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Chloroprene – Evaluation of a BAR

Assessment Values in Biological Material – Translation of the German version from 2014

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BAR (2013)	**400 µg 3,4-dihydroxybutyl mercapturic acid (DHBMA)/g creatinine**[a)] Sampling time: end of exposure or end of shift; for long-term exposures: at the end of the shift after several previous shifts
Synonyms	2-chloro-1,3-butadiene 2-chloroprene β-chloroprene
CAS number	126-99-8
Formula	$H_2C=CCl-CH=CH_2$ C_4H_5CI
Molar mass	88.54 g/mol
Melting point	−130 °C
Boiling point	59.4 °C
Vapour pressure at 20 °C	267 hPa
Density at 20 °C	0.958 g/cm³
MAK value (2001)	**not established**
Peak limitation	–
Absorption through the skin (2001)	H
Sensitization	–
Carcinogenicity (2001)	Category 2
Prenatal toxicity	–
Germ cell mutagenicity	–

a) evaluated for non-smokers

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Chloroprene [126-99-8] is produced by the chlorination of 1,3-butadiene. The monomer is almost exclusively used for the production of the elastomer polychloroprene. Only a small quantity is used in the production of 2,3-dichlorobutadiene to manufacture special copolymers. Chloroprene rubber has excellent insulation properties and is, for example, used as material for thermoprotective suits, in electric insulators, and in the automotive industry. A relevant natural occurrence of chloroprene in the environment is not known (IARC 1999; NTP 2011).

1 Metabolism and Toxicokinetics

1.1 Absorption and Distribution

Chloroprene is well absorbed, both through the skin and via the lungs and the gastrointestinal tract. Exposure at the workplace occurs mainly during production or polymerization of 2-chloroprene, where inhalation and dermal absorption are assumed to be of major importance (NTP 2011). As the substance is lipophilic, distribution via the blood into all organs is to be considered likely (Greim 2001).

1.2 Metabolism

Several in vitro studies are available for the metabolism of 2-chloroprene (Cottrell et al. 2001; Himmelstein et al. 2001; Hurst and Ali 2007; Munter et al. 2002, 2003, 2007; Wadugu et al. 2010). In addition, an in vivo study in rats is available (Summer and Greim 1980) and an occupational-medical study with workers exposed to 2-chloroprene (Eckert et al. 2013). In the phase I metabolism, chloroprene is bioactivated by cytochrome P450-dependent monooxygenases leading to an epoxidation on one of the two double bonds. In vitro, a preferred formation of the intermediary epoxide (1-chloroethenyl)oxirane (1-CEO) could be observed (Cottrell et al. 2001; Himmelstein et al. 2001). In vitro, 1-CEO forms adducts with biological macromolecules such as DNA or haemoglobin (Hurst and Ali 2007; Munter et al. 2002; Wadugu et al. 2010).

The intermediary epoxides can be further metabolised by hydrolysis via epoxide hydrolases or by conjugation with glutathione (GSH) via glutathione S-transferases (phase II metabolism). In the human organism, hydrolysis is considered as the predominant pathway for the inactivation of the epoxides (Cottrell et al. 2001; Munter et al. 2007). When chloroprene was orally administered to rats, a dose-dependent increase in thioethers was observed, which indicates the formation of GSH conjugates and mercapturic acids (Summer and Greim 1980). Different GSH conjugates as metabolites of chloroprene were also found in in vitro studies (Munter et al. 2003, 2007). In an occupational-medical study, it was shown that mainly 3-chloro-2-hydroxy-3-butenyl mercapturic acid (Cl-MA-III) and 3,4-dihydroxybutyl mercapturic acid (DHBMA) are formed in humans after exposure to 2-chloroprene (Eckert et al. 2013). Figure 1 summarises the most recent data on the metabolic pathways of 2-chloroprene.

