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Identification and quantitative determination of 3-chloro-2-hydroxypropylmercapturic acid and α-chlorohydrin in urine of rats treated with epichlorohydrin

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Abstract

Epichlorohydrin (ECH) is used in many industrial processes. Different toxic effects of ECH were found in rodents. The metabolism of ECH was investigated before in rats using [^{14}C]ECH. The aim of this investigation was the development of non-radioactive quantitative analytical methods for measuring two urinary metabolites of ECH, namely 3-chloro-2-hydroxypropylmercapturic acid (CHPMA) and α-chlorohydrin (α-CH). The identity of CHPMA and α-CH excreted in urine of rats treated with 5 to 35 mg/kg ECH was confirmed by GC–MS. The quantitative analysis of CHPMA, involving ethyl acetate extraction from acidified urine and subsequent methylation and analysis by gas chromatography–flame photometric detection (GC–FPD), showed a method limit of detection of 2 μg/ml. The analysis of α-CH, based on ethyl acetate extraction and subsequent analysis by GC–ECD, showed a method limit of detection of 2 μg/ml. CHPMA and α-CH derivatives could be determined quantitatively down to concentrations of 0.5 and 0.4 μg/ml urine, respectively, by selected-ion monitoring GC–MS under EI conditions. Cumulative urinary excretion of CHPMA and α-CH by rats treated with ECH were found to be 31 ± 10 and $1.4\pm0.6\%$ ($n=13$) of the ECH dose, respectively. For CHPMA, the dose–excretion relationship suggested partially saturated ECH metabolism. For α-CH, the dose–excretion relationship was linear. With fractionated urine collection it was found that approximately 74 and 84% of the total cumulative excretion of CHPMA and α-CH, respectively, took place within the first 6 h after administration of ECH. From these investigations it is concluded that the GC–FPD and GC–ECD based methods developed are sufficiently sensitive to measure urinary excretion of CHPMA and α-CH in urine from rats administered 5 to 35 mg/kg ECH. It is anticipated that the analysis of CHPMA and α-CH based on GC–MS may be sufficiently sensitive to investigate urinary excretion from humans occupationally exposed to ECH.

Keywords: 3-Chloro-2-hydroxypropylmercapturic acid; α-Chlorohydrin; Epichlorohydrin

1. Introduction

Epichlorohydrin (ECH, 1,2-epoxy-3-chloropropane, CAS 106-89-8) is widely used in chemical industries, e.g. in the production of epoxy-resins, of agricultural chemicals and of drugs [1,2]. For rodents, several toxic effects of ECH have been described, such as renal toxicity [3], impaired sperm

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fertility [4,5] and carcinogenicity [1,6]. Radiolabelled ECH has been used to study the metabolic fate of ECH in rats [7]. It is important to know quantitative aspects of the metabolic fate of ECH, since ECH and some of its metabolites have been associated with particular toxic effects [1,8].

In rats, an oral dose of [^{14}C]ECH was rapidly and almost completely absorbed from the gastro-intestinal tract [7,9,10]. After an oral dose of 200 mg/kg to rats, ECH was eliminated from the blood with a half-life of 5.5 min [10]. 3-Chloro-2-hydroxypropylmercapturic acid (CHPMA) was identified in the urine of rats treated with 6 mg/kg of 2-[^{14}C]ECH by NMR and direct insertion (EI) mass spectrometry [7] and α-chlorohydrin (α-CH) was identified by chemical ionization GC–MS. The excreted amounts of CHPMA and α-CH were quantified by scintillation counting of the urinary metabolites isolated by TLC and found to be 36 and 4% of the ECH dose [7], respectively (Fig. 1). Minor urinary metabolites were 2,3-dihydroxypropyl-L-cysteine (DHPC) and 2,3-dihydroxypropylmercapturic acid (DHPMA), both excreted for approximately 2% of the ECH dose [7]. At higher and repeated doses (e.g. 50 mg/kg ECH for five days), rats excreted oxalic acid, β-chlorolactic acid and 1,3-(bis-cysteinyl)-propane-2-ol or the corresponding propane-2-ol-1,3-bis-mercapturic acid into urine [11]. Dose excretion relationships for the urinary metabolites of ECH have not been reported for animals. For humans, no data concerning the metabolic fate of ECH are available.

