Collective expert appraisal: summary and conclusions

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

Evaluation of biomarkers and recommendation of biological limit values and biological reference values for dichloromethane

[CAS n°:75-09-2]

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

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 dichloromethane.

The European expert committee in charge of expert appraisals on occupational exposure limits for chemical agents (Scientific Committee on Occupational Exposure Limits, SCOEL) submitted for consultation a report on the health effects of dichloromethane in November 2007 (see SCOEL/SUM/130 of November 2007). This expert committee recommended, on the basis of an analysis of health effects, an 8 hour-TWA of 100 ppm and a 15 minute exposure limit (STEL) of 200 ppm. It also recommended assigning a "skin" notation and a biological limit value of 4% for carboxyhaemoglobin levels.

The Directorate General for Labour asked the agency to undertake a critical review of the SCOEL report on dichloromethane and take a position regarding the occupational exposure limits recommended by this committee. In the event of disagreement, the agency was to propose new occupational exposure values based on health considerations.

This request was entrusted to ANSES's OEL Committee which, in June 2009, issued a report for recommending for dichloromethane:

- establishing an 8h-OEL of 50 ppm (178 $mg.m^{-3}$)
- establishing a short-term limit value (STEL) of 100 ppm (356 $mg.m^{-3}$)
- assigning a "skin" notation.

The OEL Committee decided to supplement its appraisal by assessing the data concerning biological monitoring in the workplace for dichloromethane, in order to assess the suitability of

recommending monitoring one or more biomarkers in addition to the atmospheric OEL, possibly including elaboration of biological limit values for the biomarker(s) chosen.

It should be noted that further to a public consultation phase, the SCOEL published in June 2009 a final report recommending an 8h-OEL of 100 ppm and a STEL (15min) of 200 ppm. In addition to assigning a "skin" mention, three biological limit values were recommended, namely: 4 % for carboxyhaemoglobin levels, 0.3 mg. L^{-1} for dichloromethane in urine and 1 mg L^{-1} for dichloromethane in blood.

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.

OEL 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 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 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.

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 the work of this group were regularly submitted to the OEL 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

A rapporteur of the biomarkers WG was appointed by the Agency to produce a summary report on biomarkers of exposure and the recommendation of biological limit values (BLVs) and biological reference values (BRV) for the BME(s) considered relevant. Two ANSES employees also contributed to this report.

The summary report on the BMEs for dichloromethane was based on bibliographical information taking into account the scientific literature published on this substance until 2012.

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 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 08 March 2016.

The collective expert appraisal work and the summary report were submitted to public consultation from 19/01/2017 to 19/03/2017. No comments were received. The OEL Committee adopted this version on 15 May 2017.

Result of the collective expert appraisal

Introduction

The scientific articles selected for evaluating biomonitoring data on dichloromethane were identified using the following keywords: “dichloromethane”, “methylene chloride”, “biomarker”, “biomonitoring”, “biological monitoring”, “urine”, “blood”, “occupational” and “analysis method”, while limiting the search to human data.

Toxicokinetics data

In humans, dichloromethane is absorbed by inhalation and by the dermal route.

A study undertaken in human volunteers exposed to dichloromethane in liquid form reports dermal absorption equivalent to 10% of pulmonary absorption (Stewart and Dodd, 1964). The dermal absorption of dichloromethane vapours at exposure levels around the OEL is thought to be negligible.

In humans, the pulmonary absorption of dichloromethane is rapid and ranges from 31% to 75% depending on the level and duration of exposure. The steady state between the concentration of dichloromethane in exhaled air (and therefore in blood) and the concentration in inhaled air is reached within two to four hours (DiVincenzo and Kaplan, 1981a; McKenna *et al.*, 1980). A correlation was found between concentrations of dichloromethane in blood and those in the air in a study of volunteers (exposure to concentrations ranging from 50 to 200 ppm for 7.5 hours (DiVincenzo and Kaplan, 1981a).

Few data are available on the digestive absorption of dichloromethane in humans. In animals, it is readily absorbed by the gastrointestinal tract. Studies in mice show that absorption is practically complete in aqueous solutions and is reduced by 50% in the presence of fat (Angelo *et al.*, 1986a and b; 1987).

