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 reference values for 1,3-butadiene

[CAS No: 106-99-0]

This document summarises the work of the Expert Committees on health reference values (HRV Committee) and 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

On 12 June 2007, AFSSET, which became ANSES in July 2010, was requested by the Directorate General for Labour to carry out the necessary assessment for setting occupational exposure limits for 1,3-butadiene.

This request was entrusted to ANSES's OEL Committee which, in June 2010, issued a report in which it estimated the additional risk of leukaemia deaths (for a scenario of occupational exposure to 1,3-butadiene based on 8 hours per day, 240 days per year over 45 years of employment; probability calculated up to 70 years of age) was estimated to be:

- 10^{-4} for 45 years of exposure to a concentration of 0.08 $mg.m^{-3}$
- 10^{-5} for 45 years of exposure to a concentration of 0.008 $mg.m^{-3}$
- 10^{-6} for 45 years of exposure to a concentration of 0.0008 $mg.m^{-3}$.

France currently has no occupational exposure limits (over 8 hours or 15 minutes) for this substance.

ANSES decided to supplement its expert appraisal by assessing the biological monitoring data in the occupational environment for 1,3-butadiene, in order to establish the relevance of recommending monitoring of one or more biomarkers in addition to the OEL and the establishment of biological limit values for the selected biomarker(s).

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 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 meaning:

- 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 (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 last 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 biomarkers of exposure) 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 Expert Committee on expert appraisal for recommending occupational exposure limits for chemical agents (OEL Committee) and then 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 Committees. The report produced by the working group takes account of observations and additional information provided by the members of the Committees.

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 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 1,3-butadiene was based on bibliographical information taking into account the scientific literature published on this substance until 2017. The bibliographical research was conducted in the following databases: Medline, Scopus. The scientific articles selected for evaluating biomonitoring data on 1,3-butadiene were identified using the following keywords: “butadiene”, “biomarker”, "biomonitoring", “biological monitoring”, “urine”, “blood”, “occupational” while limiting the search to human data.

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 Expert Committee on expert appraisal for recommending occupational exposure limits for chemical agents on 4 July 2017.

This collective expert appraisal work and the summary report were submitted to public consultation from 30/01/2018 to 30/03/2018. The people or organizations that contributed to the public consultation are listed in appendix 1 of the report (only available in French). The comments received were reviewed by the Committee on Health Reference Values (term of office 2017-2020) who finally adopted this version on the 9 may 2019.

Result of the collective expert appraisal

Toxicokinetics data

1,3-butadiene (BD) enters the body mainly via the respiratory tract. Absorption is rapid and occurs through passive diffusion from the lungs to the blood. The blood:air partition coefficient (women: 1.46 and men: 1.62) and alveolar ventilation are the major determinants of absorption. The absorbed fraction of 1,3-butadiene is 45.6 ± 13.9% for men and 43.4 ± 2.9% for women (Lin *et al.* 2001). Furthermore, age and tobacco smoking reduce lung absorption. In addition, the blood:air partition coefficient (1.57) increases by an average of 20% in subjects with high blood triglyceride levels following the ingestion of a high-fat meal, which can have a significant influence on the dose of butadiene absorbed in the event of exposure (Lin *et al.* 2002). The other routes of absorption (oral and dermal) have not been documented.

According to studies in rodents, 1,3-butadiene and its metabolites are distributed extensively in the tissues from the start of exposure. The highest concentrations, one hour after exposure ended, are measured in the blood, respiratory tract, intestines, liver, kidneys, bladder and pancreas. There are no data available on humans.

For all species, 1,3-butadiene mainly seems to be oxidised by cytochromes P450 action, and then hydrolysed by epoxide hydrolase or oxidised on the second double bond (by CYP2E1s). The metabolites formed can be detoxified via glutathione S-transferases (GSTs) to form mercapturic acids likely to be eliminated in urine (DHBMA: 3,4-dihydroxybutylmercapturic acid, MHBMA: monohydroxybutenylmercapturic acid and THBMA: 1,3,4-trihydroxybutylmercapturic acid). MHBMA is classically considered to be a mixture of two isomers, 1-MHBMA and 2-MHBMA (N-acetyl-S-1-(hydroxymethyl-2-propenyl)-l-cysteine and N-acetyl-S-2-(hydroxymethyl-3-propenyl)-l-cysteine respectively). The existence of a third isomer was recently demonstrated, 3-MHBMA (N-acetyl-S-4-(hydroxy-2-buten-1-yl)-l-cysteine), which seems to predominate compared to 1-MHBMA and 2-MHBMA (Jain *et al.* 2015, Boyle *et al.* 2016, Alwis *et al.* 2012). Haemoglobin adducts in humans, MHBVal (N-(1- and N-(2-hydroxy-3-butenyl)valine), and THBVal (N-(2,3,4-trihydroxybutyl)valine) have also been observed (Osterman-Golkar *et al.* 1993; Albertini *et al.* 2003). The formation of a third haemoglobin adduct, Pyr-Val (N,N(2,3-dihydroxy-1,4-butadiyl)valine), was also demonstrated in humans by Boysen *et al.* (2012). The THBVal adduct largely predominates and represents 99.6% of the total of the three types of adducts. Many DNA adducts have been described *in vitro*; in humans, there are fewer data. Three DNA adducts have been described in humans: N1-THB-Ade (N-1-(2,3,4-trihydroxybutyl)adenine) (Zhao *et al.* 2000), N7-THB-Gua (N-7-(2,3,4-trihydroxybut-1-yl)guanine), the predominant adduct in the *in vivo* studies, and the adduct N7-HB-Gua (N-7-(1-hydroxy-3-buten-2-yl)guanine) in very small quantities (below the limit of quantification) with low stability (Sangaraju *et al.* 2014).

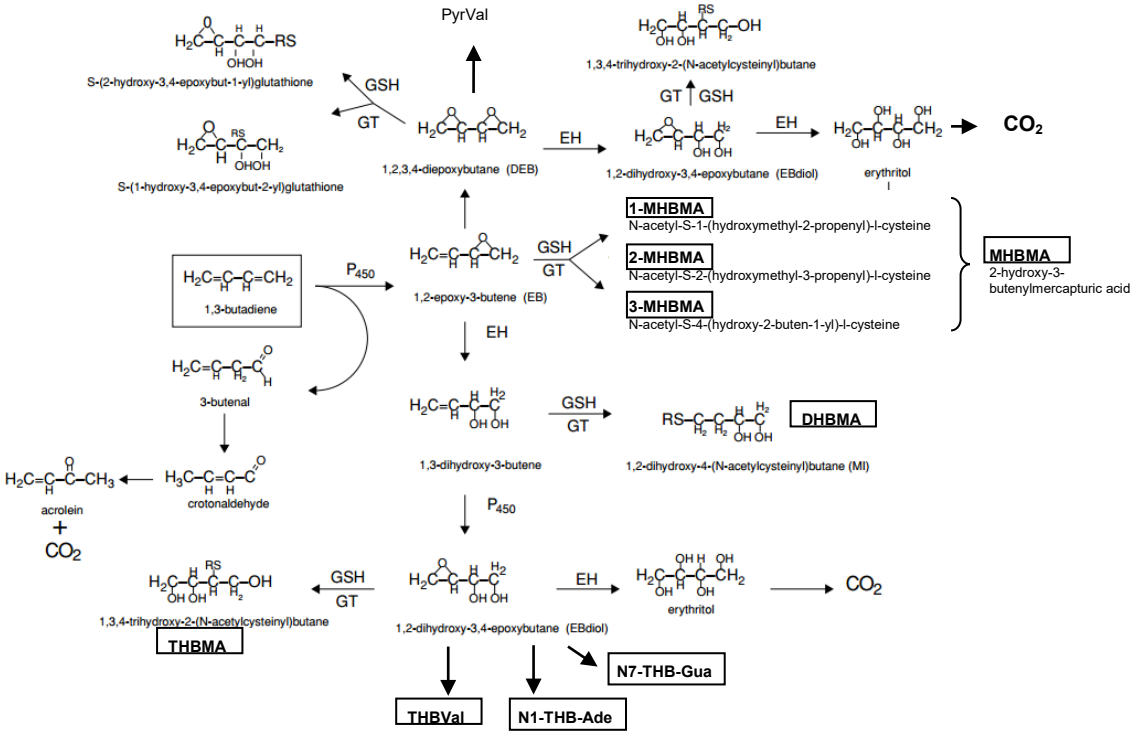

Figure 1 : metabolism of 1,3-butadiene (adapted from Health Canada 2000)

