workers in a boat factory found an average concentration of mandelic acid and phenylglyoxylic acid at the end of the shift equal to 472 $mg.g^{-1}$ of creatinine (11 to 2399). The authors report an increase in the prevalence of dyschromatopsia among workers exposed to styrene compared to the control group (11 non-occupationally exposed controls). The authors noted that this effect is reversible after four weeks without exposure.

Study of the relationship between concentrations of biomarkers and exposure to styrene

Many studies focus on the relationship between concentrations biomarkers for styrene and atmospheric concentrations of styrene. The main data on biomarkers deemed relevant are shown in the table below.

n	***Atmos styrene (mg.m^{-3})***	***Urinary styrene (end of shift) (µg.L^{-1})***		mandelic acid **+** phenylglyoxylic acid ***(end of shift)***		***References***
	Mean a: arithmetic g: geometric m: median	Mean a: arithmetic g: geometric m: median	For 8h-OELV (100 mg.m^{-3})	Mean a: arithmetic g: geometric m: median	For 8h-OELV (100 mg.m^{-3})	
34	70.5 *(a)*	5.7 *(a)* 5.2 *(m)*	7	147.1 *(a)* mg.g^{-1} cr	193 mg.g^{-1} cr	Prieto et al., 2002[a]
22	113 *(a)* [40-230]	25.6 *(a)*	22	754 *(a)* mg.g^{-1} cr		Maestri et al., 1999[b]*
39	46 *(g)* [0.9-140]	26.3 *(g)*	45	187.3 *(g)* mg.g^{-1} cr	557 mg.L^{-1}	Ong et al., 1994[b]*
69	109 *(m)*	51 *(m)*	48			Imbriani et al. 1986[a]
65	[8–770]	63 *(g)*	42	1980 *(g)* mg.L^{-1}	1185 mg.L^{-1}	Gobba et al., 1993b[b]*
22	361 *(a)*			1135 *(a)* mg.L^{-1}	474 mg.L^{-1}	Apostoli et al., 1983[c]**
20	[22–522]			mg.g^{-1} cr Mon: 591.4 *(a)* Thur: 764.3 *(a)*	346 mg.g^{-1} cr 400 mg.g^{-1} cr	De Rosa et al., 1993[b]*
20	93.6 *(a)*			485.6 *(a)* mg.g^{-1} cr	520 mg.g^{-1} cr	De Rosa et al., 1988[b]*

[a] Atmospheric sampling over 4 hours
[b] Atmospheric sampling over 8 hours
[c] Atmospheric sampling over 4 times 15 minutes
* Passive sampling
** Active sampling

Establishment of BLVs and choice of biological reference values

Impairment of the peripheral nervous system has been extensively studied, but the results of the different studies reporting a decrease in conduction velocity are rather contradictory. When reported, the neurotoxic effects due to exposure to styrene are reversible, at least in part, after cessation of exposure. Field studies have failed to establish a dose-response relationship between urinary concentrations of styrene, mandelic acid and the sum of mandelic acid + phenylglyoxylic acid, and the health effects (impaired hearing and dyschromatopsia). It is more appropriate to take into account studies linking atmospheric concentrations of styrene to urinary concentrations of the different biomarkers selected, and to establish BLVs based on exposure to the 8h-OELV (100 mg.m^{-3}). The OEL Committee's collective expert report on styrene (ANSES, 2010) proposes an OEL for styrene that should adequately protect a majority of employees from the potential deleterious effects of styrene on the central nervous system, especially on colour vision, that this compound is capable of causing.

Urinary styrene

Urinary concentrations of styrene on cessation of exposure (5 field studies) calculated from regression equations for exposure to the 8h-OELV (100 mg.m^{-3}) are between 7 and 48 µg.L^{-1}. Specifically, three studies give results of the order of 40 to 50 µg.L^{-1}, while the other two studies produce values of 7 and 22 µg.L^{-1} for styrene concentrations in urine collected at the end of the shift.

The two studies reporting measurements of atmospheric samples for 4 hours (Prieto et al., 2002 and Imbriani et al., 1986) were not used to calculate the concentration of urinary styrene at the end of the shift based on the atmospheric concentration, because the reported correlations and regression equations are only valid for exposures of 4 hours or less.

The average concentrations calculated for exposure to the 8h-OELV, using the studies by Maestri et al., 1999, Ong et al., 1994 and Gobba et al., 1993b, are around 40 µg.L^{-1}. The urinary concentration of styrene of 40 µg.L^{-1} in samples taken at the end of the shift can be used for the establishment of a BLV based on exposure to the 8h-OELV of 100 mg.m^{-3}.