Dichloromethane is rapidly distributed in all tissues and the parent substance and/or its metabolites do not accumulate in tissues. In humans, distribution in fat (the only available quantitative data) shows a higher total body burden in obese people than in thin people in relation to adipose mass (Engström and Bjurstrom, 1977).

The oxidation pathway is the primary route of metabolism for dichloromethane (CYP4502E1-dependent). It leads to the formation of carbon monoxide (CO) through an unstable intermediate, formyl chloride (HCOCl). CO binds to haemoglobin to form carboxyhaemoglobin (HbCO). This reaction results in cellular hypoxia. The oxidative metabolic pathway has high affinity but only low capacity for dichloromethane. The oxidative metabolic pathway is predominant at low concentrations (<100 ppm). It is rapidly saturated at concentrations that vary depending on the individual and species. Saturation is thought to begin at 200 pm and be complete from 500 ppm (Bos *et al.*, 2006; ACGIH, 2015b). However, the conjugation pathway by glutathione-S-transferases (GST) is not saturable and leads to the formation of reactive compounds such as formaldehyde. It is dependent on GSTT1 which has significant genetic polymorphism (20% of the population has an inactive gene). The biotransformation of dichloromethane is dose-dependent (Green, 1991; Green, 1997; Reitz *et al.*, 1989; ATSDR, 2000). At high exposure levels, a relatively low proportion is metabolised and a high proportion of the solvent is exhaled in unchanged form. In non-smokers, the level of HbCO measured at the end of exposure is dependent on the concentration and duration of exposure on the same

day, but is independent of exposure on previous days (IPCS, 1996; Amsel *et al.*, 2001; Soden *et al.*, 1996).

Several PBPK models have been published in the literature including that of David *et al.*, 2006. It shows high inter-individual variability.

From the end of exposure, the concentration of dichloromethane in the various biological fluids decreases rapidly. In humans, approximately 5% of the total amount of dichloromethane absorbed by inhalation is exhaled in unchanged form. The rest, i.e. 95%, is metabolised and a 25% to 34% fraction is exhaled during and after exposure in the form of CO and CO_2 (Di Vincenzo and Kaplan, 1981a). The majority is eliminated within five hours (after the end of the exposure period, for exposure to 90, 100 or 210 ppm) (Di Vincenzo *et al.*, 1972, 1971). Urinary elimination of unchanged dichloromethane is relatively low (<1%) and Sakai *et al.* (2002) report a urinary elimination half-life of 210-400 minutes (determined in three workers). In humans, following exposure by inhalation, elimination half-lives are estimated at 5 to 40 minutes in blood, 50 to 60 minutes in richly perfused tissues, 50 to 80 minutes in muscle and 240 to 400 minutes in adipose tissue (Riley *et al.*, 1966 cited in ACGIH, 2015b). In the study by Astrand *et al.*, 1975, after the end of exposure, the blood concentration quickly decreases: the graphical estimate of the half-life is around 15-20 minutes.

Selection of biomarkers of exposure and effect

HbCO resulting from exposure to dichloromethane is due to haemoglobin binding to CO, itself arising from the biotransformation of dichloromethane. As such, it can be considered as a biomarker of exposure to dichloromethane. However, the limit of 3.5% HbCO is also considered by several organisations (AFSSET, 2009) as a value not to be exceeded to avoid nervous system impairment and cardiovascular effects. Therefore, HbCO is sometimes considered as a marker of effects. In this document, HbCO is considered as a BME reflecting exposure to dichloromethane. However, HbCO cannot be used as a marker of exposure to dichloromethane for smokers. In the study by Di Vincenzo *et al.* (1981a) undertaken in volunteers, the levels of HbCO associated with exposure to 50 or 100 ppm dichloromethane are respectively 1.9% and 3.4%, which are below the average levels of HbCO usually found in subjects who smoke one pack a day (approximately 5-6% (average), ACGIH, 2015a). The measurement of HbCO for the monitoring of occupational exposure to dichloromethane is therefore not suitable for smokers.