In monkeys, it has been estimated that 39% of total metabolites are eliminated in the urine, 0.8% in the faeces and 56% in the form of CO_2 exhaled in the 70 hours post-inhalation exposure (Dahl *et al.* 1990). In humans, the rate of excretion of the DHBMA and MHBMA metabolites is classically reported to be > 97% and < 3% respectively (INRS 2012), but the fraction of the inhaled dose is unknown. More recently, it has been shown among smokers that the relative excretion of mercapturic acids is broken down as follows: 93% for DHBMA, 5% for THBMA and 2% for MHBMA (Kotapati *et al.* 2014). Although the elimination kinetics of mercapturic acids in humans are unclear, field studies indicate prolonged elimination that can lead to accumulation over consecutive days of exposure (Albertini et al. 2001, Van Sittert et al. 2000).

Selection of biomarkers of exposure and effect

Biomarkers of exposure (BME)

The analysis of the data in the literature led to nine potential BMEs being identified:

- butadiene in exhaled air, blood and urine
- mercapturic acids in urine: MHBMA, DHBMA and THBMA
- haemoglobin adducts in blood: MHBVal, THBVal
- DNA adducts in blood: N1-THB-Ade, N7-THB-Gua and N7-HB-Gua

The advantages and disadvantages of each BME have been identified.

The 1,3-butadiene measured in exhaled air, blood or urine is specific to exposure to 1,3-butadiene but the correlations with atmospheric concentrations of 1,3-butadiene are low and the data are limited.

Concerning urinary mercapturic acids, correlations with atmospheric 1,3-butadiene have been described. People that have not been exposed to 1,3-butadiene have lower baseline levels of MHBMA than DHBMA, which could partly be due to endogenous sources. However, MHBMA includes different isomers that may cause differences in quantification depending on the analytical techniques used. THBMA is a BME representative of a metabolic pathway that leads to the formation of DNA adducts but about which there are few data.

Haemoglobin adducts exhibit correlations with atmospheric concentrations of 1,3-butadiene. MHBVal has very low levels that require a sensitive technique. THBVal is found at higher levels (for the same reasons as DHBMA as well as the potential existence of endogenous sources). These two BMEs are interesting as indicators of cumulative exposure. However, the associated analytical technique is cumbersome.

Concerning DNA adducts, they offer good specificity but the data are limited.

Consequently, the BMEs selected as relevant for the biological monitoring of occupational exposure are the **three mercapturic acids (MHBMA, DHBMA and THBMA)** and the **haemoglobin adducts (MHBVal and THBVal).**

Biomarkers of effect

The main biomarkers of effect studied for 1,3-butadiene are mutations at the HPRT[1] locus and micro-nuclei. No data are available regarding the link between these indicators and the risk of leukaemia, the critical effect chosen for 1,3-butadiene, moreover there are few usable data on the link between these markers and atmospheric exposure to 1,3-butadiene.

[1] *Hypoxanthine-guanine phosphoribosyl transferase*

In view of these data and the invasive nature of the measurement of these parameters in blood, no biomarker of effect was selected.

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

Name	Urinary MHBMA (monohydroxybutenylmercapturic acid)
Other substances giving rise to this biomarker of exposure	Chloroprene (Eckert *et al.* 2013)

Concentrations measured in exposed workers or volunteers (with the exposures and sampling times) **Mean or Median[a] (Min-Max) or ± SD**	Fustinoni *et al.* 2004 Production of BD and polymers: 29 exposed subjects E, 18 non-exposed subjects NE Atmospheric BD: E: 11.5 (< 0.1 - 220.6) µg/m^3, NE: 0.9 (< 1 - 3.8) µg/m^3 MHBMA End of Shift (ES): E: **10.5 ± 13.7** µg/L, NE: **7.5 ± 7.0** µg/L Albertini *et al.* 2007 Production of BD: 53 exposed subjects E (23 women, 30 men), and 51 non-exposed subjects NE (26 women, 25 men) Atmospheric BD: exposed women: 56[a] (4 - 219) µg/m^3, exposed men: 241[a] (4 - 12,583) µg/m^3, non-exposed women: 4[a] (4 - 219) µg/m^3, non-exposed men: 4[a] (4 - 157) µg/m^3 MHBMA ES: exposed women: **19.2 ± 27.5** µg/L, E M: **47.9 ± 44.3** µg/L, non-exposed women: **8.3 ± 10.1** µg/L, non-exposed men: **14.9 ± 10.3** µg/L Van Sittert *et al.* 2000 Production of BD: 5 high-exposed men HE, and 16 low-exposed men LE Atmospheric BD: HE: 9460[a] (1584 - 27,500) µg/m^3, LE: 26.4[a] (0 - 440) µg/m^3 MHBMA ES: HE: **97[a] (7.8 - 464)** µg/L, LE: **4.2[a] (< 0.1 - 16)** µg/L Albertini *et al.* 2001 Production of BD: 25 NE, 24 E monomers M, 34 E polymers P Atmospheric BD: NE (n = 28): 0.026 (0.002 - 0.125) µg/m^3, EM (n = 217): 0.643 (0.002 - 19.909) µg/m^3, EP (n = 319): 1.76 (0.002 - 39.030) µg/m^3 MHBMA ES: NE: **1.70 ± 1.54** µg/L, EM: **9.44 ± 12.97** µg/L, EP: **120.17 ± 228.17** µg/L Sapkota *et al.* 2006 9 waste collectors Atmospheric BD: 2.38[a] (0.51 - 8.12) µg/m^3 MHBMA ES: **9.7 ± 9.5** µg/L Kotapati *et al.* 2015 BD production: 32 exposed subjects (16 women, 16 men), 40 non-exposed subjects (19 women, 21 men) Atmospheric BD: exposed women: 320 ± 340 µg/m^3, EM: 680 ± 410 µg/m^3, non-exposed women NEF: 7 ± 5 µg/m^3, NEM: 7 ± 5 µg/m^3 MHBMA ES: exposed women: **8.3 ± 8.1** µg/L, EM: **95.9 ± 111.4** µg/L, non exposed women: **3.1 ± 4.8** µg/L, NEM: **9.9 ± 11.2** µg/L Arayasiri *et al.* 2010 Urban pollution: 24 Bangkok traffic policemen TP, 24 office policemen OP Atmospheric BD: TP: 3.15 ± 0.16 µg/m^3, OP: 0.40 ± 0.05 µg/m^3 MHBMA start of shift SS: TP: **75.07 ± 7.89** µg/g creatinine, OP: **61.91 ± 6.82** µg/g creatinine MHBMA ES: TP: **80.90 ± 11.00** µg/g creatinine, OP: **54.21 ± 4.59** µg/g creatinine Borgie *et al.* 2014 Traffic police TP (n = 24) and office police OP (n = 23) MHBMA ES: TP: **18.7 ± 20.1** OP: **18.8 ± 31** µg/g creatinine
Concentrations measured in exposed workers or volunteers (with the exposures and sampling times) **Mean or Median[a] (Min-Max) or ± SD**	Albertini *et al.* 2001 (same data also exploited by Albertini *et al.* 2003) Production of BD: 25 NE, 24 E monomers M, 34 E polymers P Atmospheric BD: NE (n = 28): 0.026 (0.002 - 0.125) µg/m^3, EM (n = 217): 0.643 (0.002 - 19.909) µg/m^3, EP (n = 319): 1.76 (0.002 - 39.030) µg/m^3 MHBMA ES: NE: **1.70 ± 1.54** µg/L, EM: **9.44 ± 12.97** µg/L, EP: **120.17 ± 228.17** µg/L Sapkota *et al.* 2006 USA, 7 non-smoking volunteers in an urban area UA, 7 non-smoking volunteers in a suburban area SA Atmospheric BD: UA: 1.62[a] (0.23 - 3.66) µg/m^3, SA: 0.88[a] (0.23 - 4.36) µg/m^3 MHBMA: UA: **6.0 ± 4.3** µg/L, SA: **6.8 ± 2.6** µg/L
Conversion factor (with molecular weight)	MW: 233 g.mol^{-1} 1 µg.L^{-1} = 4.3 × 10^{-3} µmol.L^{-1} 1 µmol.L^{-1} = 233 µg.L^{-1}