Urinary mandelic and phenylglyoxylic acid

The combined measurement of both metabolites is preferred because this summation overcomes the instability problems with phenylglyoxylic acid in urine converting to mandelic acid. Thus the sum total of both acids remains stable in urine after sampling.

Among the field studies shown in the previous table, the values extrapolated from the reported regression equations (7 values) result in quite different urinary concentrations for the sum of mandelic and phenylglyoxylic acids at the end of the shift (193 to 520 mg.g^{-1} cr and 474 to 1185 mg.L^{-1}) for exposure to the 8h-OELV of 100 mg.m^{-3}. As with urinary styrene, studies showing regressions calculated for exposure measured for 4 hours or 15 minutes (Apostoli et al., 1983 and Prieto et al., 2002) were not used. Average urinary concentrations of mandelic acid + phenylglyoxylic acid calculated from other studies were close to 500 mg.g^{-1} of creatinine. However, this concentration is not really consistent with those calculated for mandelic acid and phenylglyoxylic acid separately using the regression equations found in the literature. The average concentrations calculated for exposure to the 8h-OELV, excluding the studies by Apostoli et al. (1983) and Prieto et al. (2002), are 448 µg.g^{-1} of creatinine for mandelic acid and 163 µg.g^{-1} of creatinine for phenylglyoxylic acid (for samples taken at the end of the shift). The daily values reported by De Rosa in 1993 seem abnormally low in relation to the average values published by the same author in 1988 for the same group of workers. Concentrations calculated from the equations indicated in the publication, for exposure to 100 mg.m^{-3}, lead to concentrations of mandelic acid + phenylglyoxylic acid (end of shift and start of shift) that are systematically lower than those calculated from other studies. Thus, only the mean values of 1988 were used, the values from 1993 were not considered for calculating the BLV.

Based on the non-excluded study data and by normalising the concentrations relative to creatinine to the value of 1.4 g of creatinine.L^{-1}, the average concentration corresponding to the sum of urinary mandelic and phenylglyoxylic acids at the end of the shift is equal to 588 mg.g^{-1} of creatinine (846 mg.g^{-1} of creatinine for Gobba et al., 1993b, and 398 mg.g^{-1} of creatinine for Ong et al., 1994). The urinary concentration for the sum of mandelic and phenylglyoxylic acids of 600 mg.g^{-1} of creatinine in samples taken at the end of the shift can be used for the establishment of a BLV based on exposure to the 8h-OELV of 100 mg.m^{-3}.

For comparison, the value proposed by the ACGIH is 400 mg.g^{-1} of creatinine and the one proposed by the MAK Committee in Germany is 600 mg.g^{-1} of creatinine for OELVs for styrene at 86 mg.m^{-3}.

In the absence of data in France, the study conducted in Italy in the general population may be used to define a biological reference value for the sum of mandelic acid + phenylglyoxylic acid (Manini et al., 2004). The urinary concentration of mandelic acid + phenylglyoxylic acid, corresponding to the 97th percentile of the distribution in this study is 3.03 mg.g^{-1} of creatinine, rounded to 3 mg.g^{-1} of creatinine.

Conclusions of the collective expert appraisal

The biological limit values (BLVs) proposed for monitoring exposure to styrene are:

Urinary styrene at the end of the shift

BLV based on exposure to the 8h-OEL (100 $mg.m^{-3}$): **40 $\mu g.L^{-1}$**

BLV based on a health effect: None

Biological reference values: NS

Sum of mandelic and phenylglyoxylic acids at the end of the shift

BLV based on exposure to the 8h-OEL (100 $mg.m^{-3}$): **600 $mg.g^{-1}$ of creatinine**

BLV based on a health effect: None

Biological reference values: 3 $mg.g^{-1}$ of creatinine

Sampling method and factors that may affect the interpretation of results

Urinary styrene

Since the BLV was calculated from assays performed at the end of the shift, it is advisable to take samples at the end of the shift, regardless of which day of the working week.

Samples should be taken from workers outside the workplace after a shower and a change of clothing, to avoid contamination of samples by ambient air. Samples must be collected in glass vials that are almost completely filled and sealed with a polytetrafluoroethylene cap. A minimum volume of 20 mL is required. The samples can be stored for 15 days at 4°C.

Urinary mandelic acid + phenylglyoxylic acid

Mandelic acid has two phases of elimination, the second of which has a half-life of between 17 and 26 hours. It is possible that mandelic acid accumulates during a working week, and it is therefore preferable to take urine samples at the end of the shift at the end of the working week to measure concentrations of mandelic acid + phenylglyoxylic acid.