No non-radioactive quantification methods for the urinary metabolites of ECH have been described as yet. Urinary metabolites are often used as non-invasive biomarkers for the assessment of exposure to potentially toxic xenobiotic chemicals [12,13]. The primary aim of the present study was therefore to develop non-radioactive quantitative analytical methods for the analysis of CHPMA and α-CH in urine. The usefulness of the analytical methods developed was verified with urine samples of rats treated with ECH. Dose–excretion relationships were also investigated.

2. Experimental

2.1. Chemicals

Epichlorohydrin (ECH, >99%), α-chlorohydrin (α-CH, 3-chloro-1,2-dihydroxypropane, >98%) and 3-chloropropanol (>96%) were obtained from

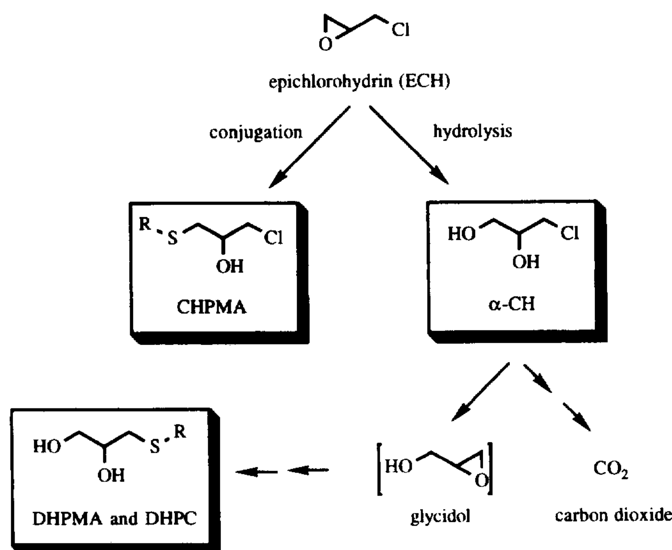

Fig. 1. Proposed metabolic scheme for ECH. (CHPMA, 3-chloro-2-hydroxypropylmercapturic acid; α-CH, α-chlorohydrin; DHPMA, 2,3-dihydroxy-propylmercapturic acid; DHPC, 2,3-dihydroxypropyl-cysteine. R, glutathionyl for GSH-conjugates; cysteinyl for L-cysteine-conjugates and N-acetyl-L-cysteinyl for mercapturic acids).

Merck (Schuchardt, Germany) and N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) was obtained from Pierce (Rockford, IL, USA). All chemicals used were of the highest purity available.

2.2. Synthesis of reference compounds

The synthesis of CHPMA was described previously [7], but slight modifications were made in this work. ECH (3.8 ml, 50 mmol) was slowly added (10 h) to a well-stirred solution of N-acetyl-L-cysteine (8.2 g, 50 mmol) in 150 ml of ethanol–water (1:2, v/v) brought to pH>9 with 13.9 ml of triethylamine and cooled to 4°C. Thereafter, the mixture was acidified to pH 6 by addition of 2 *M* HCl. The ethanol was then rotary evaporated and, after acidification to pH 2, the product was isolated from the water layer by three extractions with 150 ml of ethyl acetate. The combined ethyl acetate layers were rotary evaporated to dryness and the product appeared as a colorless oil, in an overall yield of 80%.

Trichlorovinylmercapturic acid (TCVMA) was synthesized by reacting tetrachloroethene and N-acetyl-L-cysteine as described previously [14].

The mass spectrometric and ^1H NMR data used for the identification of the reference compounds of the urinary ECH metabolites α-CH [15] and CHPMA [7] are summarized in Table 1.

2.3. Animal experiments

Male Wistar rats (200–220 g) were obtained from Harlan CPB (Zeist, Netherlands). The animals had free access to tap water and food during the whole experiment. The animals were housed in stainless steel metabolism cages, allowing the separate collec-

Table 1
^1H NMR and electron-impact (EI) mass spectrometric data of the reference compounds of the urinary metabolites of ECH and some of their derivatives

