COLLECTIVE EXPERT APPRAISAL:

SUMMARY AND CONCLUSIONS

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

on the evaluation of biomarkers of exposure and recommendation of biological limit values and biological reference values for styrene oxide

[CAS No 96-09-3]

This document summarises the work of the Expert Committee on Health Reference Values (HRV) and the Working Group on biomarkers (Biomarkers WG).

Presentation of the issue

On 12 June 2007, AFSSET, which became ANSES in July 2010, was requested by the Directorate General for Labour (DGT) to carry out the necessary assessment for setting occupational exposure limits for styrene oxide.

France does not currently have any occupational exposure limits for styrene oxide.

The DGT asked the Agency to propose occupational exposure limits based on health considerations.

The Agency thus assessed the available data to decide on the relevance of recommending the monitoring of one or more biomarkers and to establish, where appropriate, biological values for the biological monitoring of occupational exposure to styrene oxide.

Scientific background

Biological monitoring of exposure in the workplace 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 body (lung, skin, digestive tract). It is particularly worthwhile 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 (personal protection equipment, 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.

Committee definitions

Biomarker of exposure (BME): parent substance, or one of its metabolites, determined in a biological matrix, whose variation is associated with exposure to the targeted agent. 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 meaning:

- if the body of scientific evidence is sufficient to quantify a dose-response relationship with certainty, the BLVs will be established on the basis of health data (no effect for threshold substances or risk levels for non-threshold carcinogens);
- in the absence of such data for substances with threshold effects, BLVs are calculated on the basis of the expected concentration of the biomarker of exposure (BME) when the worker is exposed to the 8-hour OEL. For carcinogens, in the absence of sufficient quantitative data, the biological limit value is calculated on the basis of another effect (pragmatic BLV). These latter values do not guarantee the absence of health effects, but aim to limit exposure to these substances in the workplace.

Whenever possible, the 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 BMEs) or in a control population not occupationally exposed to the substance under study (preferentially for biomarkers of effects).

These BRVs 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 particularly useful in cases where it is not possible to establish a BLV (ANSES, 2017).

Organisation of the expert appraisal

ANSES entrusted examination of this request to the “health reference values” Committee. The Agency also mandated the Working Group on biomarkers (Biomarkers WG) for this expert appraisal. The methodological and scientific aspects of the work of this group were regularly submitted to the Expert Committee. The report produced by the working group takes account of 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

One ANSES employee and one rapporteur of the Biomarkers WG produced a summary report on biomarkers of exposure and the recommendation of biological limit values (BLVs) and biological reference values for the BME(s) considered relevant.

The summary report on the BMEs for styrene oxide was based on bibliographical information taking into account the scientific literature published on this substance until 2019. The bibliographical research was conducted in the following databases: Medline, Scopus and the Public Health Database. The scientific articles selected for evaluating biomonitoring data on styrene oxide were identified using the following keywords: "styrene oxide", "biomarker", "biomonitoring", "biological monitoring", "urine", "blood", "occupational", "analysis method".

The rapporteur reassessed the original articles or reports cited as references whenever he considered it necessary, or whenever the Committee requested it.

The report, the summary and conclusions of the collective expert appraisal work were adopted by the health reference values Committee (term of office 2017-2020) on 21 November 2019. Two experts abstained. Their position is set out in the Annex 4 of the French report.

Result of the collective expert appraisal

Toxicokinetics data

The toxicokinetics data on styrene oxide are limited, because most studies have focused on understanding the metabolism and distribution of styrene, which is 95% metabolised into styrene oxide. The main available data come from studies undertaken in animals (primarily dealing with the intraperitoneal route).

Absorption

Gaté et al. 2012 measured blood concentrations of styrene oxide in Fischer rats following exposure to 25, 50 and 75 ppm styrene oxide by inhalation (whole body). These concentrations were 0.24 ± 0.10 µg/g of blood at 25 ppm, 0.60 ± 0.20 µg/g of blood at 50 ppm and 0.86 ± 0.43 µg/g of blood at 75 ppm. In comparison, in the same study, the average blood concentration of styrene oxide measured in rats for exposure to 1000 ppm styrene was 0.37 ± 0.08 µg/g of blood.

