Allylmercapturic acid as urinary biomarker of human exposure to allyl chloride

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Abstract

***Objective*—To evaluate the use of urinary mercapturic acids as a biomarker of human exposure to allyl chloride (3-chloropropene) (AC). During three regular shut down periods in a production factory for AC, both types of variables were measured in 136 workers involved in maintenance operations.**

***Methods*—Potential airborne exposure to AC was measured by personal air monitoring in the breathing zone. In total 205 workshifts were evaluated. During 99 workshifts no respiratory protection equipment was used. Mercapturic acid metabolites were measured in urinary extracts by gas chromatography-mass spectrometry (GC-MS).**

***Results*—During 86 work shifts when no respiratory protection was used the air concentrations of AC were below the Dutch eight hour time weighted average (8h-TWA) occupational exposure limit (OEL) of AC (3 mg/m³), whereas in 13 workshifts the potential exposure, as measured by personal air monitoring, exceeded the OEL (3.3 to 17 mg/m³). With the aid of GC-MS, 3-hydroxypropylmercapturic acid (HPMA) was identified as a minor and allylmercapturic acid (ALMA) as a major metabolite of AC in urine samples from the maintenance workers exposed to AC. The concentrations of ALMA excreted were in a range from < 25 µg/l (detection limit) to 3550 µg/l. The increases in urinary ALMA concentrations during the workshifts correlated well with the 8h-TWA air concentrations of AC (r = 0.816, P=0.0001, n = 39). Based on this correlation, for AC a biological exposure index (BEI) of 352 µg ALMA/g creatinine during an eight hour workshift is proposed. In some urine samples unexpectedly high concentrations of ALMA were found. Some of these could definitely be attributed to dermal exposure to AC. In other cases garlic consumption was identified as a confounding factor.**

***Conclusion*—The mercapturic acid ALMA was identified in urine of workers occupationally exposed to airborne AC and the increase in ALMA concentrations in urine during a workshift correlated well with the 8h-TWA exposure to AC. Garlic consumption, but not smoking, is a potential confounding factor for this biomarker of human exposure to AC.**

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Allyl chloride (3-chloropropene) (AC, CAS number 107–05–01) is a colourless liquid with a pungent odour. Allyl chloride is often used as an intermediate in the synthesis of epichlorohydrin, a precursor used in the production of epoxy resins.[1] In most countries, the eight hour time weighed average occupational exposure limit (8h-TWA OEL) for AC is 1 ppm (3 mg/m³).[2]

In humans exposed to high concentrations of AC, several toxic effects have been found. In workers in a sodium allyl sulphonate production unit, exposed to extremely high concentrations AC (up to 6650 mg/m³ air), peripheral neurotoxic effects were found.[3] Reversible liver damage determined by increased serum enzyme activities[4] was found in workers exposed to AC concentrations up to 350 mg/m³ air. In more recent studies in an AC production plant with airborne exposures up to 4.8 mg AC/m³ air (8h-TWA); however, no impaired liver or kidney functions were found.[5]

In rodents several subchronic effects of AC have been found.[6 7] Guinea pigs and rats, during one month exposed to air concentrations of 25 mg AC/m³, showed histologically observable adverse effects on liver and kidneys.[7] After high semichronic AC exposures (200 mg AC/m³ for three months) of rabbits and cats, neurotoxic effects were identified from changes in electromyograms and these effects were accompanied by degeneration of myelin sheaths in peripheral nerves and by impaired motor functions. These effects were not found in rabbits and rats at lower exposures—for example, 17 mg AC/m³ for five months.[6] Allyl chloride has been shown to be mutagenic in various *Salmonella typhimurium* strains.[8–10] In mice tumours were only seen at the site of application of AC when combined with a tumour promoter[11] or when mice were treated intraperitoneally with maximal tolerated doses of AC.[12] There are no indications that AC is a human carcinogen.[1]

The biotransformation of AC has been investigated almost exclusively in rats.[13–15]

Table 1 ^{1}H-NMR, electron impact (EI) mass spectrometric (MS), and gas chromatographic (GC) retention time data of mercapturic acids of interest in this study