While dichloromethane does have a low level of elimination in urine (<1%), the analytical methods used to determine urinary concentrations are sensitive and specific and do not require invasive sampling. Moreover, several studies (Ghittori *et al.*, 1993; Ukai *et al.*, 1998 and Sakai *et al.*, 2002) report a close correlation between urinary and atmospheric concentrations of dichloromethane.

Several studies also report measurements of atmospheric and blood concentrations of dichloromethane (Perbellini *et al.*, 1977; McCammon *et al.*, 1991; Astrand *et al.*, 1975 and Di Vincenzo *et al.,* 1981a). However, among these studies, only the field study by Perbellini *et al.* (1977) reports a correlation (r = 0.76). Considering the short half-life of dichloromethane in blood (Di Vincenzo *et al.*, 1972 and 1981), which does not seem as long (5 to 40 minutes) as the urinary elimination half-life (210-400 minutes)[1], and the fact that blood concentrations are not as closely correlated with atmospheric concentrations as urinary concentrations, blood

[1] Few data to that end

levels of dichloromethane have not been retained as a BME. Furthermore, in addition to being invasive, analyses using blood samples do not have any specific advantages over urine assays.

Since dichloromethane is mainly eliminated by the pulmonary route (Di Vincenzo *et al.*, 1972), measurements in exhaled air could be worthwhile. However, due primarily to disadvantages related to sampling difficulties, the measurement of dichloromethane in exhaled air cannot reasonably be proposed for the biological monitoring of workers exposed to this solvent. Moreover, the rapid decrease in levels in the first few minutes after the end of exposure makes it difficult to interpret measurements taken at the end of the work shift.

The measurement of CO in exhaled air could have been an alternative, but it is not specific and few field data are available. The study by Ghittori *et al.* (1993) undertaken with 20 workers does not show any significant correlation between concentrations of CO in exhaled air and the atmospheric concentration of dichloromethane (r=0.3). When only non-smokers are considered, the correlation is high (r=0.87), but this applied to a very small number of workers (n=8). The concentration of CO in exhaled air peaked after one to two hours of exposure and this concentration was proportional to the degree of exposure to dichloromethane (during and after exposure). For high levels of exposure (over 174 $mg.m^{-3}$), the proportional relationship is no longer valid. The authors of this publication report occupational co-exposure to perchloroethylene. Moreover, disadvantages related to sampling difficulties are also described here.

Therefore, urinary concentrations of dichloromethane and HbCO (for non-smokers only) have been retained as BMEs for the monitoring of occupational exposure to dichloromethane. **Although HbCO concentrations above 3.5% also pose a health risk to smokers, in this case they cannot be attributed only to occupational exposure to dichloromethane.**

Studies undertaken in humans and animals show that the central nervous system is one of the main targets of dichloromethane. Furthermore, exposure to dichloromethane induces the formation of HbCO (non-functional form for the transport of oxygen resulting from the metabolism of this substance), leading to hypoxia.

Based on the described and studied effects, it is not possible to propose one or more relevant biomarkers of effects for biomonitoring.

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

Name	**URINARY DICHLOROMETHANE**
Other substances giving rise to this biomarker	None
Concentrations found in exposed workers or volunteers	-Field studies: Ghittori *et al.* 1993 Exposure (4 hours): from 3.4 to 200.8 mg.m^{-3} (1 to 57 ppm), mean: 50.3 mg.m^{-3}, SD: 55.8 mg.m^{-3} (n=20) Urinary DCM: 0.0042 to 0.788 mg.L^{-1} (4 hours after the start of exposure) Urinary DCM estimated for exposure to 50 ppm: 0.60 mg.L^{-1} (co-exposure to perchloroethylene, mean: 114.9 mg.m^{-3} [13.9 - 315.3] SD=89.9) r=0.90 Ukai *et al.* 1998 Exposure from 1 to 180 ppm (n=61) Urinary DCM: 0.01 to 0.821 mg.L^{-1} (end of shift) Urinary DCM estimated for exposure to 50 ppm: 0.17 mg.L^{-1} r=0.911 Sakai *et al.* 2002 Exposure from 5 to 270 ppm (n=95) Urinary DCM: 0.01 to 1.12 mg.L^{-1} (after 4 hours of exposure) Urinary DCM estimated for exposure to 50 ppm: 0.24 mg.L^{-1} r=0.924 -Studies in volunteers: Di Vincenzo *et al.* 1972 Exposure: 100 ppm for 120 minutes at rest (n=4) Mean quantity of DCM collected in urine: 0.0226 mg Considering the quantity of DCM in urine and the volume of urine collected for each worker, the mean urinary concentration of DCM is 0.091 ± 0.024 mg.L^{-1} (end of shift) r: not specified in the article Exposure: 200 ppm for 120 minutes at rest (n=7) Mean quantity of DCM collected in urine: 0.0815 mg within 24 hours Considering the quantity of DCM in urine and the volume of urine collected for each worker, the mean urinary concentration of DCM is 0.210 ± 0.066 mg.L^{-1} (end of shift)
Conversion factor	MW: 84.93 1 mg.L^{-1} = 0.0118 mmol.L^{-1} 1 mmol.L^{-1} = 84.93 mg.L^{-1}
Concentrations in the general population[2]	Poli *et al.*, 2005, mean: 0.78 µg.L^{-1}, SD of 0.44 (median of 0.64 µg.L^{-1}) (n=120 control subjects not occupationally exposed)