Whereas dermal absorption appears slow (IARC, 1994), oral absorption seems to be rapid (Langvardt and Nolan, 1991). In a study in Fischer 344 rats exposed to oral doses of 275 and 550 mg/kg body weight [bw], maximum blood concentrations of styrene oxide were observed after five to 15 minutes, with values ranging from 0.27 to 8.84 µg/mL and from 2.1 to 32.4 µg/mL respectively for 275 and 550 mg/kg bw (Langvardt and Nolan, 1991). Studies by intraperitoneal

injection of the product showed similar blood plasma kinetics with a maximum blood concentration reached within seven minutes in CD2F1 mice exposed by injection of 200 mg/kg styrene oxide (Bidoli et al., 1980).

Distribution

Styrene oxide is rapidly distributed throughout the body with higher concentrations in the liver, brain, kidneys and duodenum than in the blood, lungs and spinal cord, in male rats exposed by the intraperitoneal route (Savolainen and Vainio, 1976). However, in mice (exposed by the same route), maximum concentrations of styrene oxide were reached two hours after injection and were higher in the subcutaneous adipose tissue than in the other tissues studied (blood, liver, kidneys, lungs, brain) (Nordqvist et al., 1983; Löf et al., 1984).

Metabolism

Styrene oxide is the main metabolite of styrene. Figure 1 shows the pattern by which styrene oxide is metabolised from styrene: in humans, styrene is 95% metabolised into styrene oxide by cytochrome P450 action, mainly by CYP2E1 (ANSES, 2010).