Compound	^1H NMR δ in ppm[a] [multiplicity, intensity, proton]	MS (EI) *m/z* (fragment, intensity)	Retention time (min)
α-CH	3.50–3.76 [m, 4H, $CH_2(OH)CH(OH)CH_2Cl$] 3.83–4.00 [m, 1H, $CH_2(OH)CH(OH)CH_2Cl$]	bis-TMS[b] derivative: 239 (Cl[c], $[M-CH_3]^+$, 18%) 205 ($[M-ClCH_2]^+$, 5%) 147 ($[M-Cl-Me_2SiCH_2]^+$, 100%) 116 ($[205-OSiMe_3]^{+\circ}$, 65%) 101 ($[116-CH_3]^+$, 48%)	8.25
CHPMA	2.04 [s, 3H, $-COCH_3$] 4.55–4.65 [m, 1H, $-SCH_2CH$] 2.90–3.20 [m, 2H, $-SCH_2CH$] 2.67–2.90 [m, 2H, $CH_2(Cl)CH_2(OH)CH_2S-$] 3.95–4.09 [m, 1H, $CH_2(Cl)CH(OH)CH_2S-$] 3.60–3.78 [m, 2H, $CH_2(Cl)CH_2(OH)CH_2S-$]	methylester: 251 (Cl, $[M-H_2O]^{+\circ}$, 25%) 192 (Cl, ($[251-COOCH_3]^+$ or $[251-NH_2COCH_3]^{+\circ}$), 100%) 176 (($[M-ClCH_2CHOHCH_2]^+$ or $[M-Cl\text{-}NHCOCH_3]^{+\circ}$), 16%) 157 ($[192-Cl]^{+\circ}$ or $^+$, 66%) 132 (($[M-ClCH_2COH\text{-}COOCH_3]^+$ or $[M-ClCH_2COH\text{-}NH_2COCH_3]^{+\circ}$), 53%)	16.4
		TMS derivative of methylester: 326 (Cl, $[M-CH_3]^+$, 5%) 251 (Cl, $[M-HOSiMe_3]^{+\circ}$, 48%) 192 (Cl, ($[251-COOCH_3]^+$ or $[251-NH_2COCH_3]^{+\circ}$, 95%) 151 (Cl, $[M-SiMe_3\text{-}COOCH_3$ $\text{-}NHCOCH_3]^+$, 72%) 144 ($[M-ClCH_2CH(OSiMe_3)CH_2S]^+$, 56%) 73 ($[SiMe_3]^+$, 100%)	16.9

[a] Relative to tetramethylsilane, NMR in $^2H_2O-NaCO_3$ or ^2H_2O, s=singlet, d=doublet, t=triplet, m=multiplet.
[b] TMS=trimethylsilyl.
[c] Cl denotes a chloro-isotope pattern was found for the fragment.

tion of urine and faeces. ECH was administered intraperitoneally (i.p.) in doses of 0, 5, 15, 25 or 35 mg/kg (54, 163, 272 or 380 μmol/kg) in arachis oil (1 ml/kg). At an ECH dose of 15 mg/kg (163 μmol/kg) urine was collected in vials cooled to 4°C, in fractions from 0–6, 6–24 and 24–48 h after administration of ECH. In the other cases, urine was collected for 24 h after the administration of ECH. After collection of urine, the cages were rinsed with 15 ml of distilled water. Urine fractions were stored at −18°C until analysis. After the collection of urine, the rats were killed by decapitation.

2.4. Isolation of CHPMA from urine

A 0.1-ml portion of the internal standard solution (TCVMA) in methanol (100 μg/ml) was transferred into a test tube and the methanol was evaporated. A 1.0-ml volume of rat urine was added and the pH was adjusted to 0.8–1.1 by addition of 0.2–0.3 ml of 2 *M* HCl. Extraction was performed twice with 4 ml of ethyl acetate; a clear phase separation was obtained by centrifugation at 800 *g* for 30 min. The organic layers were pooled and dried under a gentle stream of N_2 and elevated temperature (50°C). The residues were redissolved in 0.5 ml of methanol and methylated by treatment with 2.0 ml of ethereal diazomethane for 1 h [16]. After evaporation of the organic solvents, the residues were redissolved in 0.5 ml of ethyl acetate. Routine analysis of the methylated urine samples was performed by GC–FPD in the sulphur-mode. Alternatively, the redissolved samples were silylated by addition of 0.5 ml of BSTFA and were subsequently heated to 70°C for 30 min. Analysis of 1 μl of the trimethylsilylated/methylated samples was performed by GC–MS in the SIM mode (see below). Calibration curves were constructed in control urine that had been spiked with synthetic CHPMA.

2.5. Isolation of α-CH from urine

To 0.9-ml samples of rat urine, 0.1 ml of 3-chloropropanol (10 mg/ml water) was added as the internal standard. Extraction was performed three times with 1 ml of ethyl acetate; a clear phase separation was obtained by centrifugation at 800 *g* for 30 min. The organic layers were pooled and, without evaporation, subjected to GC–ECD analysis. Alternatively, 0.5 ml of the ethyl acetate layer was silylated as described above and a 1-μl portion was subjected to GC–SIM-MS analysis (see below). Calibration curves were constructed in control urine that had been spiked with authentic α-CH.