Concentrations in the general population[2] **Mean or Median[a] (Min-Max) or ± SD**	Sarkar *et al.* 2013 37 adult Caucasian smokers in the USA MHBMA: **2.55 ± 1.72** µg/g creatinine Boyle *et al.* 2016 United States, 488 pregnant women including 33 smokers 3-MHBMA: 75th percentile: **12.1** µg/L Urban *et al.* 2003 Germany, 10 non-smokers NS, 10 smokers S (mean = 16.3 cig/d) MHBMA: NS: 12.5 ± 1.0 µg/24h, S: 86.4 ± 14.0 µg/24h, or NS: **7.4 ± 0.6** µg/g creatinine, S: **51.4 ± 8.3** µg/g creatinine Yuan *et al.* 2012 343 cases of lung cancer LC, 392 controls CON smokers MHBMA: LC: **2.6[a] (2.3 - 3.1)** µg/g creatinine CON: **1.9[a] (1.7 - 2.3)** µg/g creatinine Eckert *et al.* 2011 Germany, 54 NS, 40 S MHBMA: NS: **< 5.0[a] (95th percentile < 5.0)** µg/g creatinine S: **< 5.0[a] (95th percentile 9.5)** µg/g creatinine Pluym *et al.* 2015 25 S and 25 NS Germany 1-MHBMA: S ≥ 10 cig/d (n = 12): **< LQ[a] (< LD - 0.52)** µg/g creatinine, S > 10 cig/d (n = 13): **0.28[a] (< LD - 0.66)** µg/g creatinine, NS: **< LD[a] (< LD - 0.15)** µg/g creatinine 2-MHBMA: S ≥ 10 cig/d (n = 12): **0.53[a] (< LQ - 0.96)** µg/g creatinine, S > 10 cig/d (n = 13): **0.80[a] (0.095 - 1.30)** µg/g creatinine, NS: **< LD[a] (< LD - 0.11)** µg/g creatinine Schettgen *et al.* 2009 Germany, 210 subjects aged 19-80 years divided into four tobacco exposure groups (passive and active) on the basis of urine cotinine. 1 (n = 73): no exposure to passive smoking, 2 (n = 38): low exposure to passive smoking, 3 (n=18): high exposure to passive smoking, 4 (n=81): active smokers MHBMA: 1: **< 2[a] (95th percentile < 2)** µg/L, 2: **< 2[a] (95th percentile 2.4)** µg/L, 3: **< 2[a] (95th percentile < 2)** µg/L, 4: **< 2[a] (95th percentile 8.6)** µg/L Zhang *et al.* 2015 China, 1: 55 NS, 2: 61 S (8 mg of tar/cig), 3: 74 S (10 mg of tar/cig) MHBMA (publication data adjusted for creatinine): 1: **30.3 (8.7 - 68.1)** µg/g creatinine, 2: **68.1 (10.0 - 147)** µg/g creatinine, 3: **68.5 (15.1 - 165.7)** µg/g creatinine

[2] 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)

Concentrations in the general population **Mean or Median[a] (Min-Max) or ± SD**	Alwis *et al.* 2012 NHANES, USA, 1203 NS and 347 S (multi-ethnic, men and women, age > 12 years) 1-MHBMA: NS: **< LD**, S: **< LD** 2-MHBMA: NS: **< LD**, S: **1.80 ± 2.10** µg/L 3-MHBMA: NS: **6.40 ± 10** µg/L, S: **36 ± 34** µg/L Sarkar *et al.* 2008 USA, 25 non-smokers NS1, 20 non-smokers NS2, 25 smokers S1, 20 smokers S2 MHBMA: NS1: **0.09 ± 0.10** µg/g creatinine, NS2: **0.006 ± 0.10** µg/g creatinine, S1: **2.70 ± 1.59** µg/g creatinine, S2: **3.64 ± 3.12** µg/g creatinine Roethig *et al.* 2009 USA, 1077 NS, 3585 S MHBMA: weighted mean (standard error): NS: **0.30 (0.02)** µg/24h**,** S: **3.61 (0.1)** µg/24 h. Ding *et al.* 2009 USA, 59 NS, 61 S MHBMA: NS: **Not Detected (ND)- 122** µg/g creatinine, S: **ND - 59.7** µg/g creatinine Carmella *et al.* 2009 USA, 17 S MHBMA: **66.1 ± 69.4** nmol/24h Kotapati *et al.* 2014 36 S (20 ± 7 cig/d) MHBMA: **11 ± 12** µg/g creatinine CDC 2019a (Volume 1) NHANES (2013-2014 campaign), USA: 2639 subjects were divided by age group from 6 years. Analysis according to age, sex and ethnicity Age group ≤ 20 years (n = between 1702 and 1783): Geometric mean (95% confidence interval): 1-MHBMA: **Not Recorded (NR)**, 2-MHBMA: **NR**, 3-MHBMA: **6.03 (5.42-6.70)** µg/L ou **6.85 (6.09-7.70)** µg/g creat. CDC 2019b (Volume 2) NHANES (2013-2014 campaign), USA: analysis according to smoking status Smokers ≤ 20 years (n= between 884 and 905): Geometric mean (95% confidence interval): 1-MHBMA: **NR**, 2-MHBMA: **NR**, 3-MHBMA: 25.6 (21.4-30.8) µg/L ou 26.2 (21.0-32.6) µg/g creat. Non-smokers ≤ 20 years (n= between 1296 and 1369): Geometric mean (95% confidence interval): 1-MHBMA: **NR**, 2-MHBMA: **NR**, 3-MHBMA: **4.3 (3.90-4.74)** µg/L ou **4.96 (4.39-5.61)** µg/g creatinine BAR[3] value (2012): < **2 µg/g creatinine (assessment for non-smokers)**	
Recommended limit values for exposed workers	USA - ACGIH (BEI)	NR
	Germany - DFG (BAT)	BD atmospheric equivalents - MHBMA (2012) ES after several days of exposure: 0.45 mg/m^3 - **10 µg/g creatinine** 1.1 mg/m^3 - **20 µg/g creatinine** 2.3 mg/m^3 - **40 µg/g creatinine** 4.5 mg/m^3 - **80 µg/g creatinine** 6.8 mg/m^3 - **120 µg/g creatinine**
	Quebec - IRSST (BIE)	NR
	Finland - FIOH (BAL)	NR
	Other value(s) (Swiss, etc.)	NR