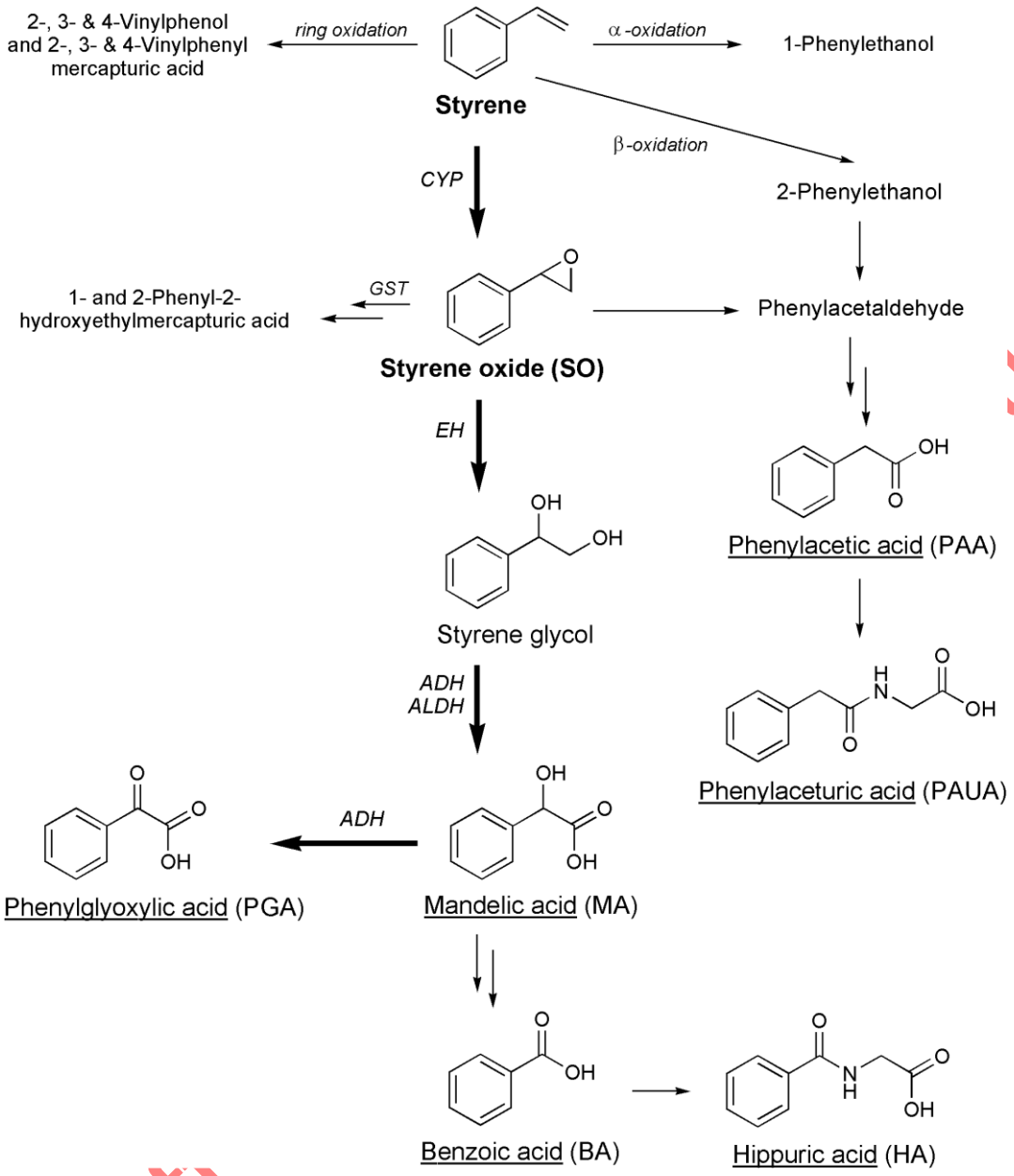

Figure 1: Metabolic pattern of styrene and styrene oxide in humans and animals (Cosnier et al., 2012)

The enzymes that metabolise styrene oxide are stereoselective. During hydrolysis by epoxide hydrolase, the *(S)* enantiomer is favoured over the *(R)* enantiomer (Watabe et al., 1981), keeping in mind that *(R)*styrene 7,8-oxide has proven to be more toxic than (*S*)styrene 7,8-oxide (Vodicka et al., 2006).

Mendrala et al. (1993) showed similarities and differences between the metabolism of styrene in humans, rats and mice. The affinity of cytochromes P450 for styrene was similar in humans and mice; the Vmax was similar between rats and mice but was lower in humans (with respective values of 9.3, 13 and 2.1 nmol/mg proteins per minute). The Km of epoxide hydrolase for the

conversion of styrene oxide into styrene glycol was lower in humans than in mice (it was respectively 0.01 versus 0.74 mM). As for the Vmax value, it did not differ between the species. Glutathione-S-transferase activity for the conversion of styrene oxide into glutathione conjugates was lower in humans than in animals. The authors concluded that, qualitatively, these data indicate that the mouse has the greatest capacity and the human the lowest capacity to form SO from styrene. In addition, human liver should be more effective than rodent liver in hydrolyzing low levels of SO.

Adduct formation

In humans

Yeowell-O'Connell et al., (1996) measured haemoglobin and albumin adducts in 48 workers exposed to styrene oxide and styrene (n=20 for styrene oxide and n=48 for styrene). The samples were subjected to base hydrolysis to release styrene glycol, representing the binding of styrene oxide to carboxylic acid in the proteins. The proteins were also treated with Raney nickel, a catalyst, to release 1-phenylethanol (1-PE) and 2-phenylethanol (2-PE), representing the binding of styrene oxide to cysteine, at the β and α positions respectively. The authors studied correlations between the adducts and atmospheric levels of styrene and styrene oxide (Table 1).

Table 1: Determination coefficients concerning albumin adducts (Yeowell-O'Connell et al., 1996)

Albumin adducts (nmol/g of albumin)	Correlation with atmospheric styrene oxide (n=20)	Correlation with atmospheric styrene (n=48)
1-PE 0.29 ± 0.038	r2 = 0.128 (p = 0.122)	r2 = 0.050 (p = 0.126)
2-PE 1.68 ± 0.116	r2 = 0.312 (p = 0.010)	r2 = 0.118 (p = 0.017)
Styrene glycol 1.80 ± 0.191	r2 = 0.070 (p = 0.259)	r2 = 0.000 (p = 0.965)