2.6. Apparatus

Gas chromatography was performed on different HP 5890 gas chromatographs (Hewlett-Packard, Palo Alto, CA, USA) equipped with a split/splitless injector and with either an HP 5970 quadrupole mass selective detector, a flame photometric detector (used with a sulphur selective filter, $\lambda = 393 \pm 5$ nm) or an electron-capture detector. Slightly different columns and GC conditions were used with the different detectors; column and conditions are described in the appropriate section (see below).

2.7. Gas chromatography with flame photometric detection (GC–FPD)

A gas chromatograph with a flame photometric detector was used to detect urinary sulphur containing metabolites of ECH. A 1-μl sample of the methylated organic fraction was injected in the split mode (split ratio 1:15) into the gas chromatograph. The analysis was performed using a Cp Sil 5 CB column (25 m×0.25 mm I.D., stationary phase thickness 0.2 μm; Chrompack, Bergen op Zoom, Netherlands) and helium was used as the carrier gas (2.5 ml/min). The temperatures of the injection port and the detector were 250°C. The oven was programmed from 50°C (1 min) to 250°C at a rate of 10°C/min. The final temperature was kept for another 10 min. The retention times of CHPMA and the internal standard, TCVMA, measured as their methyl esters, were 16.9 and 16.4 min, respectively.

2.8. Gas chromatography with electron-capture detection (GC–ECD)

Analysis of α-CH was performed by GC–ECD. A 2-μl volume of the untreated ethyl acetate layer was injected in the split mode (split ratio 1:20) on an HP-5 column (30 m×0.32 mm I.D., stationary phase thickness 0.25 μm; Hewlett-Packard, Amstelveen,

Netherlands). Helium was used as the carrier gas (1.2 ml/min) and nitrogen was used as the detector make up gas. The temperature of the injection port was 200°C and that of the detector was 300°C. The oven was programmed from 50°C (1 min) to 250°C at a rate of 10°C/min. The final temperature was kept for another 10 min. The retention times of underivatized α-CH and the internal standard, 3-chloropropanol, were 4.9 and 2.9 min, respectively.

2.9. Gas chromatography and mass spectrometry (GC–MS)

GC–MS analysis was performed by injection of 1 to 3 μl samples of the above mentioned trimethylsilylated solutions. The injector was used in splitless mode at injection (switched to split mode after 0.5 min). A Cp Sil 5 CB column (50 m×0.25 mm I.D., stationary phase thickness 0.2 μm; Chrompack) was used and helium was used as the carrier gas (2 ml/min). The temperatures of the injection port and the transfer line were 250°C. The oven was programmed from 50°C (1 min) to 250°C at a rate of 15°C/min. The final temperature was kept for another 10 min. The mass spectrometer was operated in the EI mode (70 eV). Full scanning (m/z 50–550, 1.1 scan/s) was used for identification purposes and for the selection of signals appropriate for selected-ion monitoring (SIM) experiments. Quantitative detection was performed under SIM conditions (in so-called low resolution mode) of three or four characteristic ions (see Table 1) per analyte and using a dwell time of 65 ms; this resulted in more than eighteen data points per compound in the chromatographic peaks. The observed retention time of the CHPMA derivative was 16.9 min and that of the internal standard, TCVMA, was 15.6 min. The retention time of the TMS derivative of α-CH was 8.2 min and that of the internal standard, 3-chloropropanol, was 5.9 min.

3. Results

3.1. Identification of urinary metabolites of ECH

Two metabolites of ECH were identified in the 24 h urine samples from rat, after i.p. administration of 25 mg/kg ECH. CHPMA was identified by GC–MS, after methylation and trimethylsilylation, by its retention time (16.9 min) and by its characteristic ions; ion chromatograms are given in Fig. 2A. This identification was confirmed by the nearly identical GC–MS characteristics of the TMS derivative of synthetic CHPMA methyl ester (Table 1). From experiments with sulphur-selective GC–FPD, of which results are shown in Fig. 3, it is clear that only one quantitatively important sulphur-containing ECH metabolite was extracted from rat urine. α-CH was identified, by GC–MS, in the same urine extract used for the identification of CHPMA. Five characteristic ions of the bis-TMS derivative of α-CH were found at 8.25 min in the corresponding ion chromatograms (Fig. 2B). Similar results were obtained when authentic α-CH was trimethylsilylated and subjected to GC–MS (Table 1).