Name	**Urinary DHBMA (3,4-dihydroxybutylmercapturic acid)**

[3] *Biologischer Arbeitsplatz Referenzwert*

Other substances giving rise to this biomarker of exposure	Chloroprene (Eckert *et al.* 2013)
Concentrations measuredin exposed workers or volunteers (with the exposures and sampling times) **Mean or Median[a] (Min-Max) or ± SD**	Bechtold *et al.* 1994 Production of BD: 7 exposed subjects E, 3 low-exposed subjects LE, 10 non-exposed subjects NE, 9 controls outside the plant C Atmospheric BD: estimated at 6600-8800 µg/m^3 for exposed subjects and < 220 µg/m^3 for non-exposed subjects DHBMA ES: E: **3200 ± 1600** µg/L, LE: **1390 ± 550** µg/L, NE: **630 ± 190** µg/L, C: **320 ± 70** µg/L Kelsey *et al.* 1995 BD production: 44 exposed subjects Atmospheric BD: 484 ± 836 µg/m^3 DHBMA ES: **1206.6 ± 2604.4** µg/g creatinine Ward *et al.* 1996 BD production: 3 exposure groups (1, 2, 3) Atmospheric BD: 1: 660 ± 1298 µg/m^3 (n = 7), 2: 462 ± 462 µg/m^3 (n = 7), 3: 264 ± 594 µg/m^3 (n = 8) DHBMA ES: 1: **761 ± 245** µg/g creatinine, 2: **596 ± 155** µg/g creatinine, 3: **684 ± 176** µg/g creatinine Hallberg *et al.* 1997 BD production: 24 exposed subjects E, 19 non-exposed subjects NE Atmospheric BD: E: 5280 ± 3960 µg/m^3, NE: 660 ± 0.00 µg/m^3 DHBMA ES: E: **2429 ± 1877** µg/L, NE: **694 ± 365** µg/L Hayes *et al.* 2000 BD production: 39 exposed subjects E, 14 non-exposed subjects NE Atmospheric BD: E: 4400[a] (interquartile range 45320) µg/m^3, NE: 0 µg/m^3 DHBMA ES: E: **1300[a] (interquartile range 5200)** µg/g creatinine (n = 17), NE (n = 4): **600[a] (interquartile range 700)** µg/g creatinine Fustinoni *et al.* 2002 BD production: 30 exposed subjects E, 10 non-exposed subjects NE Atmospheric BD: E: 55 ± 53 µg/m^3, NE: Not Determined DHBMA ES: E: **1800 ± 940** µg/g creatinine, NE: **1610 ± 600** µg/g creatinine Fustinoni *et al.* 2004 Production of BD and polymers: 29 exposed subjects E, 18 non-exposed subjects NE Atmospheric BD: E: 11.5 (< 0.1 - 220.6) µg/m^3, NE: 0.9 (< 1 - 3.8) µg/m^3 DHBMA ES: E: **605 ± 409** µg/L, NE: **602 ± 207** µg/L Albertini *et al.* 2007 Production of BD: 53 exposed (23 women, 30 men) and 51 non-exposed (26 women, 25 men) Atmospheric BD: exposed women: 56[a] (4 - 219) µg/m^3, exposed men: 241[a] (4 - 12,583) µg/m^3, non-exposed women: 4[a] (4 - 219) µg/m^3, non-exposed men: 4[a] (4 - 157) µg/m^3 DHBMA ES: exposed women: **508.1 ± 597.4** µg/L, exposed men: **854.1 ± 567.0** µg/L, non exposed women : **331.6 ± 284.9** µg/L, non-exposed men: **512.8 ± 272.1** µg/L

Concentrations measured in exposed workers or volunteers (with the exposures and sampling times) **Mean or Median[a] (Min-Max) or ± SD**	Ammenheuser *et al.* 2001 Styrene-butadiene rubber plant: 24 high-exposed HE, 25 low-exposed LE Atmospheric BD: HE: 3256 ± 814 µg/m^3, LE: 330 ± 44 µg/m^3 DHBMA ES: HE: **2046 ± 348** µg/g creatinine, LE: **585 ± 98** µg/g creatinine Van Sittert *et al.* 2000 Production of BD: 5 high-exposed men HE, and 16 low-exposed men LE Atmospheric BD: HE: 9460[a] (1584 - 27,500) µg/m^3, LE: 26.4[a] (0 - 440) µg/m^3 DHBMA ES: HE: **2719[a] (342 - 20,213)** µg/L, LE: **669[a] (52 - 2947)** µg/L Albertini *et al.* 2001 (same data also exploited by Albertini *et al.* 2003) Production of BD: 25 NE, 24 E monomers M, 34 E polymers P Atmospheric BD: NE (n = 28): 0.026 (0.002 - 0.125) µg/m^3, EM (n = 217): 0.643 (0.002 - 19.909) µg/m^3, EP (n = 319): 1.76 (0.002 - 39.030) µg/m^3 DHBMA ES: NE: **353 ± 157** µg/L, EM: **764 ± 728** µg/L, EP: **4647 ± 6630** µg/L Sapkota *et al.* 2006 9 waste collectors Atmospheric BD: 2.38[a] (0.51 - 8.12) µg/m^3 DHBMA: **378.5 ± 196.0** µg/L Kotapati *et al.* 2015 BD production: 32 exposed subjects (16 women, 16 men), 40 non-exposed subjects (19 women, 21 men) Atmospheric BD: exposed women: 320 ± 340 µg/m^3, exposed men: 680 ± 410 µg/m^3, non-exposed women NEW: 7 ± 5 µg/m^3, non-exposed men: 7 ± 5 µg/m^3 DHBMA ES: EW: **716.1 ± 830.7** µg/L, EM: **3136.1 ± 2560.3** µg/L, NEW: **561.2 ± 531.5** µg/L, NEM: **1480.6 ± 968.5** µg/L Borgie *et al.* 2014 Traffic police TP (n = 24) and office police OP (n = 23) DHBMA ES: TP: **207.5 ± 112.2** OP: **73.3 ± 45.3** µg/g creatinine Sapkota *et al.* 2006 USA, 7 volunteers in an urban area UA, 7 volunteers in a suburban area SA Atmospheric BD: UA: 1.62[a] (0.23 - 3.66) µg/m^3, SA: 0.88[a] (0.23 - 4.36 µg/m^3 DHBMA: UA: **257.8 ± 133.2** µg/L, SA: **306.5 ± 242.7** µg/L
Conversion factor (with molecular weight)	MW: 251 g.mol^{-1} 1 µg.L^{-1} = 4.0 × 10^{-3} µmol.L^{-1} 1 µmol.L^{-1} = 251 µg.L^{-1}
Concentrations in the general population **Mean or Median[a] (Min-Max) or ± SD**	Urban *et al.* 2003 Germany, 10 NS, 10 S (mean = 16.3 cig/d) DHBMA: NS: 459 ± 72 µg/24h, S: 644 ± 90 µg/24h, or NS: **273.2 ± 42.9** µg/g creatinine, S: **383.4 ± 53.2** µg/g creatinine Eckert *et al.* 2011 Germany 54 NS, 40 S DHBMA: NS: **159[a] (95th percentile 329)** µg/g creatinine, S: **211[a] (95th percentile 417)** µg/g creatinine Schettgen *et al.* 2009 Germany 210 subjects aged 19-80 years divided into four tobacco exposure groups (passive and active) on the basis of urine cotinine. 1 (n = 73): no exposure to passive smoking, 2 (n = 38): low exposure to passive smoking, 3 (n=18): high exposure to passive smoking, 4 (n=81): active smokers DHBMA: 1: **289[a] (95th percentile 760)** µg/L, 2: **384[a] (95th percentile 1113)** µg/L, 3: **250[a] (95th percentile 759)** µg/L, 4: **398[a] (95th percentile 1071)** µg/L