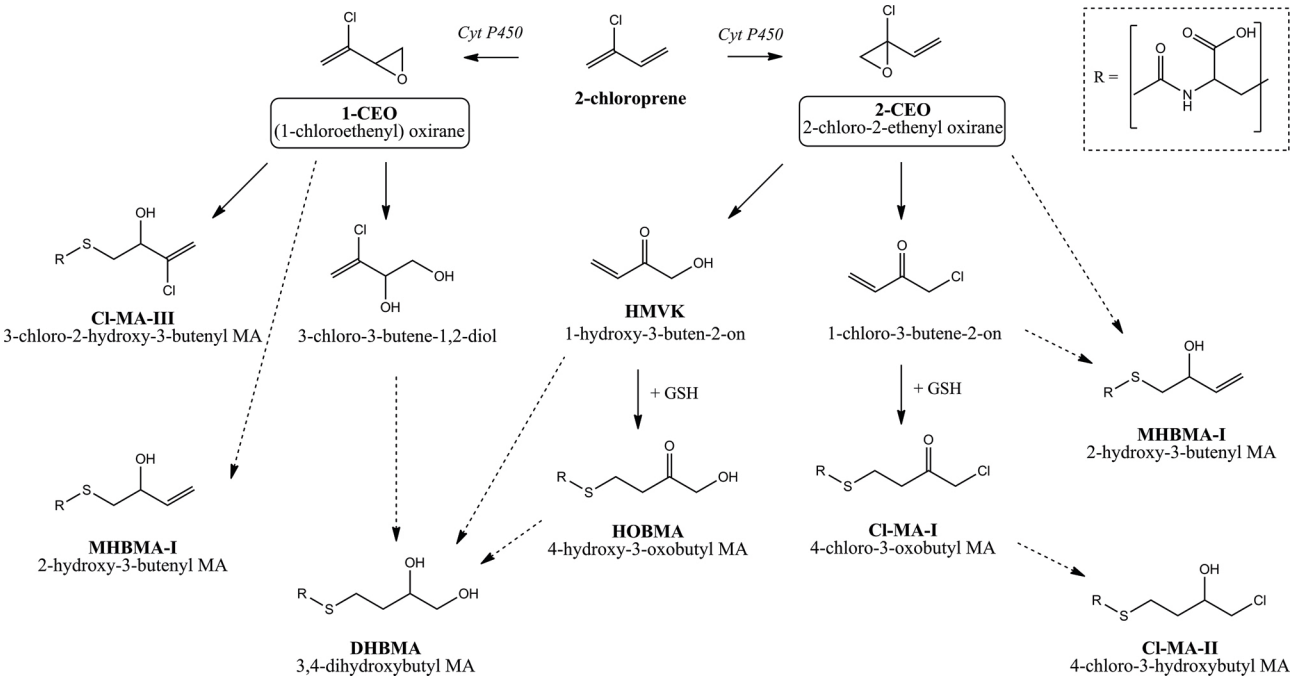

Fig. 1 Metabolic pathways of chloroprene according to Eckert et al. (2013), reprinted by permission of Springer Nature, https://www.springernature.com. MA: mercapturic acid

2 Critical Toxicity

Exposure to chloroprene at high levels causes acute irritation to eyes, skin and airways, leading to damage of the lungs, liver and kidneys (Valentine and Himmelstein 2001). The symptoms of chronic toxicity are headaches and tiredness, as well as various diseases of the skin and airways (Greim 2001; IARC 1999). Carcinogenicity is considered to be the most critical toxicity after occupational exposure. In animals, chloroprene was shown to be carcinogenic after inhalation. Its carcinogenicity is attributed to the formation of reactive epoxide compound intermediates (Greim 2001).

The data on the toxicology and especially on the carcinogenicity of chloroprene are comprehensively summarised and discussed in the MAK documentation of 2001 (Greim 2001) as well as in the monographs by the IARC (1999) and the NTP (2011).

3 Exposure and Effects

3.1 Relationship between external and internal exposure

So far, no human studies are available that enable the derivation of a correlation between external and internal exposure to chloroprene. Owing to its good dermal penetration, skin absorption is assumed to make a considerable contribution to the total exposure to chloroprene. Measurement of the concentration in the air at the workplace alone is therefore not sufficient for the assessment of the total exposure to chloroprene.

In a field study by Eckert et al. (2013), increased levels of four potential mercapturic acids of chloroprene could be determined in post-shift urine samples of 14 persons occupationally exposed to chloroprene. As a specific biomarker, the chlorinated mercapturic acid Cl-MA-III (see Figure 1) could be detected (median concentration:

6.1 µg/g creatinine) in 11 urine samples of the 14 investigated occupationally exposed persons. In the urine samples of a control group (n = 30), however, the analyte Cl-MA-III was not detectable. In addition to this, in the exposed group, statistically significantly increased levels of three non-chlorinated mercapturic acids were found: DHBMA, 4-hydroxy-3-oxobutyl mercapturic acid (HOBMA) and 2-hydroxy-3-butenyl mercapturic acid (MHBMA). DHBMA was determined to be the main metabolite and showed an 18-fold increased median concentration of 3255 µg/g creatinine compared with the control group. Furthermore, the DHBMA level in the urine samples of the exposed persons was statistically significantly associated with Cl-MA-III excretion whereas the excretion rate for DHBMA was about 400-fold higher than that of the chlorinated mercapturic acid. HOBMA, showed a statistically significant though only slight increase of the median level in the exposed group compared with the control group level (111 µg/g creatinine versus 214 µg/g creatinine). For MHBMA, the concentrations in both groups were much lower. Nevertheless, the median concentration in the exposed group (3.5 µg/g creatinine) was still statistically significantly elevated compared with the MHBMA level of the control group (< 0.5 µg/g creatinine). On the other hand, the determination of chloroprene in the workplace air showed only very low concentrations (< 0.1 ml/m^3). A potential dermal exposure of the occupationally exposed persons to chloroprene is therefore more than likely.

3.2 Relationship between internal exposure and effects

There are no relevant human studies available for chloroprene.

4 Selection of Indicators

The results of the study by Eckert et al. (2013) showed that the mercapturic acids Cl-MA-III, DHBMA, HOBMA and MHBMA are formed and excreted with the urine in the human organism after exposure to 2-chloroprene. Cl-MA-III is a 2-chloroprene-specific biomonitoring parameter, whereas the non-chlorinated mercapturic acids may also be formed physiologically or from other reactive compounds, especially 1,3-butadiene. Such an exposure can, for example, occur via tobacco smoke. However, the level of formed DHBMA is much higher than that of Cl-MA-III (see Section 3.1), leading to the conclusion that the parameter DHBMA provides a far higher sensitivity for exposure to 2-chloroprene.

5 Analytical Methods

The mercapturic acids Cl-MA-III, DHBMA and HOBMA in urine are determined using liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) after online enrichment of the analytes. Evaluation is carried out using isotope-labelled standards, and the detection limits are between 1.4 and 4.2 µg/l (Eckert et al. 2012).

The determination of the mercapturic acids DHBMA and MHBMA has already been described by several authors. Usually the two mercapturic acids are analysed together using LC-MS/MS with the addition of isotope-labelled internal standards after solid phase extraction (Carmella et al. 2009; Eckert et al. 2010; Roethig et al. 2009; Urban et al. 2003), or after online enrichment of the analytes (Schettgen et al. 2009). The detection limits are approximately between 0.1 and 5 µg/l.

For the determination of MHBMA and DHBMA in urine, an analytical method has been developed and was published by the MAK Commission's working group "Analyses in Biological Materials", in which the two mercapturic acids are extracted from the biological matrix using solid phase extraction and are subsequently quantified using LC-MS/MS (Scherer et al. 2008).

6 Background Exposure

6.1 3,4-Dihydroxybutyl mercapturic acid (DHBMA)

DHBMA is an established biomarker of exposure to 1,3-butadiene. For this parameter, numerous studies are available in which the background exposure in the general population was investigated. For non-smokers, the median concentrations were generally found to be in the range of 100 to 300 µg/l creatinine. The DHBMA levels in smokers were usually slightly increased (median values 150–400 µg/g creatinine). In two studies on the DHBMA excretion in persons of the German general population, the 95th percentile was given in addition as 760 µg/l (Schettgen et al. 2009) or 329 µg/g creatinine (Eckert et al. 2011) in non-smokers.

For a detailed review of the literature data, see the BAR documentation of 1,3-butadiene (translated in Göen 2021).

6.2 2-Hydroxy-3-butenyl mercapturic acid (MHBMA)

Like DHBMA, MHBMA is also an established parameter for exposure to 1,3-butadiene. The median background level of MHBMA in the urine of the general population is < 2 µg/g creatinine. The concentrations in the urine samples of smokers are usually significantly higher (median values up to 10 µg/g creatinine). A detailed review of the literature data can be found in the BAR documentation of 1,3-butadiene (Göen 2021).

6.3 4-Hydroxy-3-oxobutyl mercapturic acid (HOBMA)

HOBMA is described as a metabolite of 1,3-butadiene (Barshteyn and Elfarra 2009; Sprague and Elfarra 2004). To date, there is no information available neither for the background exposure nor for the suitability of this parameter as biomarker of 1,3-butadiene. HOBMA was described as a metabolite of chloroprene in humans (Eckert et al. 2013). In this study, a median level of 111 µg HOBMA/g creatinine and a 95th percentile of 305 µg HOBMA/g creatinine was determined in a control group (30 persons) of persons not exposed to chloroprene or to 1,3-butadiene (see Table 1 for individual results).