3.2. Quantification of urinary metabolites of ECH

CHPMA was measured accurately as its methyl ester, by GC–FPD, in a concentration range from 25 to 250 μg/ml ($y=0.16+0.15x$, $r^2=0.993$). Due to the non-linear response of the FPD in sulphur mode, double logarithmic calibration curves were constructed by plotting the quotient of the natural logarithm of the analyte and the natural logarithm of the internal standard response against the natural logarithm of the analyte concentration. When the sample was injected splitless, concentrations in the range from 2 to 15 μg of CHPMA/ml of urine could also be measured accurately ($y=0.70+0.15x$, $r^2=0.980$). The method limit of detection of CHPMA, by GC–FPD, was 2 μg/ml ($S/N=10$:1, $n=2$). Sometimes peak tailing was observed when the methyl ester of CHPMA was measured. This problem could be solved by derivatization of methylated CHPMA with BSTFA. The trimethylsilyl derivatives were subjected to GC–MS analysis. The ion at m/z 151 was used for CHPMA and the ion at m/z 211 was used for the internal standard, TCVMA, to quantify the urinary CHPMA concentrations. Calibration curves obtained with GC–MS were linear from 0.5 to 10 μg/ml urine, ($y=-0.0048+0.14x$, $r^2=0.999$). The method limit of detection for CHPMA was found to be 0.5 μg/ml ($S/N=10$:1, $n=2$).

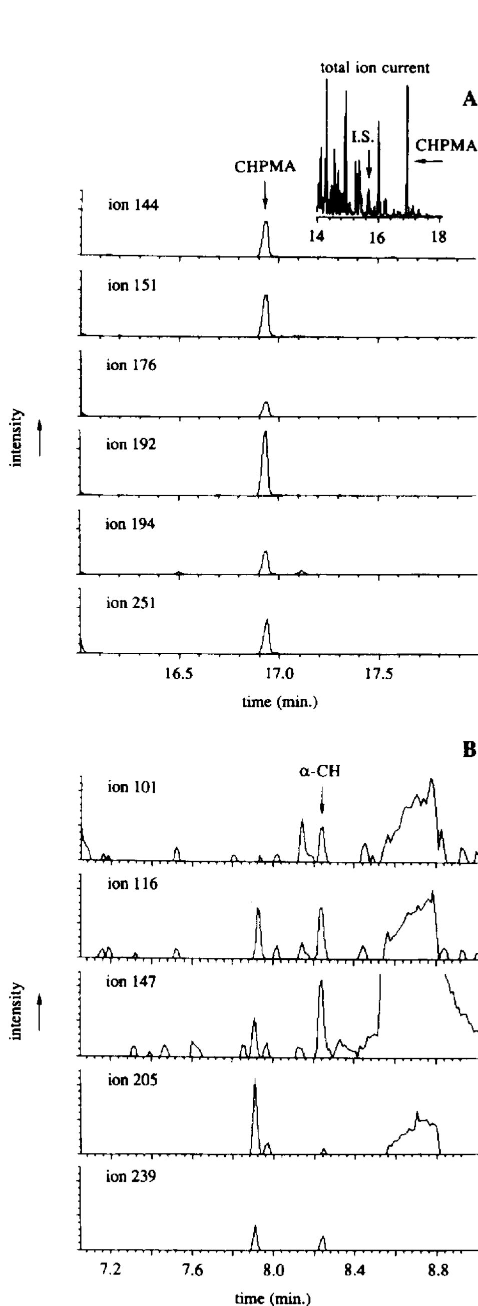

Fig. 2. Identification of CHPMA and α-CH in urine of a rat treated i.p. with 25 mg/kg ECH. CHPMA was identified as the TMS derivative of its methyl ester, by comparison of retention times and the intensity of six characteristic ions with synthetic CHPMA (A). α-CH was identified similarly by comparison with commercial α-CH in the same urine extract as was used for the identification of CHPMA (B). I.S. denotes the internal standard, TCVMA.