<table>
<tr><td>Concentrations in the general population
Mean or Median[a] (Min-Max) or ± SD</td><td colspan="2">Alwis et al. 2012
NHANES, USA, 1203 NS and 347 S
DHBMA: NS: 331 ± 279 µg/L, S: 440 ± 311 µg/L

Zhang et al. 2015
China, 1: 55 NS, 2: 61 S (8 mg of tar/cig), 3: 74 S (10 mg of tar/cig)
DHBMA: 1: 187.56 (68.2 - 344.0) µg/L, 2: 219.31 (65.20 - 396.0) µg/L, 3: 219.47 (66.40 - 400.0) µg/L

Pluym et al. 2015
Germany 25 S and 25 NS
DHBMA: S ≥ 10 cig/d: 112[a] (65.5 - 243) µg/g creatinine, S > 10 cig/d: 122[a] (52.9 - 244) µg/g creatinine, NS: 76.2[a] (47.4 - 349) µg/g creatinine

Roethig et al. 2009
USA, 1077 NS, 3585 S
DHBMA: weighted mean (standard error): NS: 391 (5.5) µg/24 h, S: 556 (4.9) µg/24 h

Ding et al. 2009
USA, 59 NS, 61 S
DHBMA: NS: Not Detected (ND) - 582 µg/g creatinine, S: ND - 1092 µg/g creatinine

Carmella et al. 2009
USA, 17 S
DHBMA: 1038 ± 514 nmol/24 h

Carrieri et al. 2009
Italy, 33 NS
DHBMA: 166 (16 - 599) µg/L

Kotapati et al. 2014
36 smokers (20 ± 7 cig/d)
DHBMA: 631 ± 452 µg/g creatinine

CDC 2019a (Volume 1)
NHANES (2013-2014 campaign), USA: 2639 subjects were divided by age group from 6 years old. Analysis according to age, sex and ethnicity
Age group ≤ 20 years (n = 1791- 1792): Geometric mean (95% confidence interval): DHBMA: 242 (223-261) µg/L or 283 (260-307) µg/g creatinine

CDC 2019b (Volume 2)
NHANES (2013-2014 campaign), USA: analysis according to smoking status
Smokers ≤ 20 years (n= 913- 914): Geometric mean (95% confidence interval): DHBMA: 360 (327-397) µg/L ou 366 (332-403) µg/g creatinine
Non-smokers ≤ 20 years (n=1374- 1375): Geometric mean (95% confidence interval): DHBMA: 223 (206-240) µg/L or 267 (245-290) µg/g creatinine

BAR value (2012): 400 µg/g creatinine (assessment for non-smokers)</td></tr>
<tr><td rowspan="6">Recommended limit values for exposed workers</td><td>USA - ACGIH (BEI)</td><td>2.5 mg/L ES (eq. 2 ppm or 4.4 mg/m^3 BD) (2006)</td></tr>
<tr><td>Germany - DFG (BAT)</td><td>BD atmospheric equivalents – DHBMA (2012) ES after several days of exposure:
0.45 mg/m^3 - 600 µg/g creatinine
1.1 mg/m^3 - 1000 µg/g creatinine
2.3 mg/m^3 - 1600 µg/g creatinine
4.5 mg/m^3 - 2900 µg/g creatinine
6.8 mg/m^3 - 4200 µg/g creatinine</td></tr>
<tr><td>Quebec - IRSST (BIE)</td><td>NR</td></tr>
<tr><td>Finland - FIOH (BAL)</td><td>NR</td></tr>
<tr><td>Other value(s) (Swiss, etc.)</td><td>NR</td></tr>
</table>

Name	Urinary THBMA (1,3,4-trihydroxybutylmercapturic acid)	
Other substances giving rise to this biomarker of exposure	None	
Concentrations measuredin exposed workers or volunteers (with the exposures and sampling times) **Mean ± SD**	Kotapati *et al.* 2015 BD production: 32 exposed subjects (16 women, 16 men), 40 non-exposed subjects (19 women, 21 men) Atmospheric BD: exposed women: 320 ± 340 µg/m^3, exposed men: 680 ± 410 µg/m^3, non-exposed women: 7 ± 5 µg/m^3, non-exposed men: 7 ± 5 µg/m^3 THBMA ES: exposed women: **47.4 ± 70.9** µg/L, exposed men: **139.3 ± 104.7** µg/L, non-exposed women: **24.2 ± 16.6** µg/L, non-exposed men: **57.1 ± 33.5** µg/L	
Conversion factor (with molecular weight)	MW: 267 g.mol^{-1} 1 µg.L^{-1} = 3.74 nmol.L^{-1} 1 µmol.L^{-1} = 267 µg.L^{-1}	
Concentrations in the general population	Kotapati *et al.* 2011 USA, 19 NS, 27 S THBMA: NS: **13.7 ± 7.9** µg/g creatinine, S: **21.6 ± 10.2** µg/g creatinine Kotapati *et al.* 2014 36 smokers (20 ± 7 cig/d) THBMA: **31 ± 20** µg/g creatinine	
Recommended limit values for exposed workers	USA - ACGIH (BEI)	NR
	Germany - DFG (BAT)	NR
	Quebec - IRSST (BIE)	NR
	Finland - FIOH (BAL)	NR
	Other value(s) (Swiss, etc.)	NR

Name	Blood MHBVal (N-(1- and N-(2-hydroxy-3-butenyl)valine)
Other substances giving rise to this biomarker of exposure	None