For the same workers exposed jointly to styrene and styrene oxide, Rappaport et al. 1996 reported levels of albumin adducts (α and β, corresponding respectively to 2-PE and 1-PE) and DNA adducts (positions 1 and 2) based on their smoker or non-smoker status and job category. For all of the subjects for whom measurements of atmospheric styrene oxide were available (n=20), only the correlation between 2-PE adducts and concentrations of styrene oxide was significant (Pearson's r = 0.559, 0.01 ≤ p < 0.05). In non-smokers (n=8), albumin adducts were correlated with atmospheric concentrations of styrene oxide (Pearson's r = 0.811, 0.01 ≤ p < 0.05 and Pearson's r = 0.667, 0.05 ≤ p < 0.1 respectively for 2-PE and 1-PE), but not DNA adducts. In smokers, no significant correlation between adducts formation and exposure to styrene oxide was reported. When the subjects were divided into job groups (eight groups such as laminating, painting, service and mould repair, six of which had styrene oxide measurements), only the correlation between 2-PE adducts and concentrations of styrene oxide was significant (Pearson's r = 0.863, 0.01 ≤ p < 0.05). Additional analyses were undertaken by category of exposure levels to styrene oxide (low (81.8 µg/m^3 (min-max: 13.4-123 µg/m^3, n=10) and high (236 µg/m^3 (min-

max: 142-525 µg/m^3, n=10)). Albumin adducts (2-PE) were statistically higher for the highly exposed category (p=0.019) and for smokers (p=0.006). The other adducts (1-PE and DNA-1 and DNA-2 adducts) did not show any statistically significant differences. According to the authors, the difference in levels of albumin adducts between smokers and non-smokers might be explained by the fact that cigarette smoke might contain styrene oxide; alternatively, there might be a difference in metabolism between smokers and non-smokers, in particular with depletion of epoxide hydrolases.

Fustinoni et al. (2008) measured albumin (Alb) and haemoglobin (Hb) adducts in workers exposed to styrene and styrene oxide. The study included 21 workers, eight of whom were involved in the production of reinforced plastics and 13 in varnishes (exposure by joint inhalation of styrene and styrene oxide), as well as 22 control subjects working as automotive mechanics. Overall, albumin adduct levels in the control subjects were half those in the exposed workers, whereas the levels were similar for DNA adducts. The authors reported that levels of albumin adducts were significantly different between the exposed and unexposed (control) subjects but did not differ between the varnish and plastics workers. Moreover, no difference was observed for haemoglobin adducts between the exposed and unexposed workers.

Rappaport et al. (1993) showed that the reactivity with styrene oxide was 13 times higher for albumin than for haemoglobin in humans. According to Osterman-Golkar et al. (1995), this difference was due to the high reactivity of serum albumin cysteine 34. The authors also reported that serum albumin has a half-life of around 20 days in humans; therefore, adducts of this protein may enable biomonitoring of integrated exposure over several days.

In animals

Styrene oxide binds to cysteine groups of plasma proteins and to haemoglobin histidine. The amino acids to which it binds are, in order of preference, cysteine, histidine, lysine and serine (IARC, 1994). Female Wistar rats having received doses of styrene oxide by the intraperitoneal route showed carboxylic acid ester haemoglobin adducts of styrene oxide, with levels that increased with the dose, even more so at higher doses (IARC, 1994). Another study on the covalent binding of styrene and styrene oxide to albumin and haemoglobin was undertaken in Sprague-Dawley rats, following the intraperitoneal injection of radiolabelled products (styrene and styrene oxide). A linear relationship was observed between the adduct levels and the doses administered (0.5 to 3 mmol styrene/kg bw and 0.1 to 1 mmol styrene oxide/kg bw). Comparison of the curves for the two products showed that the production of protein adducts was much higher following exposure to styrene oxide. The slope of the adduct formation curve following exposure to styrene accounted for only 2% of that obtained after exposure to styrene oxide (IARC, 1994).

The IARC mentioned that styrene oxide can also form DNA adducts via alkylation. It was shown that the relative adduct formation yields of alkylated deoxynucleotides in an aqueous buffer were, in increasing order, deoxyguanosine, deoxycytidine, deoxyadenosine and thymidine. The dominant product of the styrene oxide reaction with DNA was 7-alkylguanine. N7-alkylguanosine adducts of styrene oxide were found in five organs of mice (liver, brain, lung, spleen and testis) after intraperitoneal injection of styrene oxide. Covalent binding of styrene oxide to DNA was also detected in the forestomach of rats (IARC, 1994). Koskinen et al. (2000) suggested that N7-guanosine adducts should be targeted when conducting biomonitoring in humans. This study also showed that the lifetimes of styrene oxide adducts varied depending on the DNA base involved (they were longer for N7-guanosine than for N7-adenosine). These adducts accounted for around 1% of adduct formation.