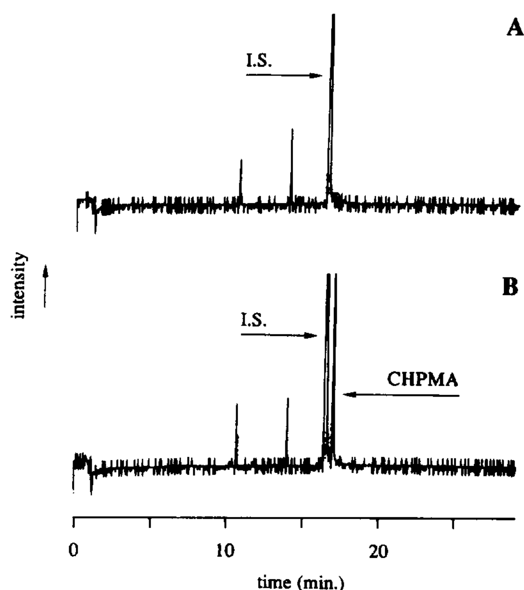

Fig. 3. Determination of the methyl ester derivative of CHPMA by gas chromatography with sulphur selective detection (GC–FPD). Urine of a rat after i.p. administration of arachis oil (A). Urine of a rat after i.p. administration of 5 mg/kg (54 μmol/kg) ECH (B). I.S. denotes the internal standard, TCVMA.

α-CH was isolated from rat urine by repeated ethyl acetate extractions and was subjected to GC–ECD analysis without derivatization. It was not possible to concentrate the organic phase by evaporation without serious losses of α-CH. However, α-CH was never completely lost and even when ethyl acetate was evaporated to dryness, residual α-CH was found. Without success, different evaporation temperatures (e.g. 4, 20 and 50°C) and different (combinations of) solvents (e.g. dichloromethane, ether, petroleum ether and butanol) were used to find a way to concentrate α-CH without significant loss. Only when butanol was added to ethyl acetate were slight improvements observed. Calibration curves for α-CH on GC–ECD were linear from 2 to 60 μg of α-CH/ml of urine ($y=0.11+0.14x$, $r^2=0.998$). The method limit of detection of underivatized α-CH on GC–ECD was 2 μg/ml urine ($S/N=10{:}1$, $n=2$). For the analysis of the bis-TMS derivative of α-CH by GC–MS with SIM acquisition, the ions at m/z 116 and 123 were used for α-CH and the internal standard, 3-chloropropanol, respectively. Calibration curves for the TMS derivative of α-CH obtained

with GC–MS were linear from 0.4 to 20 μg/ml urine ($y=0.0085+0.054x$, $r^2=0.999$). The method limit of detection was 0.4 μg/ml urine ($S/N=10:1$, $n=2$). When higher injection volumes were used (up to 3 μl), α-CH could de detected down to concentrations of 0.05 μg/ml urine ($S/N=3:1$, $n=1$).

3.3. Dose–excretion relationships

The excretion of CHPMA in urine of rats treated i.p. with ECH was quantified by GC–FPD (Fig. 3). The animals excreted 31±10% (±S.D., $n=13$) of the ECH dose as CHPMA within 24 h. The excreted percentage of the ECH dose declined from 45±2% ($n=3$) to 27±4% ($n=3$) with increasing dose. This resulted in the non-linear dose–excretion relationship in the dose range used (5 to 35 mg/kg), which is shown in Fig. 5. From measurements in fractionated urine samples, it appeared that at a dose of 15 mg/kg ECH, 74±31% ($n=4$) of the cumulative amount of CHPMA was excreted within 6 h of administration (Fig. 6). After 24 h no more CHPMA was excreted.

The excretion of α-CH in urine of rats treated i.p. with ECH was quantified by GC–ECD (Fig. 4). The urinary excretion of α-CH was substantially lower than the urinary excretion of CHPMA. In total,