Concentrations measured in exposed workers or volunteers (with the exposures and sampling times) **Mean or Median[a] (Min-Max) or ± SD**	Sorsa *et al.* 1996 2 plants, 3 sampling campaigns BD production plant 1: 10 production workers P1, 7 maintenance and laboratory workers ML, 9 controls C1 BD production plant 2, campaign 1: 12 polymerisation and production workers PP, 14 controls C2 BD production plant 2, campaign 2: 4 production workers P2, 8 controls C3 Atmospheric BD (personal sensors, measurement on the work shift corresponding to the day of the blood sample): Plant 1: 70% < 440 µg/m^3 Plant 2 (campaigns 1 and 2): 50% between 440 and 4400 µg/m^3, and 10% > 22,000 ppm with a few isolated samples of more than 1100 mg/m^3 MHBVal (no information on the period of exposure 3 months before the blood sample, no quantified data available to differentiate non-smokers from smokers): Plant 1: P1: **0.16 ± 0.10** pmol/g globin, ML: **< 0.1** pmol/g globin, C1: **< 0.13** pmol/g globin Plant 2 (campaign 1): PP: **2.0 ± 3.6** pmol/g globin, C2: **0.13 ± 0.35** pmol/g globin Plant 2 (campaign 2): P2: **0.54 ± 0.33** pmol/g globin, C3: **0.12 ± 0.05** pmol/g globin Osterman-Golkar *et al.* 1993 BD production: 4 exposed non-smoker workers ENS, 5 non-exposed non-smoker workers NENS, 1 university employee U, 2 non-exposed smokers NES (>30 cigarettes/day) Atmospheric BD: ENS: Mean: 3.5 ppm MHBVal (no information on the period of exposure 3 months before the blood sample): ENS: **1.8 ± 0.7** pmol/g globin, NENS + U **< 0.5** pmol/g globin (except for one subject whose level was not reported), NES: **0.8** pmol/g globin Osterman-Golkar *et al.* 1996 BD production: 10 exposed workers E (7 smokers, 3 non-smokers), 7 maintenance and laboratory workers ML (3 smokers, 4 non-smokers), 10 controls C (5 smokers, 5 non-smokers) Atmospheric BD (measurement on one or three consecutive work shifts close to the day of the blood sample): E: 11,200 ± 18,600 µg/m^3, ML: 600 – 900 µg/m3 MHBVal ES (no information on the period of exposure 3 months before the blood sample, no quantified data available to differentiate non-smokers from smokers): E: **0.16 ± 0.099** pmol/g, ML and C: **~0.05** pmol/g Van Sittert *et al.* 2000 Study 1: BD production: 36 loading workers L (21 non-smokers LNS, 15 smokers LS), 16 controls C1 (12 non-smokers C1NS, 4 smokers C1S) Study 2: 24 monomer workers M, 34 styrene-butadiene rubber workers (SBR), 25 controls C2 Atmospheric BD (10 measurements distributed over a period of 60 days before the blood sample): Study 1: Truck loading (n = 4): 2090 (902 - 5280) µg/m^3, ship loading (n = 9): 8140 (< 440 - 20,900) µg/m^3 Study 2: M: 638 (44 - 3520) µg/m^3, SBR: 1804 (44 - 9240) µg/m^3, C2: 22 (0 - 83.6) µg/m^3 MHBVal: L: **1.3 (0.6 - 3.8)** pmol/g, LNS: **1.3 (0.6 - 3.8)** pmol/g, LS: **1.3 (0.6 - 2.0)** pmol/g: C1: **0.27 (<0.1 - 0.3)** pmol/g, C1NS: **0.33 (<0.1 - 1.2)** pmol/g, C1S: **0.08 (<0.1 - 0.3)** pmol/g. M**: 0.47 (0.1 - 2.1)** pmol/g, SBR: **2.2 (0.6 - 6.2)** pmol/g, C2: **0.2 (0.1 - 1.0)** pmol/g Boogaard *et al.* 2002 77 workers in BD companies Atmospheric BD (according to graphical data, 10 measurements distributed over a period of 2 months before the blood sample): 10 - 10,000 µg/m^3 MHBVal (according to graphical data): **0.06 - 6.3** pmol/g globin. Albertini *et al.* 2001 (same data also exploited by Albertini *et al.* 2003) Production of BD: 25 NE, 24 E monomers EM, 34 E polymers EP Atmospheric BD (10 measurements distributed over a period of 28 to 84 days before the blood sample depending on the subjects): NE (n = 28): 0.026 (0.002 - 0.125) µg/m^3, EM (n = 217): 0.643 (0.002 - 19.909) µg/m^3, EP (n = 319): 1.76 (0.002 - 39.030) µg/m^3 MHBVal: NE: **0.224 ± 0.205** pmol/g, EM: **0.466 ± 0.452** pmol/g, EP: **2.23 ± 1.399** pmol/g

Conversion factor (with molecular weight)	MW: NR 1 µg.L^{-1} = NR 1 µmol.L^{-1} = NR	
Concentrations in the general population	NR	
Recommended limit values for exposed workers	USA - ACGIH (BEI)	2.5 pmol/g of haemoglobin ES after 4 months of exposure (eq. 2 ppm or 4.4 mg/m^3 BD) (2006)
	Germany - DFG (BAT)	Calculation of 2.1 pmol/g of haemoglobin ES (eq. 1 ppm or 2.2 mg/m^3 BD after 18 weeks of exposure) but not selected officially (2005)
	Quebec - IRSST (BIE)	NR
	Finland - FIOH (BAL)	NR
	Other value(s) (Swiss, etc.)	NR
	Germany - DFG (BAT)	NR
	Quebec - IRSST (BIE)	NR
	Finland - FIOH (BAL)	NR
	Other value(s) (Swiss, etc.)	NR

Name	**Blood THBVal (N-(2,3,4-trihydroxybutyl)valine)**
Other substances giving rise to this biomarker of exposure	None
Concentrations measured in exposed workers or volunteers (with the exposures and sampling times) **Mean or Median[a] (Min-Max) or ± SD**	Perez *et al.* 1997 Petrochemical plant: 2 workers E, 2 controls NE Atmospheric BD (estimated): E: ~ 2200 µg/m^3 THBVal (no information on the period of exposure 3 months before the blood sample): E: **10.15** pmol/g and **14.2** pmol/g, NE: **1.85** pmol/g and **3.32** pmol/g Albertini *et al.* 2001 (same data also exploited by Albertini *et al.* 2003) Production of BD: 25 NE, 24 E monomers EM, 34 E polymers EP Atmospheric BD (10 measurements distributed over a period of 28 to 84 days before the blood sample depending on the subjects): NE (n = 28): 0.026 (0.002 - 0.125) µg/m^3, EM (n = 217): 0.643 (0.002 - 19.909) µg/m^3, EP (n = 319): 1.76 (0.002 - 39.030) µg/m^3 THBVal: NE: **94.8 ± 38.7** pmol/g, EM: **178.7 ± 101.3** pmol/g, EP: **717.3 ± 425.7** pmol/g Hayes *et al.* 2000 BD production: 39 exposed subjects E, 14 non-exposed subjects NE Atmospheric BD (measurement on the work shift corresponding to the day of the blood sample): E: 4400[a] (IQ 45320) µg/m^3, NE: 0 µg/m^3 THBVal (no information on the period of exposure 3 months before the blood sample): E: **74.0[a] (interquartile range 30.9)** pmol/g (n = 33), NE (n = 25): **37.6 (interquartile range 9.2)** pmol/g Swenberg *et al.* 2000 BD Production, China: polymerisation workers P (n = 24), workers BD (n= 7), maintenance workers M (n= 7), non-exposed US workers NE (7 NS and 4 S), controls C (n = 25) Atmospheric BD: P: 2200 µg/m^3, BD: 7700 µg/m^3, M: 2420 µg/m^3, C: 0 µg/m^3 THBVal (no information on the period of exposure 3 months before the blood sample): P: **71 ± 24** pmol/g, BD: **140 ± 94** pmol/g, M: **78 ± 48** pmol/g, C: **39 ± 13** pmol/g, NENS: **36 ± 23** pmol/g, NES: **40 ± 9** pmol/g

Concentrations measuredin exposed workers or volunteers (with the exposures and sampling times) **Mean or Median[a] (Min-Max) or ± SD**	Begemann *et al.* 2001 BD production: 10 monomer workers M, 10 polymer workers P, 10 copolymer workers CoP, 10 controls C, 14 workers exposed to diesel D Atmospheric BD (measurement on the work shift corresponding to the day of the blood sample): 31 (4 - 201) µg/m^3 in the BD plant THBVal (no information on the period of exposure 3 months before the blood sample): M: **44.8[a] (30.3 - 61.4)** pmol/g, P: **41[a] (22.1 - 48.2)** pmol/g, CoP: **33[a] (25 - 43.9)** pmol/g, C: **34.7[a] (22.7 - 44.9)** pmol/g, D: **43.5[a] (22.7 - 57)** pmol/g Vacek *et al.* 2010 Polymerisation unit, 25 control men CM (administrative) and 30 exposed men EM, and 26 control women CW and 23 exposed women EW Atmospheric BD (10 measurements distributed over a period of 120 days before the blood sample): CM: 0.007 ± 0.012 mg/m^3, EM: 0.808 ± 1.663 mg/m^3, CW: 0.008 ± 0.015 mg/m^3, EW: 0.397 ± 1.094 mg/m^3 THBVal: CM-smokers: **501.9 ± 436.6** pmol/g, CM-non-smokers: **179.1 ± 40.4** pmol/g, EM-smokers: **± 931.3 448.3** pmol/g, EM-non-smokers: **909.6 ± 353.9** pmol/g CW-smokers: **189.2 ± 48.5** pmol/g, CW-non-smokers: **180.2 ± 93.3** pmol/g, EW-smokers: **294.8 ± 249.6** pmol/g, EW-non-smokers: **199.6 ± 85.8** pmol/g	
Conversion factor (with molecular weight)	MW: NR 1 µg.L^{-1} = NR 1 µmol.L^{-1} = NR	
Concentrations in the general population	NR	
Recommended limit values for exposed workers	Germany - DFG (BAT)	NR
	Quebec - IRSST (BIE)	NR
	Finland - FIOH (BAL)	NR
	Other value(s) (Swiss, etc.)	NR

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

According to the analysis of the existing scientific literature, no study reporting correlation was identified between the biological concentrations of the selected BMEs and the health effects.