[2] Or failing that, in a non-occupationally exposed control population; 95th percentile, or failing that the median or the mean (number of people in the study if this information is available)

Recommended limit values for exposed workers	USA - ACGIH (BEI)	0.3 $mg.L^{-1}$ (end of shift) (ACGIH, 2015b)
	Germany - DFG (BAT)	NS
	Quebec - IRSST (IBE)	NS
	Finland - FIOH (BAL)	NS
	Other value(s) (Swiss, etc.)	NS

DCM : dichloromethane; SD : standard deviation; MW : molecular weight, NS : not specified

Name	**CARBOXYHAEMOGLOBIN (HbCO)**
Other substances giving rise to this biomarker	Carbon monoxide, trichloroethylene, perchloroethylene, triiodoethylene, tribromoethylene
Concentrations found in exposed workers or volunteers	- <u>Field studies:</u> Ghittori *et al.* 1993 Exposure to 174 $mg.m^{-3}$ (48 ppm), non-smokers (n=20) HbCO: 1.9% (end of shift) Exposure to 348 $mg.m^{-3}$ (96.7 ppm), non-smokers (n=20) HbCO: 3.25% (end of shift) r: not specified in the article Soden *et al.* 1996 Exposure to 25-500 ppm=> mean exposure of 99 ppm, 8 hours (number of subjects not specified) Non-smokers, HbCO: 1.77% - 4.0% (end of shift) Smokers, HbCO: 4.95 - 6.35% (end of shift) r=0.99 - <u>Studies in volunteers:</u> Di Vincenzo *et al.* 1981a Exposure: 50 ppm for 7.5 hours at rest, non-smokers (n=14) HbCO: 1.9% (end of shift) Exposure: 100 ppm for 7.5 hours at rest, non-smokers (n=14) HbCO: 3.4% (end of shift) Exposure: 150 ppm for 7.5 hours at rest, non-smokers (n=14) HbCO: 5.3% (end of shift) Exposure: 200 ppm for 7.5 hours at rest, non-smokers (n=14) HbCO: 6.8% (end of shift) r: not specified in the article
Conversion factor	MW: 64 kDa
Concentrations in the general population	1.5% in non-exposed subjects (FIOH, 2015), ACGIH (2001) mentions : 1 to 2% (mean or 95th percentile not specified) for non-smokers (urban population),(ACGIH, 2015a) 5 to 6% (mean) for smokers, (1 pack/day) (ACGIH, 2015a)

Recommended limit values for exposed workers	USA - ACGIH (BEI)	NS
	Germany - DFG (BAT)	NS
	Quebec - IRSST (IBE)	NS
	Finland - FIOH (BAL)	HbCO: 4% immediately at the end of shift (last modified in 2013)
	Other value(s) (Swiss, etc.)	Swiss value: HbCO: 5% at the end of shift (last modified before 2007)

Study of the relationship between BME concentrations and health effects

Peterson (1978) (cited in ATSDR, 2000) showed that in the event of exposure to dichloromethane concentrations of 50 to 500 ppm for five weeks, HbCO can be predicted based on exposure parameters. However, correlations with atmospheric concentrations of dichloromethane are stronger with concentrations of dichloromethane in exhaled air than with HbCO. For exposure to 50 ppm of dichloromethane, the primary effects are neurological.