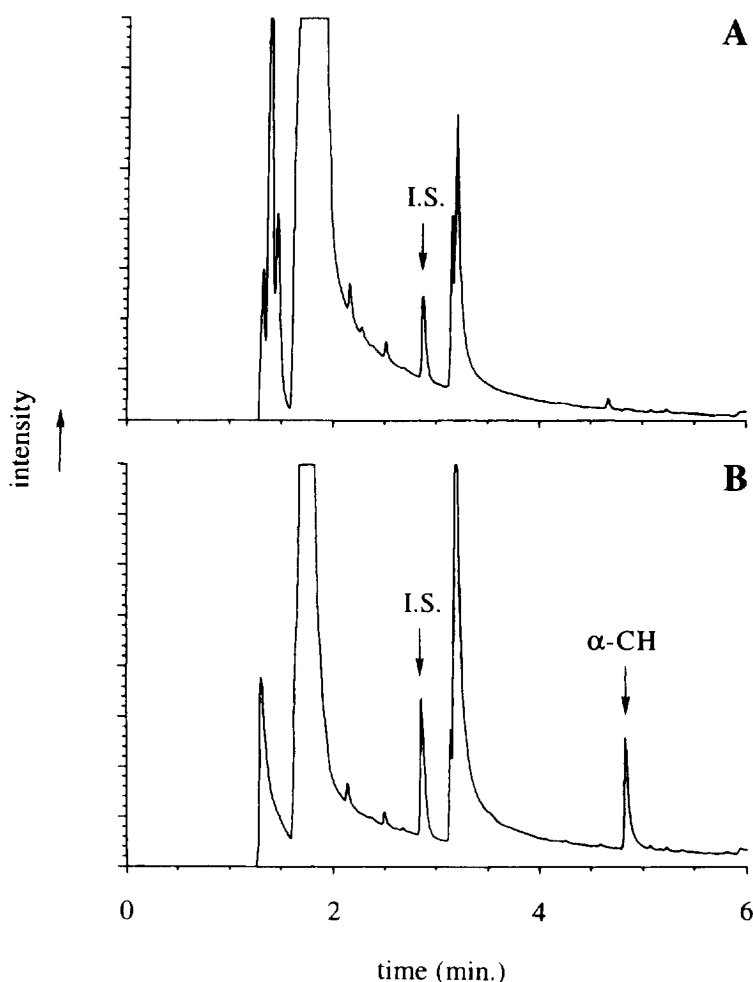

Fig. 4. Determination of underivatized α-CH by gas chromatography with electron-capture detection (GC–ECD). Urine of a rat after i.p. administration of arachis oil (A). Urine of a rat 0 to 6 h after i.p. administration of 15 mg/kg ECH (163 μmol/kg) in arachis oil (B). I.S. denotes the internal standard, 3-chloropropanol.

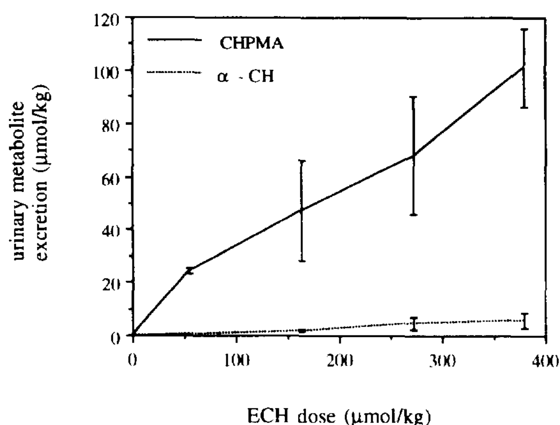

Fig. 5. Dose–excretion relationship between the ECH dose and the urinary metabolites CHPMA (solid line) and α-CH (dotted line). Rats were i.p. administered 0 or 5 to 35 mg/kg ECH in arachis oil. Urine was collected for 24 h following administration. For every dose, at least three rats were used.

1.4±0.6% ($n=13$) of the ECH dose was excreted into urine as α-CH. In the dose range used, the averaged excretion of α-CH was linear ($r^2=0.975$) (Fig. 5). From measurements in the fractionated urine samples, it appeared that at the dose of 15 mg/kg ECH, 84±19% ($n=4$) of the cumulative amount of α-CH was excreted within 6 h of i.p. administration of ECH (Fig. 6).

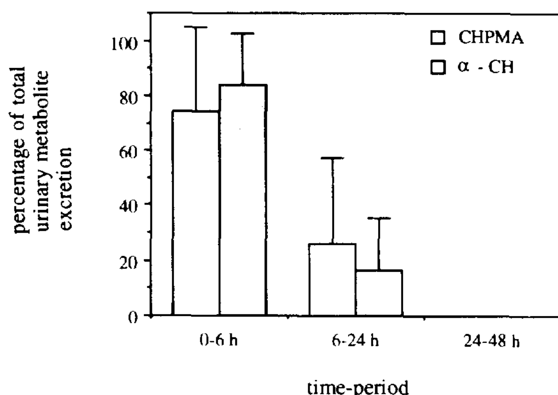

Fig. 6. Fractionated urine samples of rats treated i.p. with 15 mg/kg ECH ($n=4$). The excreted amounts of CHPMA (open) or α-CH (filled) are given as percentages of the total cumulative amount of the metabolite excreted in 48 h.