Samples should be taken using glass, plastic or polyethylene vials (100 mL). The ACGIH (2007) recommends acidifying samples with 1% acetic acid (v/v). The IRSST (1995a and b) advocates acidification followed by extraction with ethyl acetate. A sample volume of between 10 and 20 mL is required.

Consumption of alcohol and certain medications can inhibit metabolism and thus interfere with the interpretation of measurement results (ACGIH, 2007; INRS, 2010).

Competitive inhibition of the enzyme systems metabolising styrene occurs during co-exposure to acetone (quite low), methanol, benzene, toluene, xylene, ethylbenzene and phenylglycol. Exposure to these last two substances also leads to the urinary excretion of mandelic and phenylglyoxylic acids.

Biometrology

Urinary styrene			
	Method 1	**Method 2**	**Method 3**
Analytical technique	Headspace injection-gas chromatography and mass spectrometry detection (HS-GC-MS)	Headspace injection-gas chromatography-purge and trap sample concentrator and flame ionisation detection (PT-HS-GC-FID)	Headspace injection-gas chromatography and flame ionisation detection (HS-GC-FID)
Limit of detection	0.01 $\mu g.L^{-1}$	0.4 $\mu g.L^{-1}$	3 $\mu g.L^{-1}$
Limit of quantification			
Fidelity		< 3%	2.5 %
Precision	12 to 22%		
Reference standard			
Existence of an inter-laboratory quality control programme	no	no	no
Processing of sample / duration	Rapid SPE	Purge and trap	no
Sample volume	4 to 10 ml	5 ml	200 µl to 5ml
References	Pezzagno et al., 1985 Wang et al., 2007 Prado et al., 2006	Prieto et al., 2002 Periago et al., 1996	Ghittori S et al., 1987 Ong CN et al., 1994 INRS, 2010

Urinary Mandelic acid and Phenylglyoxylic acid			
	Method 1	**Method 2**	**Method 3**
Analytical technique	High-performance liquid chromatography with ultraviolet detector (+/- diode array) (HPLC-UV or HPLC-UV/DAD)	Gas chromatography and flame ionisation detection (GC-FID)	Gas chromatography and tandem mass spectrometry detection (GC-MS/MS)
Limit of detection	0.5 to 10 $mg.L^{-1}$ (phenylglyoxylic acid) 5 to 25 $mg.L^{-1}$ (mandelic acid)	10 $mg.L^{-1}$ for each acid	0.16 $mg.L^{-1}$ (phenylglyoxylic acid) 0.115 $mg.L^{-1}$ (mandelic acid)
Limit of quantification	20 $mg.L^{-1}$ (phenylglyoxylic acid) 50 $mg.L^{-1}$ (mandelic acid)	20 $mg.L^{-1}$ (phenylglyoxylic acid) 30 $mg.L^{-1}$ (mandelic acid)	
Fidelity	CV 7 to 10 %	CV = 0.94% (phenylglyoxylic acid) CV = 1.03% (mandelic acid)	
Precision	Bias < 5% to 20 %	NS	
Reference standard	Commercial solution		
Existence of an inter-laboratory quality control programme	G-EQUAS		
Processing of sample / duration	Simple dilution and centrifugation Liquid/liquid extraction	Liquid/liquid extraction then derivatisation	Derivatisation then solid-phase microextraction by direct immersion (DI-SPME)
Sample volume	200 µl to 1 ml	1 to 5 ml	2 ml
References	Ogata et al., 1988 Sperlingova et al., 2004 Chua et al., 1993 Papaleo et al., 2011	Flek et al., 1980 Lanchote et al., 1994	Pacenti et al., 2008

Urinary Mandelic acid and Phenylglyoxylic acid		
	Method 4	**Method 5**
Analytical technique	Gas chromatography and mass spectrometry detection (GC-MS)	Liquid chromatography and tandem mass spectrometry detection (LC-MS/MS)
Limit of detection	2 $mg.L^{-1}$ (phenylglyoxylic acid) 1 $mg.L^{-1}$ (mandelic acid)	0.01 $mg.L^{-1}$
Limit of quantification	40 $mg.L^{-1}$ (phenylglyoxylic acid) 10 $mg.L^{-1}$ (mandelic acid)	
Fidelity		
Precision	< 6% (phenylglyoxylic acid) < 19% (mandelic acid)	< 8%
Reference standard	Commercial solution	
Existence of an inter-laboratory quality control programme	G-EQUAS	
Processing of sample / duration	Solid-phase extraction (SPE) then derivatisation	Simple dilution to 1/10 and filtration Deuterated internal standard (d6-mandelic acid and d5-phenylglyoxylic acid)
Sample volume	10 ml	20 µl
References	Szucs et al., 2002	Manini et al., 2002 Marchese et al., 2004

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