Tab. 1 Individual results of the determination of HOBMA in urine samples of persons occupationally not exposed to 2-chloroprene

Test person No.	HOBMA in urine	
	[µg/l]	[µg/g creatinine]
1	16.0	46.8
2	130.3	140.7
3	27.1	60.8
4	127.1	122.4
5	161.8	128.2
6	459.1	255.8
7	13.9	26.8
8	638.2	775.5
9	13.9	31.0
10	230.6	148.7
11	90.8	106.9
12	202.6	180.4
13	253.0	224.3
14	122.3	49.7

Tab. 1 *(continued)*

Test person No.	HOBMA in urine	
	[µg/l]	[µg/g creatinine]
15	36.3	53.2
16	326.6	293.2
17	98.2	51.7
18	32.0	88.4
19	84.9	80.8
20	30.6	69.6
21	181.1	106.4
22	52.8	105.7
23	157.8	114.3
24	107.1	62.5
25	51.3	142.4
26	28.8	57.6
27	258.1	314.8
28	113.7	186.4
29	199.5	162.2
30	118.1	125.6

6.4 3-Chloro-2-hydroxy-3-butenyl mercapturic acid (Cl-MA-III)

Cl-MA-III is a specific biomarker of chloroprene (see Figure 1). In the field study by Eckert et al. (2013), Cl-MA-III could only be found in the urine samples of persons exposed to chloroprene. In the urine samples of the occupationally not exposed control group, Cl-MA-III was not detectable (< 1.4 µg/g creatinine).

7 Evaluation

For HOBMA and Cl-MA-III, no sufficient human data for the derivation of a biological reference value (BAR) are available. On the basis of the existing data, the derivation of a BAR is only feasible for the mercapturic acids DHBMA and MHBMA. In the study by Eckert et al. (2013), MHBMA could be demonstrated in low concentrations only in the group of chloroprene-exposed persons. Further studies are necessary in order to confirm MHBMA as a relevant metabolite of chloroprene. DHBMA, on the other hand, was clearly confirmed to be the main metabolite of chloroprene in humans (Eckert et al. 2013) and is therefore suitable as a sensitive parameter for occupational exposure to chloroprene.

The BAR describes the background level of a biomonitoring parameter in the working age population who are not occupationally exposed to chloroprene independently of the cause of the occurrence of this parameter. Therefore, for the derivation of a BAR, also those studies can be used which were already decisive in the derivation of the BAR for 1,3-butadiene (Göen 2021). In the corresponding studies, only those persons were included in the reference populations who were occupationally not exposed to alkylating substances such as 1,3-butadiene and 2-chloroprene. For the derivation of the BAR, only data from non-smokers are used. On the basis of these data, analogous to the BAR for 1,3-butadiene,

a BAR of 400 µg 3,4-dihydroxybutyl mercapturic acid (DHBMA)/g creatinine

is derived for 2-chloroprene as background exposure of non-smokers. The values of smokers can be higher. For this reason, an occupational exposure is not necessarily implied, if the BAR is exceeded.

Sampling should be carried out at the end of exposure or end of shift, for long-term exposures after several shifts.

8 Interpretation of Results

When interpreting the results, personal factors, especially smoking habits, as well as passive smoke exposure, are to be taken into account. Apart from this, it must be borne in mind that DHBMA is a main metabolite both of chloroprene and of 1,3-butadiene. This has to be taken into consideration particularly at workplaces where, as with the chlorination of 1,3-butadiene to 2-chloroprene, exposure to both substances may be present. In these cases, as well as in the case of unclear exposure conditions, determination of the chloroprene-specific biomarker Cl-MA-III is recommended in addition.

The BAR relates to normally concentrated urine, in which the creatinine concentration should be in the range of 0.3–3 g/l. In order to further improve the validity of the analyses, the Commission considers it useful, to select an even narrower target range of 0.5–2.5 g/l for urine samples. As a rule, where urine samples are outside the above limits, a repetition of the measurement in normally hydrated test persons is recommended (Bader et al. 2016).

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (https://www.dfg.de/en/dfg_profile/statutory_bodies/senate/health_hazards/conflicts_interest/index.html) ensure that the content and conclusions of the publication are strictly science-based.

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