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A method for the simultaneous determination of mercapturic acids as biomarkers of exposure to 2-chloroprene and epichlorohydrin in human urine

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ABSTRACT

We developed and validated an analytical method for the simultaneous determination of several chlorine and non-chlorine containing mercapturic acids in urine as specific metabolites of the hazardous chemicals 2-chloroprene and epichlorohydrin. The method involves an online column switching arrangement for online solid phase extraction of the analytes with subsequent analytical separation and detection using LC–MS/MS. The developed method enables for the first time the determination of Cl-MA-I (4-chloro-3-oxobutyl mercapturic acid), Cl-MA-II (4-chloro-3-hydroxybutyl mercapturic acid), Cl-MA-III (3-chloro-2-hydroxy-3-butenyl mercapturic acid) and HOBMA (4-hydroxy-3-oxobutyl mercapturic acid) as potential biomarkers of 2-chloroprene in urine. Additionally, CHPMA (3-chloro-2-hydroxypropyl mercapturic acid) as a specific metabolite of epichlorohydrin in urine and DHBMA (3,4-dihydroxybutyl mercapturic acid) can be determined. The analytical method proved to be both sensitive and reliable with detection limits ranging from 1.4 μg/L (for Cl-MA-III) to 4.2 μg/L (for HOBMA). Intra- and interday imprecision was determined to range from 4.7 to 11.8%. Due to the good accuracy and precision and the low limits of detection the developed method is well suited for application in biomonitoring studies in order to determine occupational exposure to 2-chloroprene and epichlorohydrin.

1. Introduction

The alkylating agents epichlorohydrin and 2-chloroprene are important high production volume chemicals mainly used as intermediates in the industrial synthesis of polymers.

The worldwide production volume of 2-chloroprene totals 350,000 t per year (in 2004) [1] which is almost exclusively used for the synthesis of polychloroprene. The synthetic rubber (also known as Neopren® or Baypren®) exhibits excellent insulation properties and is used in manifold applications, e.g. for thermal protection suits [1,2]. 2-Chloroprene is classified as probably carcinogenic to humans (Group 2) by the Deutsche Forschungsgemeinschaft (DFG) and accordingly, as possibly carcinogenic to humans (Group 2B) by the International Agency for Research on Cancer (IARC) [2,3]. In vivo, 2-chloroprene is apparently detoxified by the formation and excretion of mercapturic acids [4]. However, there are few studies on the biotransformation of 2-chloroprene. The most relevant ones are the in vitro-studies by Munter et al. [5,6] who investigated the metabolism of 2-chloroprene in liver microsomes of humans and rodents. They proposed a detailed metabolism scheme of 2-chloroprene that is shown in part in Fig. 1. According to that scheme, the biotransformation of 2-chloroprene involves the intermediate formation of two reactive epoxide forms, 1-CEO ((1-chloroethenyl)oxirane) and 2-CEO (2-chloro-2-ethenyl oxirane). These highly reactive intermediates are likely metabolised by epoxide hydrolases and conjugation with glutathione (GSH) to several chlorine and non-chlorine containing mercapturic acids [5,6].

The annual worldwide production of epichlorohydrin adds up to about 700,000 t (in 1999) [1]. The alkylating substance is used primarily as a monomer for the synthesis of epoxy resins and polymer additives [1,7]. The DFG and the IARC classified epichlorohydrin as probably carcinogenic to humans (Group 2 and Group 2A) [3,7]. In vivo, the formation of DNA and protein adducts has already been shown. Besides, due to its bifunctionality, epichlorohydrin can cause crosslinks between nucleophilic side chains of biological molecules, which illustrates the major genotoxic potential of this substance [8–10].

The proposed biotransformation pathway of epichlorohydrin according to Gingell et al. [11] is illustrated in part in Fig. 2. A direct conjugation of epichlorohydrin with GSH leads to an urinary excretion of the mercapturic acid CHPMA (3-chloro-2-hydroxypropyl