Excretion

The main route of excretion for styrene oxide and its metabolites in animals is renal (80% of the oral exposure dose was found in urine in rabbits), but excretion of the mercapturic acid metabolites of styrene oxide and glutathione conjugate derivatives appears to depend on the species (IARC, 1994). The in vitro half-life of styrene oxide in blood was estimated at 0.70 ± 0.03 h in two human subjects (Rappaport et al., 1993). In rats under the same conditions, it was 0.36 ± 0.02 h. In orally exposed mice, styrene oxide was detected in blood 5h after exposure to trace levels (Langvardt and Nolan, 1991). According to the authors, there might be a saturation of the process of styrene oxide elimination. When urinary metabolites were compared following exposure to styrene and styrene oxide, the metabolites specific to exposure to styrene were 4-vinylphenol, 1-phenylethanol and 2-phenylethanol. The urinary metabolites common to both products were hydroxyphenethyl mercapturic acid, monoglucuronide of phenylethylene glycol, mandelic acid, hippuric acid and phenylglyoxylic acid (Leibman, 1975). In humans, there do not seem to be any studies on the urinary excretion of mandelic acid or phenylglyoxylic acid following exposure to styrene oxide. However, the available data on exposure to styrene show that the half-life of mandelic acid is biphasic, with an initial 4 to 9 h elimination phase and a 17 to 26 h terminal phase. The half-life of phenylglyoxylic acid is 8 to 10 h following exposure to styrene (ANSES, 2014). Following exposure to styrene, the excretion of mercapturic acids is minor compared to that of mandelic and phenylglyoxylic acids; however, no studies have been undertaken in humans enabling urinary concentrations of mercapturic acids to be quantified following exposure to styrene oxide.

Selection of biomarkers of exposure and effect

Biomarkers of exposure (BME)

Name of the biomarker of exposure	Sampling matrix
Styrene oxide	Blood
Mandelic acid	Urine
Phenylglyoxylic acid	Urine
1-phenyl-2-hydroxy-ethylmercapturic acid	Urine
2-phenyl-2-hydroxy-ethylmercapturic acid	Urine
Haemoglobin adducts	Blood
Albumin adducts	Blood
DNA adducts	Blood

The advantages and disadvantages of each BME have been identified.

There do not seem to be any studies in humans quantifying the excretion of urinary metabolites following exposure to styrene oxide.

None of the described BMEs are specific to exposure to styrene oxide, because they may be present after exposure to styrene. Moreover, concomitant occupational exposure to styrene and styrene oxide is common, as shown in the field studies by Rappaport et al. (1996), Yeowell-O'Connell et al. (1996) and Tornero-Velez et al. (2001). It is therefore difficult to establish correlations between the BMEs and atmospheric concentrations of styrene oxide.

Mandelic and phenylglyoxylic acids are the main metabolites found in urine following exposure to styrene oxide. The challenge is determining urinary quantities of metabolites due to exposure to styrene oxide in the concomitant presence of styrene or other substances that generate these metabolites. Regarding mercapturic acids, it seems that these BMEs cannot be recommended for monitoring exposure to styrene, due in particular to the role of polymorphic enzymes in the interindividual variability of levels (De Palma et al., 2001). The same conclusion can be drawn for exposure to styrene oxide.

With regard to the possible BMEs, the following should be noted:

- Styrene oxide in blood would seem *a priori* to be an interesting BME. However, its very short half-life in blood of 0.7 h (Rappaport et al., 1993) and its high reactivity with the blood components limit the implementation of this BME.

- In the sector of reinforced plastics production, a correlation was found between exposure to styrene oxide and albumin adducts but not haemoglobin adducts. Although this correlation was not found in another study where exposure was lower, probably due to a small number of subjects and high background noise and despite the influence of smoking, this BME was identified as relevant for the biological monitoring of exposure to styrene oxide.

Styrene oxide DNA adducts also seem to have good potential as a BME. This is another BME for which studies are available in workers and show a correlation between exposure to the product and adduct formation; as with albumin adducts, the influence of smoking should be noted.