Study of the relationship between concentrations of biomarkers and atmospheric concentration

Many studies report correlations between the selected BMEs (MHBMA, DHBMA, THBMA, MHBVal and THBVal) and the atmospheric concentration of 1,3-butadiene. Concentrations of each BME were calculated for each available correlation equation and for three atmospheric concentrations of 1,3-butadiene (0.08 mg.m^{-3}, 0.008 mg.m^{-3} and 0.0008 mg.m^{-3}) associated respectively with the three levels of additional risk of leukaemia deaths, 10^{-4}, 10^{-5} and 10^{-6}. The field studies selected and the calculations of BME concentrations performed are described in the table below (Table 1).

Table 1: Summary of BMEs concentrations, calculated from data enabling the biological concentrations to be linked with the atmospheric concentrations

IBE	Equations linking the exposure to BME concentrations (ES)	Level	BME concentrations
MHBMA	log MHBMA (µg/L) = 1,591(0,202)* + 0,655(0,142)* X log (BD + 0,007) (ppm) r^2 = 0,542, p < 0,001 *(Van Sittert et al. 2000)*	10^{-4} 10^{-5} 10^{-6}	5 µg.L^{-1} 2 µg.L^{-1} 1,6 µg.L^{-1}
	Log MHBMA (µg/L) = 0,631 X log BD (mg/m3) + 1,61 r^2 = 0,3582, p < 0,005 *(Kotapati et al. 2015)*	10^{-4} 10^{-5}	8,3 µg.L^{-1} 1,9 µg.L^{-1}

		10^{-6}	0,5 µg.L^{-1}
	Log MHBMA (µg/L) = 0,3784 X log BD (mg/m^3) + 1,0814** r^2 = 0,2154 *(Albertini et al. 2003)*	10^{-4} 10^{-5} 10^{-6}	4,6 µg.L^{-1} 1,9 µg.L^{-1} 0,8 µg.L^{-1}
DHBMA	log DHBMA (µg/L) = 3,344(0,180)* + 0,371(0,126)* X log (BD+0,007) (ppm) r^2 = 0,325, p < 0,01 *(Van Sittert et al. 2000)*	10^{-4} 10^{-5} 10^{-6}	687 µg.L^{-1} 409 µg.L^{-1} 357 µg.L^{-1}
	DHBMA (µg/g créat) = (776 ± 179) X BD (ppm) + 474 (equation established by the DFG with data provided by the author) *(Ammenheuser 2001)*	10^{-4} 10^{-5} 10^{-6}	502 µg.g^{-1} créat. 477 µg.g^{-1} créat. 474 µg.g^{-1} créat.
	Log DHBMA (ng/mL) = 0,1563 X log BD(mg/m3) + 3,146 r^2 = 0,0762, p < 0,005 *(Kotapati et al. 2015)*	10^{-4} 10^{-5} 10^{-6}	943 µg.L^{-1} 658 µg.L^{-1} 459 µg.L^{-1}
	Log DHBMA (µg/L) = 0,2232 X log BD(mg/m3) + 3,1704** r2 = 0,3184 (Albertini 2003)	10^{-4} 10^{-5} 10^{-6}	842 µg.L^{-1} 504 µg.L^{-1} 301 µg.L^{-1}
THBMA	Log THBMA (µg/L) = 0,1814 x log BD (mg/m3) + 1,903 r^2 = 0,19, p < 0,005 *(Kotapati et al. 2015)*	10^{-4} 10^{-5} 10^{-6}	36,1 µg.g^{-1} créat. 23,8 µg.g^{-1} créat. 15,7 µg.g^{-1} créat.
MHBVal	Log MHBVal (pmol/g) = 0,219 (0,068)* + 0,566 (0,064)* X log (BD + 0,016) (ppm) r^2 = 0,495, p < 0,0001 *(Van Sittert et al. 2000)*	10^{-4} 10^{-5} 10^{-6}	0,31 pmol.g^{-1} 0,18 pmol.g^{-1} 0,16 pmol.g^{-1}
	Log MHBVal (pmol/g) = 0,527 (0,058)* x log BD (mg/m3) + 0,054 (0,043*) r^2 = 0,505, p < 0,0001 *(Boogaard et al. 2002)*	10^{-4} 10^{-5} 10^{-6}	0,30 pmol.g^{-1} 0,09 pmol.g^{-1} 0,03 pmol.g^{-1}
	Ln MHBVal (pmol/g) = 0,098 + 0,491 x ln BD (mg.m^{-3}) Pearson's correlation coefficient: 0,700 (r^2 = 0,491) *(Albertini et al. 2003)*	10^{-4} 10^{-5} 10^{-6}	0,32 pmol.g^{-1} 0,10 pmol.g^{-1} 0,03 pmol.g^{-1}
THBVal	Ln THBVal (pmol/g) = 6,01 + 0,395 x ln BD (mg/m3) Pearson's correlation coefficient: 0,718 (r^2 = 0,515) *(Albertini et al. 2003)*	10^{-4} 10^{-5} 10^{-6}	150,2 pmol.g^{-1} 60,5 pmol.g^{-1} 24,4 pmol.g^{-1}
	Ln THBVal (pmol/g) = 6,3999 + 0,2289 x ln BD (mg/m3)** r^2 = 0,3795 *(Vacek et al. 2010)*	10^{-4} 10^{-5} 10^{-6}	337,6 pmol.g^{-1} 199,3 pmol.g^{-1} 117,6 pmol.g^{-1}

** Standard error of the regression coefficients*
*** Estimated from graphical data*

Establishment of BLVs and choice of biological reference values

The Committee considered that the carcinogenicity of 1,3-butadiene operates in humans according to a non-threshold mechanism of action. It was not possible to use the field studies to establish a dose-response relationship between the concentrations of the different BMEs selected (MHBMA, DHBMA, THBMA, MHBVal and THBVal) and leukaemia.

Using the correlation equations linking the BME concentrations to atmospheric exposure, it is possible to calculate BME concentrations corresponding to the three atmospheric concentrations of 1,3-butadiene (0.08 $mg.m^{-3}$, 0.008 $mg.m^{-3}$ and 0.0008 $mg.m^{-3}$) associated respectively with the three levels of additional risk of leukaemia deaths, 10^{-4}, 10^{-5} and 10^{-6}. By averaging for each BME the values obtained from the available correlation equations, it would then be possible to calculate the theoretical BLVs. However, these values would be extrapolated for very low atmospheric concentrations (especially for the risk levels 10^{-5} and 10^{-6}) compared to the atmospheric concentrations used to establish the equations, which introduces too much uncertainty to be reliable. In addition, some of these theoretical BLVs are found very close to or even below the values found in non-exposed subjects. For this reason, for the five BMEs selected, the Committee considered that no BLV could be proposed.

Thus, since it is not possible to recommend a biological limit value, a biological reference value may therefore be proposed.

Proposed biological reference values

With regard to the biological reference values, the only BME for which the data are sufficient to determine a BRV are urinary mercapturic acids, **DHBMA and 3-MHBMA**. Regarding THBMA, no approach has been undertaken to determine a BRV due to the lack of data (very few studies available).