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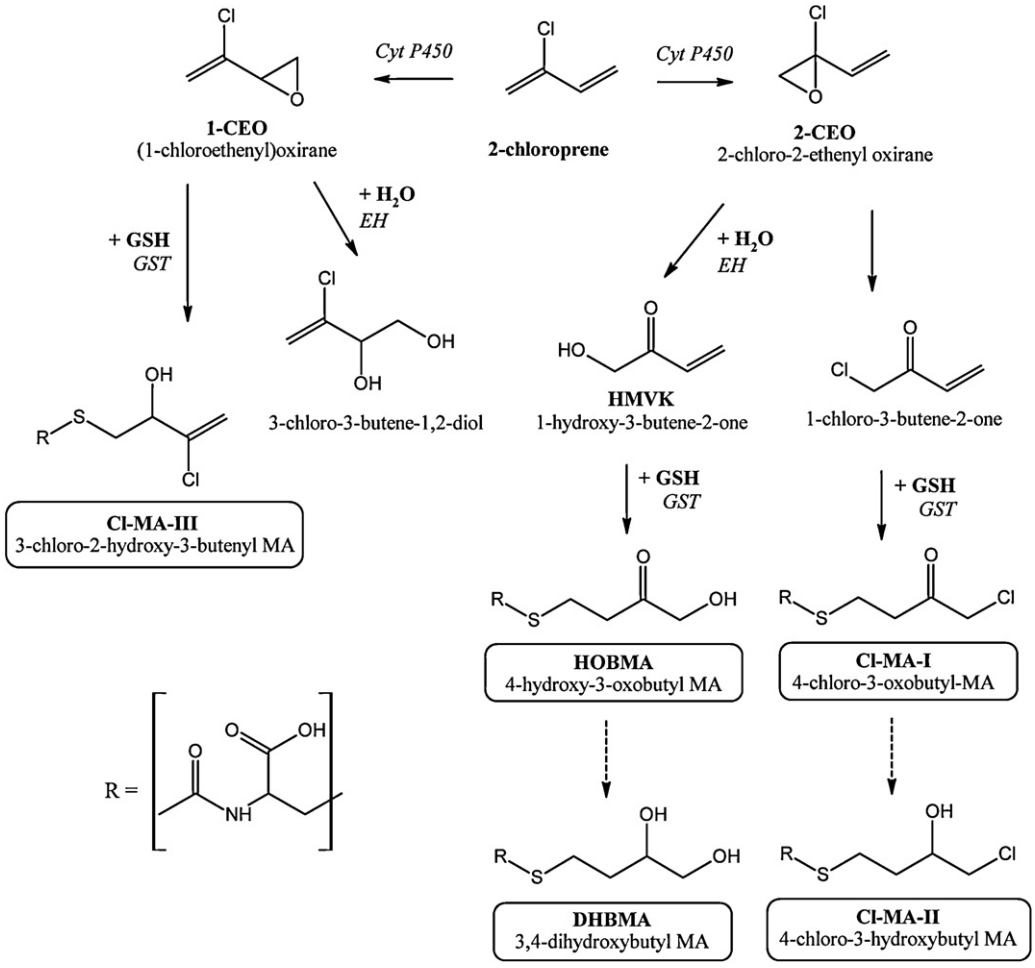

Fig. 1. Proposed biotransformation of 2-chloroprene according to Munter et al. [5,6], modified (Cyt P450 = cytochrome P450; GSH = glutathione; GST = glutathione-S-transferase; EH = epoxide hydrolase; MA = mercapturic acid).

mercapturic acid) and, following a nucleophilic dehalogenation step, of DHPMA (2,3-dihydroxypropyl mercapturic acid) [11–13]. Animal studies demonstrated that the biological half-life of epichlorohydrin averages to only 6–12 h, which lies within the half-life range of other short chain epoxides as ethylene oxide [11,13].

For the biological exposure monitoring of 2-chloroprene analytical procedures for the determination of the specific mercapturic acids do not exist. For the determination of the mercapturic acid of epichlorohydrin, CHPMA, there is only one method available, however, with limited sensitivity [12]. Our study aimed to fill this gap by the development of a suitable LC–MS/MS procedure in combination with online solid phase extraction (SPE). The use of online SPE has already been established for the analytical determination of several biomonitoring parameters [14–17]. The convenient sample cleanup procedure using column switching arrangements is also often applied for the determination of mercapturic acids in human urine [18–21]. Above all, the automation of the sample cleanup and accordingly, the feasible high sample throughput is advantageous for biomonitoring studies. To achieve high sensitivity, online sample cleanup systems are often combined with mass spectrometric detection. Because of similar physical and chemical properties of the mercapturic acids of 2-chloroprene and epichlorohydrin, we developed an analytical procedure for the simultaneous determination of these biomarkers. Thus, the presented method does not only enable further research on human metabolism of these substances but also promises to be a useful tool for a reliable monitoring of occupational exposure.

2. Experimental

2.1. Chemicals

The mercapturic acids CHPMA (N-acetyl-S-(3-chloro-2-hydroxypropyl)-L-cysteine), Cl-MA-I (N-acetyl-S-(4-chloro-3-oxobutyl)-L-cysteine), Cl-MA-II (N-acetyl-S-(4-chloro-3-hydroxybutyl)-L-cysteine), Cl-MA-III (N-acetyl-S-(3-chloro-2-hydroxy-3-butenyl)-L-cysteine) and HOBMA (N-acetyl-S-(4-hydroxy-3-oxobutyl)-L-cysteine) were custom synthesized with a stated purity of at least 95% (Institute for Organic and Biomolecular Chemistry, Göttingen, Germany). DHBMA (N-acetyl-S-(3,4-dihydroxybutyl)-L-cysteine, purity 98%) was purchased from Toronto Research Chemicals, Toronto, Canada.

The internal standard substances d_3-CHPMA (N-acetyl-d_3-S-(3-chloro-2-hydroxypropyl)-L-cysteine, purity 98%), d_3-Cl-MA-I (N-acetyl-d_3-S-(4-chloro-3-oxobutyl)-L-cysteine, purity 96%), d_3-Cl-MA-III (N-acetyl-d_3-S-(3-chloro-2-hydroxy-3-butenyl)-L-cysteine, purity 98%) and d_3-HOBMA (N-acetyl-d_3-S-(4-hydroxy-3-oxobutyl)-L-cysteine, purity 98%) were custom synthesized (Institute for Organic and Biomolecular Chemistry, Göttingen, Germany). The internal standard substance d_7-DHBMA (N-acetyl-S-(3,4-dihydroxybutyl)-L-cysteine-d_7, purity 98%) was purchased