Therefore, styrene oxide in blood, albumin adducts and DNA adducts could *a priori* be used as BMEs for exclusive exposure to styrene oxide (without any concomitant exposure to styrene).

Biomarkers of effect

A correlation between the level of sister-chromatid exchanges and exposure was observed in the study by Rappaport et al. (1996), in workers simultaneously exposed to styrene and styrene oxide. Given that sister-chromatid exchanges can also reflect exposure to several other xenobiotics (such as butadiene), this biomarker of effect is not specific. In conclusion, no biomarker of effect can be recommended.

To conclude, in light of the available data, the following three biomarkers of exposure have been selected as relevant for the biological monitoring of workers exposed to styrene oxide (without any concomitant exposure to styrene): styrene oxide in blood, styrene oxide albumin adducts and styrene oxide DNA adducts.

Information on biomarkers of exposure identified as relevant for the biomonitoring of exposed workers

Name	Styrene oxide in blood	
Other substances giving rise to this biomarker	Styrene	
Concentrations measured in exposed workers or volunteers (with exposure levels and sampling times if available)	• **Field studies:** Tornero-Velez *et al.*, 2001 – exposure from 5.7 to 119 ppb (28.1 to 585 µg/m^3) (co-exposure from 4.4 to 97 ppm styrene) Blood sampling at end of workshift (ES) and immediate extraction by n-pentane ≈0.05 – 0.5 µg/L styrene oxide in blood (n= 27) Correlation equation: ln[blood-SO (µg/L)]=-3.27+0.406 ln[SO (ppb)], r=0.62 Serdar *et al.*, 2006 – exposure to 17.1 ppb styrene oxide (median) (min-max: <1 – 138 ppb) corresponding to 0.069 µg/L styrene oxide in blood (median) (min-max: <0.05 – 0.393) for n=328 workers Co-exposure to styrene (median: 9.14 ppm, min-max: <1-117 ppm). • **Studies on volunteers**: NS	
Conversion factor and molecular weight	MW: 120.15 1 µg/L = 0.0083 µmol/L 1 µmol/L = 120.15 µg/L	
Concentration in the general population[1]	NS	
Recommended limit values for exposed workers	USA - ACGIH (BEI)	NS
	Germany - DFG (BAT)	NS
	Quebec - IRSST (IBE)	NS
	Finland - FIOH (BAL)	NS
	Other value(s) (Swiss, etc.)	NS

[1] *For this table and the following tables:* or failing this, in a non-occupationally exposed control population; 95th percentile or failing this the median or the mean (number of people in the study, if this information is available)

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Name	**Albumin adducts**	
Other substances giving rise to this biomarker	Styrene	
Concentrations measured in exposed workers or volunteers (with exposure levels and sampling times if available)	• **Field studies:** Rappaport *et al.* (1996) and Yeowell-O'Connell *et al.* (1996) Exposure from 13.4 to 525 µg/m³ styrene oxide with a mean of 159 µg/m³ ± 25.0 (n=20) (co-exposure to styrene: mean of 64.3 mg/m³) Styrene oxide-albumin (1-PE) 0.290 ± 0.038 nmol/g protein (n=48) Styrene oxide-albumin (2-PE) 1.68 ± 0.116 nmol/g protein (n=48) Styrene oxide-albumin (SG) 1.80 ± 0.191 nmol/g protein (n=48) • Fustinoni *et al.*, 2008, workers in the varnish (n=13) and reinforced plastics (n=8) industries *Varnish workers (n=13)*: • Exposure to styrene oxide (co-exposure to styrene (median 3.4 mg/m³)): 12.2 (6.7-32.0) µg/m³ (median (min-max)) • 1-PE albumin adducts (start of workshift, SS): 0.48 (0.21-0.75) (nmol/g protein) • 2-PE albumin adducts (SS): 6.18 (2.66-9.53) (nmol/g protein) *Plastics workers (n=8):* • Exposure to styrene oxide (co-exposure to styrene (median 18.2 mg/m³)): 133.5 (39.5-281.5) µg/m³ (median (min-max)) • 1-PE albumin adducts (SS): 0.23 (<0.03-1.22) (nmol/g protein) • 2-PE albumin adducts (SS): 5.91 (4.40-8.14) (nmol/g protein) *Note: PE: phenyl-hydroxyethyl mercapturic acid, SG: styrene glycol* • **Studies on volunteers**: NS	
Conversion factor (with molecular weight)	NS	
Concentration in the general population	• Fustinoni *et al.* 2008 *Control subjects (n=22 automotive mechanics)*: - Exposure to styrene oxide (co-exposure to styrene): below the limit of detection (<5 µg/m³) - 1-PE albumin adducts (SS): 0.19 (<0.03-0.53) (nmol/g protein) - 2-PE albumin adducts (SS): 3.57 (<0.90-5.18) (nmol/g protein)	
Recommended limit values for exposed workers	USA - ACGIH (BEI)	NS
	Germany - DFG (BAT)	NS
	Quebec - IRSST (IBE)	NS
	Finland - FIOH (BAL)	NS
	Other value(s) (Swiss, etc.)	NS