4. Discussion

CHPMA and α-CH, two urinary metabolites of ECH in rats, were unequivocally identified in the urine of rats i.p. treated with ECH by GC–FPD, GC–ECD and GC–MS. Both metabolites have previously been identified in urine of rats orally administered [^{14}C]ECH [7]. Two minor sulphur-containing metabolites found earlier [7], 2,3-dihydroxypropyl-cysteine (DHPC) and the corresponding mercapturic acid (DHPMA), could not be identified by GC–FPD and GC–MS. These two metabolites are probably not extracted from urine when using liquid–liquid extraction with ethyl acetate; it is noted that the structurally related 3,4-dihydroxybutylmercapturic acid could only be isolated by drying, but not by ethyl acetate extraction [17].

With the extraction method applied, the concentration of the ethyl acetate layers by evaporation lead to serious losses of α-CH. Co-evaporation of α-CH with ethyl acetate in urine extracts might be the cause of the observed losses of α-CH. In earlier studies [10,15] in which α-CH was determined in blood or foodstuff, this problem was not reported. In order to prevent evaporation losses of α-CH, the evaporation step was omitted and ethyl acetate extracts were directly submitted to GC analysis. Nevertheless, satisfactory low urinary concentrations of α-CH could be quantified; the method limit of detection was 2 μg/ml of urine by GC–ECD, and 0.4 μg/ml of urine by GC–MS. With the methyl ester of CHPMA, peak tailing was sometimes observed in GC experiments. The chromatographic behaviour of the methyl ester of CHPMA could be improved by trimethylsilylation. Low urinary concentrations of CHPMA could be determined, the method limit of detection by GC–FPD (methyl ester) and SIM GC–MS (trimethylsilyl derivative) being 2.0 and 0.5 μg/ml of urine, respectively. The analytical methods described here were sufficiently sensitive to measure the excretion of CHPMA and α-CH in urine of rats i.p. treated with 5 to 35 mg/kg of ECH (54 to 380 μmol/kg).

The percentages of the ECH dose excreted by rats as CHPMA or α-CH were 31±10% and 1.4±0.6% ($n=13$), respectively. Despite slightly different experimental circumstances (i.e. i.p. vs. oral administration and Wistar vs. Fischer 344 rats), the excretion

percentages are in good agreement with previously reported percentages, i.e. 36 and 4% of the ECH dose, respectively [7]. It is noted that in previous studies the excreted amounts of urinary metabolites of ECH were quantified by scintillation counting of radiolabelled metabolites of [^{14}C]ECH and not with non-radioactive methods. The dose–excretion relationship between ECH and its urinary metabolite, CHPMA, was non-linear and showed saturation at higher ECH doses. For α-CH, a linear dose–excretion relationship ($r^2 = 0.975$ on average) was found in the dose range tested. Rapid urinary excretion of the ECH metabolites was observed, 74±31 and 84±19% ($n = 4$) of the cumulative amounts of CHPMA and α-CH, respectively, being excreted within 6 h of ECH administration. This observation is in agreement with the rapid blood elimination of ECH and α-CH that was previously described for ECH-treated mice [10] and with the relatively short urinary half-life values described for several other mercapturic acids [12].

The limits of detection of the present method for CHPMA and α-CH are probably sufficiently low to measure the excretion of both metabolites in urine of humans exposed to the current occupational exposure limit (OEL) for ECH (OEL, 4 mg/m^3 air, [18]). When it is assumed that: (1) humans are exposed to an inhalatory air concentration of ECH of 4 mg/m^3 air during an 8 h work-shift, (2) that 10 m^3 air are inhaled in this period [19], (3) that 50% of the inhalatory ECH is absorbed by the lungs [20], (4) that 30 or 1.5% of the inhaled ECH is metabolized to CHPMA or to α-CH, respectively, (5) that the excretion rates in rat and human are equal and (6) that 2 l of urine are produced in 24 h [19], one could calculate averaged concentrations of CHPMA of 8 μg/ml urine and of α-CH of 0.2 μg/ml urine. This approximation shows that evidence for exposure close to the OEL in principle could easily be obtained by monitoring urine levels of CHPMA and α-CH.

In conclusion, the GC–FPD and GC–ECD based methods presented in this paper provide sufficiently sensitive detection for the qualitative and quantitative determination of CHPMA and α-CH in the urine of rats treated i.p. with 5 to 35 mg/kg of ECH. Urinary excretion of the largest fraction of CHPMA and α-CH occurred within 6 h of administration of ECH and the cumulative excretion was clearly related to the ECH dose administered. It is anticipated that the analytical methods for CHPMA and α-CH presented, notably the ones based on GC–MS, are sensitive enough to investigate the excretion of both metabolites in the urine of humans occupationally or otherwise exposed to ECH.

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