For the MHBVal adduct, certain field studies have measured the concentration in non-exposed subjects, but as the number of subjects was very low and, the concentrations close to the limits of detection (with unacceptably large standard deviations), it was not possible to recommend a BRV based on these values. Similarly, for the THBVal adduct, disparate data with small numbers of subjects (and an analytical method poorly suited to routine use) did not make it possible to recommend a BRV for this BME.

For MHBMA, studies usually measure this BME without distinguishing between the isomers, although a few more recent studies have measured them separately. Among these studies, the very recent data reported by the CDC[4] (CDC, 2019a and 2019b) are very comprehensive, since they concern the three isomers of MHBMA, with or without adjustment for creatinine, with or without distinction as to smoking status, for a large number of subjects, and the 95th percentile values are provided by the authors.

These data confirm that 3-MHBMA is the foremost isomer in terms of quantity, systematically detected in both smokers and non-smokers, and that this BME could be retained to propose a BRV. The adequate interpretation requires the use of quantification by a specific analysis technique, based on LC-MS-MS[5] (Alwis et al., 2012). The risk of misinterpretation of the results if the analysis technique is not specific (possible interference with the urinary matrix) is important. This BME is significantly influenced by tobacco (see Table 6, section 4.3), which requires dissociation of reference values as a function of smoking status. Jain (2015) performed a statistical analysis of the differences between smokers and non-smokers for this IBE. However, this analysis was based on an earlier version of the NHANES values that were

[4] Centers for Disease Control and Prevention
[5] Liquid chromatography tandem-mass spectrometry

subsequently modified so this statistical analysis can no longer be used for this comparison. On the NHANES values (2013-2014 campaign) reported by the CDC in 2019, smokers have higher levels than non-smokers both for women and men. The use of the 95th percentile of the NHANES data (CDC, 2019b) makes it possible to recommend BRVs for 3-MHBMA according to smoking status.

The recommended BRVs for 3-MHBMA are:

- for non-smokers: 20.9 µg/L rounded to à **20** µg.L^{-1} or 16.5 µg.g^{-1} creatinine rounded to **15** µg.g^{-1} creatinine
- **for smokers**: 119 µg/L rounded to **120 µg/L** or 110 µg.g^{-1} creatinine

Concerning DHBMA, urinary concentrations are significantly higher than those of MHBMA isomers, with high variability measured in the general population according to studies. These concentrations in the general population are close to those measured in workers occupationally exposed to 1,3-butadiene, indicating a significant background level in controls. Some studies also suggest possible endogenous sources of DHBMA (carbohydrate catabolism generating 3-butene-1,2-diol) (Fustinoni et al., 2002). In addition, urinary levels of DHBMA are significantly influenced by the smoking status of subjects (Boyle et al., 2016). Numerous studies report the measurement of the DHBMA concentration in the general population. Among these data, the NHANES study (CDC, 2019b) giving values for the 95th percentile as according to smoking status with the largest number of subjects was retained for the recommendation of BRVs. The BRVs recommended for DHBMA concentrations are:

- for non-smokers: 753 µg. L^{-1} rounded to 750 µg. L^{-1} or 565 µg.g^{-1} creatinine rounded to 550 µg.g^{-1} of creatinine)
- for smokers: 1130 µg. L^{-1} rounded to 1100 µg. L^{-1} or 768 µg/g^{-1} creatinine rounded to 750 µg/g^{-1} creatinine).

Conclusions of the collective expert appraisal

The biological values proposed for monitoring occupational exposure to 1,3-butadiene are:

Urinary DHBMA:

BLV based on a health effect	None
BLV based on the 3 atmospheric concentrations in BD (0.08mg.m^{-3}, 0.008mg.m^{-3}, 0.0008mg.m^{-3}) associated respectively with the 3 additional risks of leucemia deaths	None
Biological reference value (BRV)	Non-smokers: 750 µg. L^{-1} 550 µg.g^{-1} creatinine Smokers: 1100 µg.L^{-1} 750 µg/g^{-1} creatinine

Urinary 3-MHBMA:

BLV based on a health effect	None
BLV based on the 3 atmospheric concentrations in BD (0.08mg.m^{-3}, 0.008mg.m^{-3}, 0.0008mg.m^{-3}) associated respectively with the 3 additional risks of leucemia deaths	None
Biological reference value (BRV)	Non-smokers: 20 µg. L^{-1} or 15 µg.g^{-1} creatinine Smokers: 120 µg.L^{-1} or 110 µg.g^{-1} creatinine

These BRV can not 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.

Sampling methods and factors that may affect the interpretation of results

For DHBMA and 3-MHBMA, urine samples should be taken at the end of the shift at the end of the week.

Exposure to chloroprene, a chlorinated derivative of butadiene, has been described as leading to the formation of MHBMA and DHBMA (Eckert *et al.* 2013), but the influence of this exposure in quantitative terms on urinary DHBMA and MHBMA levels is unknown.

Competitive inhibition of the metabolism of 1,3-butadiene by styrene has been described (Laib *et al.* 1992), but the influence of this exposure in quantitative terms on urinary DHBMA and MHBMA levels is unknown.

With regard to MHBMA, the influence of polymorphism of GST (glutathione S-transferases) and EH (epoxide hydrolase) has also been described (Albertini *et al.* 2007).

Biometrology

Analysis methods described in scientific literature for measurement of DHBMA are also included in the summary report. The objective of this section is not to recommend a measurement method, but to provide succinct information on certain characteristics of the analysis methods.

DHBMA (3,4-dihydroxybutylmercapturic acid)		
	Method 1	Method 3
Method name	Albertini *et al.* 2003	Urban *et al.* 2003, Fustinoni *et al.* 2004, Sapkota *et al.* 2006, Schettgen *et al.* 2009, Kotapati *et al.* 2015
Analytical technique	GC-NECI-MS-MS	LC-MS/MS

Standardisation (ISO/AFNOR)	NEN-EN-ISO 14001	NR
Sensitivity	NR	NR
Limit of detection	5 µg/L	23 µg/L (Urban*et al.* 2003) 50 µg/L (Fustinoni *et al.* 2004) 3.7 µg/L (Sapkota *et al.* 2006) 5 µg/L (Schettgen *et al.* 2009, Kotapati *et al.* 2015)
Limit of quantification	NR	76 µg/L (Urban*et al.* 2003) 10 µg/L (Schettgen *et al.* 2009) 10 µg/L (Kotapati *et al.* 2015)
Linearity area	0 - 20 mg/L	50 - 1000 µg/L (Urban*et al.* 2003, Sapkota *et al.* 2015) 100 - 10000 µg/L (Schettgen *et al.* 2009)
If necessary, preparation of the sample and its duration	Internal standard mixed with urine, liquid/liquid extraction, evaporation to dryness, derivatisation for 1 h at 60°C, evaporation and dissolved in 100 µL of toluene	Internal standard mixed with urine, solid phase extraction (or on-line for Schettgen *et al.* 2009)
Analytical interference(s)	NR	NR
Quality control Reference standard	Internal controls of overloaded urine	Internal controls of overloaded urine

3-MHBMA (N-acetyl-S-4-(hydroxy-2-buten-1-yl)-L-cysteine)	Method
Method name	Alwis et al., 2012
Analytical technique	LC-NESI-MS/MS
Standardisation (ISO/AFNOR	NR
Sensitivity	NR
Limit of detection	0,6 µg/L
Limit of quantification	NR
Linearity area	0,6 - 44 µg/L
If necessary, preparation of the sample and its duration	N-acetyl-^{2}H3-S-(4-hydroxy-2-buten-1-yl)-L-cysteine (MHBMA3-^{2}H3) used as internal standard

Analytical interference(s)	NR
Quality control Reference standard	Internal controls of overloaded urine at 2 levels of concentration

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