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Name	DNA adducts	
Other substances giving rise to this biomarker	Styrene	
Concentrations measured in exposed workers or volunteers (with exposure levels and sampling times if available)	• **Field studies:** Rappaport *et al.*, 1996 Exposure from 13.4 to 525 µg/m^3 styrene oxide with a mean of 159 µg/m^3 ± 25.0 (n=20) (co-exposure to styrene: mean of 64.3 mg/m^3) Styrene oxide-DNA(1)[1] 15.8 ± 3.22 RAL[3] x 10^8 (n=47) Styrene oxide-DNA(2)[2] 14.2 ± 2.30 RAL[3] x 10^8 (n=47) *1. Identified as being N2-(2-hydroxy-l-phenylethyl)-2'-deoxyguanosime 3,5-biphosphate* *2. Unidentified* *3. Relative Adduct Level* • **Studies on volunteers**: NS	
Conversion factor (with molecular weight)	NS	
Concentration in the general population	NS	
Recommended limit values for exposed workers	USA - ACGIH (BEI)	NS
	Germany - DFG (BAT)	NS
	Quebec - IRSST (IBE)	NS
	Finland - FIOH (BAL)	NS
	Other value(s) (Swiss, etc.)	NS

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310 ***Study of the relationship between concentrations of BMEs and health effects***

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312 No studies in humans enable concentrations to be linked to carcinogenicity data or to other health
313 effects for the selected BMEs.

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315 ***Study of the relationship between concentrations of BMEs and atmospheric***
316 ***concentrations***

317

318 There have been very few workplace studies on exposure to styrene oxide.

319 The study by Tornero-Velez *et al*. (2001) reported correlations between concentrations of blood
320 styrene oxide and atmospheric styrene oxide (according to the following correlation equation):
321 ln[blood-SO (µg/L)]=-3.27+0.406 ln[SO (ppb)], r=0.62).

322 The study by Rappaport *et al*. (1996) measured levels of adducts (albumin and DNA) for various
323 levels of exposure to styrene oxide. The authors did not provide the correlation equations.

A major limitation of these studies was co-exposure to styrene at levels 500 to 1000 times higher (Fustinoni *et al.*, 1998 and 2008).

Moreover, since no OEL is available in France for styrene oxide, these correlation equations cannot be used to establish a biological limit value corresponding to exposure to the 8h-OEL.

Establishment of BLVs and choice of biological reference values

Given that:

- styrene oxide is a metabolite of styrene and exposure to styrene oxide is generally concomitant with exposure to styrene (occupational exposure to styrene oxide alone is uncommon);
- there are very few data on the various links between exposure and health effects, health effects and potential BMEs, or atmospheric concentrations and BMEs;
- no OEL is currently available for styrene oxide;
- the half-life of styrene oxide in blood is short (0.7 h);
- there are no values for styrene oxide DNA or albumin adducts in the unexposed general population except for a few controls from studies undertaken in workers,

no biological limit value (BLV) can be recommended for the biological monitoring of occupational exposure to styrene oxide.

Since no studies in the general population or in non-occupationally exposed control populations give any concentrations for the three BMEs selected as relevant, no BRV can be recommended.

Conclusions of the collective expert appraisal

BLV based on an effect: **None**

BLV based on an 8h-OEL: **None**

BRV: **None**

Since styrene oxide is classified as Category 1B carcinogenic and is genotoxic (IARC, 2019), exposure should be reduced to the lowest possible level.

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