Study of correlations between BME concentrations and atmospheric concentrations of dichloromethane

Urinary dichloromethane

The data in the literature report correlations between atmospheric concentrations and urinary concentrations of dichloromethane.

n	Atmospheric concentration	Urinary concentration		Urinary DCM concentration for 50 ppm or 178 $mg.m^{-3}$	Reference
20	Mean: 50.3 $mg.m^{-3}$ [3.4-200.8] (1 to 57 ppm)	0.0042 to 0.788 $mg.L^{-1}$ (4 hours after the start of exposure, end of a ½ day of work)	[DCMu] ($\mu g.L^{-1}$) = 3.266 [DCMa] ($mg.m^{-3}$) + 26.8 i.e. [DCMu] ($mg.L^{-1}$)= 0.01108 [DCMa] (ppm) + 0.0268. r = 0.9 Co-exposure to perchloroethylene	0.608 $mg.L^{-1}$ a	Ghittori *et al.* (1993)
61	[1– 180] ppm	[0.01 to 0.821 $mg.L^{-1}$] end of shift (end of an 8-hour day)	End of shift [DCMu] ($mg.L^{-1}$) = 0.00322 [DCMa] (ppm) + 0.0077 r = 0.911	0.17 $mg.L^{-1}$ a	Ukai *et al.* (1998)
95	[5– 270] ppm	[0.01 to 1.12] $mg.L^{-1}$ (after 4 hours of exposure)	[DCMu] ($mg.L^{-1}$) = 0.0037 [DCMa] (ppm) + 0.0545 r = 0.924	0.240 $mg.L^{-1}$ a	Sakai *et al.* (2002)

[DCMu]: urinary concentration of dichloromethane; [DCMa]: atmospheric concentration of dichloromethane; a : value calculated from the regression equation reported in the publication

Establishment of BLVs and choice of biological reference values

The collective expert appraisal report of the OEL Committee on dichloromethane recommends an 8h-OEL of 50 ppm, i.e. 178 $mg.m^{-3}$. The aim of this recommendation is to prevent possible effects in the workplace resulting in the over-production of carbon monoxide (CO) in the body, and genotoxicity; dichloromethane is a carcinogen that is genotoxic only from a certain threshold of exposure. In humans, the metabolic pathway that produces carcinogenic metabolites is activated between 100 and 200 ppm.

Regarding the relationship between biological effects and BME concentrations for dichloromethane, no field studies in workers exposed to dichloromethane linking urinary concentrations of dichloromethane or HbCO to health effects (neurological effects in particular) were identified in the literature.

Urinary dichloromethane

Three field studies report a close correlation between atmospheric concentrations and urinary concentrations of dichloromethane (r>0.86) (Sakai *et al.*, 2002; Ghittori *et al.*, 1993 and Ukai *et al.*, 1998). Only the studies by Ukai *et al.* (1998) and Sakai *et al.* (2002) have been chosen for the establishment of the BLV. The study by Ghittori *et al.* (1993) has not been retained due to co-exposure to perchloroethylene, which can potentially induce modifications in the metabolism of dichloromethane.

Based on the results of these studies, urinary concentrations of dichloromethane at the end of the shift for exposure to the 8h-OEL (50 ppm) tend towards 0.2 $mg.L^{-1}$. This concentration has been retained for the establishment of the BLV based on exposure to the 8h-OEL.

In the general population, urinary concentrations of dichloromethane are generally below the limits of quantification of the techniques used. Only the study by Poli *et al.* (2005) undertaken in an Italian population reports urinary concentrations of dichloromethane for 120 non-occupationally exposed control subjects. The mean urinary concentration of dichloromethane in these subjects is 0.78 $\mu g.L^{-1}$ with a standard deviation of 0.44 (median of 0.64 $\mu g.L^{-1}$). The author reports a very low limit of detection (0.005 $\mu g.L^{-1}$), far below the values published by other authors. The 95^{th} percentile can be calculated from the mean of 0.78 $\mu g.L^{-1}$ + 2 times the standard deviation of 0.44, i.e. 1.64 $\mu g.L^{-1}$ rounded to 1.6 $\mu g.L^{-1}$. This concentration of 1.6 $\mu g.L^{-1}$ has been retained as the biological reference value.

Carboxyhaemoglobin levels

The value of 3.5% for HbCO has been proposed as the BLV, given that it corresponds to the value not to be exceeded to prevent nervous system impairment and cardiovascular effects.

This BLV of 3.5% corresponds approximatively to the values measured for an exposure to 50 ppm of dichloromethane for non-smoking workers. This value of 3.5% cannot be used to assess exposure to dichloromethane in smokers. Unlike urinary dichloromethane, concentration of HbCO is influenced by co-exposure to other pollutants including carbon monoxide.

The biological reference value retained for HbCO is 1.5% for non-smokers (FIOH, 2015).

Conclusions of the collective expert appraisal

The biological values proposed for monitoring exposure to dichloromethane are:

Urinary dichloromethane:

Biological Limit Value based on an exposure to the 8h-OEL (50 ppm): 0.2 $mg.L^{-1}$ (sampling after the end of the shift or end of exposure)

Biological reference value: 1.6 $\mu g.L^{-1}$

Carboxyhaemoglobin levels

Biological Limit Value:3.5% for non-smokers (sampling immediately after the end of the shift/exposure)

Biological reference value: 1.5% for non-smokers

Sampling method and factors that may affect the interpretation of results

Urine samples should be taken after the end of the shift (within 30 minutes after the end of exposure). Special precautions should be taken to prevent the loss of dichloromethane by evaporation due to the high volatility of this substance. Urine samples should be taken away from the workplace, ideally after a shower, chang of chlothes and at the very least after handwashing, in order to reduce the risk of external contamination of the samples. For headspace analysis, it is advisable to use glass bottles that will be immediately sealed. The bottles should be kept between +2 and +8°C for two weeks. As no stability studies during transport are available, it is advisable to transport the samples between 2 and 8°C.

For non-smoking workers, HbCO can also be monitored. Blood samples should be taken immediately after the end of the shift/exposure. Samples should be kept between +2 and +8°C and analysed at the earliest possible date. The samples can be transported at +2 to +8°C and analysed the same day.

Co-exposure to perchloroethylene can influence urinary concentrations of dichloromethane, as they follow the same metabolic route.

Regarding carboxyhaemoglobinaemia, it may be generated by carbon monoxide, trichloroethylene, perchloroethylene, triiodoethylene, or tribromoethylene. Smoking induces the formation of carboxyhaemoglobin and should therefore be taken into account.

Biometrology

Urinary dichloromethane			
Existence of an interlaboratory quality control programme	NS		
	Method 1	**Method 2**	
Analytical technique	Gas chromatography with flame ionisation detection (GC-FID)	Solid phase microextraction-gas chromatography (SPME-GC): GC-MS (Poli *et al.*), GC-ECD (Hoffer *et al.*)	
Limit of detection	0.01 µg.L^{-1}	0.005 µg.L^{-1}	0.01 µg.L^{-1}
Limit of quantification	NS	0.01 µg.L^{-1}	NS
Fidelity	CV 3.7%	Reproducibility 3.8%	Reproducibility (CV%): 10
Precision	NS		
Reference standard	Standard: 2 mg.L^{-1} in distilled water	NS	Internal standard: chloroform
References	Sakai *et al.*, 2002	Poli *et al.*, 2005	Hoffer *et al.*, 2005

Carboxyhaemoglobin			
Existence of an interlaboratory quality control programme	NS		
	Method 1	**Method 2**	**Method 3**
Analytical technique	Gas chromatography with flame ionisation detection (GC-FID)	Spectrophotometry	Colorimetry
Limit of detection	0.002 volumes/100 ml	NS	NS
Limit of quantification	NS	0.1%	0.1%
Fidelity	CV = 1.08%	CV=9%	CV=3%
Precision	NS		
Reference standard	NS		
References	Collison *et al.* (1968)	Luchini *et al.* (2009)	Trinder *et al.* (1962)

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