Compound	^{1}H-NMR (δ in ppm)*	MS (EI); derivative, retention time (m/z, fragment, intensity)
ALMA	2.04 [s 3H -COCH$_3$]	Methylester, 10.4 min
	4.38 - 4.48 [m 1H -SCH$_2$CH]	217 ([M]$^{+\bullet}$, 10.2%)
	2.78 - 3.05 [m 2H -SCH$_2$CH]	176 ([M - CH$_2$=CHCH$_2$]$^+$, 28%)
	3.20 [d 2H CH$_2$=CHCH$_2$S-]	158 ([M - COOCH$_3$]$^+$ or [M - NH$_2$COCH$_3$]$^{+\bullet}$, 100%)
	5.70 - 5.93 [m 1H CH$_2$=CHCH$_2$S-]	144 ([M - CH$_2$=CHCH$_2$S]$^+$, 24%)
	5.11 and 5.20 [s + d 2H CH$_2$=CHCH$_2$S-]	117 ([176 - NH$_2$COCH$_3$]$^+$ or [M - COOCH$_3$]$^{+\bullet}$, 74%)
D$_3$-ALMA	4.27 - 4.38 [m 1H -SCH$_2$CH]	Methylester, 10.4 min
	2.70 - 3.00 [m 2H -SCH$_2$CH]	220 ([M]$^{+\bullet}$, 6%)
	3.15 [d 2H CH$_2$=CHCH$_2$S-]	179 ([M - CH$_2$=CHCH$_2$]$^+$, 26%)
	5.70 - 5.91 [m 1H CH$_2$=CHCH$_2$S-]	158 ([M - COOCH$_3$]$^+$ or [M - NH$_2$COCD$_3$]$^{+\bullet}$, 100%)
	5.12 and 5.20 [s + d 2H CH$_2$=CHCH$_2$S-]	147 ([M - CH$_2$=CHCH$_2$S]$^+$, 27%)
HPMA	2.02 [s 3H -COCH$_3$]	Methylester, 14.8 min
	4.26 - 4.36 [m 1H -SCH$_2$CH]	235 ([M]$^{+\bullet}$, 1.5%)
	2.72 - 3.06 [m 2H -SCH$_2$CH]	176 ([M - CH$_2$(OH)CH$_2$CH$_2$]$^+$, 50%)
	2.54 - 2.66 [t 2H HOCH$_2$CH$_2$CH$_2$S-]	158 ([M - H$_2$O - COOCH$_3$]$^+$ or [M - H$_2$O - NH$_2$COCH$_3$]$^{+\bullet}$, 39%)
	1.68 - 1.89 [m 2H HOCH$_2$CH$_2$CH$_2$S-]	144, ([M - CH$_2$(OH)CH$_2$CH$_2$S]$^+$, 13%)
	3.49 - 3.71 [t 2H HOCH$_2$CH$_2$CH$_2$S-]	
D$_5$-PMA	1.91 [s 3H -COCH$_3$]	Methylester, 17.2 min
	4.79 - 4.86 [m 1H -C$_6$D$_5$SCH$_2$CH]	258 ([M]$^{+\bullet}$, 15 %)
	3.34 - 3.61 [m 2H -C$_6$D$_5$SCH$_2$CH]	199 ([M - COOCH$_3$]$^+$ or [M - NH$_2$COCH$_3$]$^{+\bullet}$, 100 %)
		140 ([M - COOCH$_3$ - NH$_2$COCH$_3$]$^+$, 57 %)
		128 ([C$_6$D$_5$SCH$_2$]$^+$, 40 %)
		114 ([C$_6$D$_5$S]$^+$, 15 %)

* Relative to tetramethylsilane, NMR in D$_2$O/NaCO$_3$. s = Singlet; d = doublet; m = multiplet.

Two urinary mercapturic acid metabolites were identified in rats.[14 15] 3-Hydroxypropylmercapturic acid (HPMA) was reported to be a major and allylmercapturic acid (ALMA) a minor urinary metabolite of AC in rats. However, recently we investigated the urinary metabolite profile of AC in rats[16] after intraperitoneal administration. At doses between 5 and 45 mg/kg about 30% of the AC dose was excreted in urine as ALMA. Also, minor amounts of HPMA, 3-chloro-2-hydroxypropylmercapturic acid (CHPMA), and α-chlorohydrin (α-CH) were measured, for < 3.0%, 0.20%, and 0.13% of the AC dose, respectively. The urinary excretion of CHPMA and α-CH suggests the formation of epichlorohydrin from AC in the rat. The oxidation of AC to ECH has been suggested as playing a part in the tumorigenic activity of AC in mice.[11] The urinary excretion of ALMA has also been shown to be related to garlic consumption[17] whereas the urinary excretion of HPMA was also found in rats treated with acrolein,[13 15] a compound found in cigarette smoke.[18 19]

Mercapturic acids have often been used as biomarkers of human exposure to electrophilic chemicals.[20 21] The mercapturic acid pathway is generally considered to be a detoxification pathway.[22] However, in some cases this metabolic pathway can lead to toxicity as well.[23 24] Recent examples of the use of mercapturic acids as a biomarker of human exposure are Z- and E-3-chloropropenylmercapturic acid as biomarkers of exposure to Z- and E-1,3-dichloropropene[25] and phenylmercapturic acid as biomarker of exposure to benzene.[26 27]

The present study was undertaken to evaluate the utility of urinary mercapturic acids as biomarkers of human AC exposure. The study was undertaken in an organochlorine production plant, in which AC is produced by chlorination of propene and subsequently used in the synthesis of epichlorohydrin. The biomonitoring sessions were performed in maintenance workers during three annual maintenance shut down periods in which AC production was stopped, installations were cleaned and maintenance was performed. During these periods the potential exposure to AC was determined in the breathing zone by personal air monitoring.

Materials and methods

CHEMICALS

Allyl bromide (>99%) was obtained from Aldrich (Beerse, Belgium), benzylmercapturic acid (> 99%) and N-acetyl-L-cysteine (> 96%) were obtained from Janssen Chimica (Geel, Belgium), and creatinine (> 98%) from JT Baker (Deventer, The Netherlands). All chemicals used were of the highest purity available.

SYNTHESIS

Synthesis of allylmercapturic acid (ALMA)

ALMA was synthesised as described previously[17] with slight modifications. In short: allyl bromide (1.2 g, 10 mmol) was added to a mixture of 1.6 g (10 mmol) N-acetyl-L-cysteine in 10 ml methanol and 0.24 g (20 mmol) sodium. The mixture was allowed to stand at room temperature for four hours and was subsequently neutralised with 2 N HCl. After rotary evaporation of methanol, the residue was dissolved in 10 ml 2 N HCl and extracted with two 30 ml volumes of ethyl acetate. After rotary evaporation of the organic solvent the product appeared as a white powder and was recrystallised from hot acetone. The overall yield was about 80%. The identification data on ALMA, and the other synthetic products (described later), were obtained with ^{1}H-NMR and gas chromatography-mass spectrometry (GC-MS) (table 1).

Synthesis of 3-hydroxypropylmercapturic acid (HPMA)

HPMA was synthesised and identified as described before.[13 15 16]

Synthesis of trideuteroacetyl-allylmercapturic acid (D_3-ALMA)

D_3-ALMA was synthesised by addition of hexadeuteroacetic anhydride in an aqueous solution of S-(allyl)-L-cysteine, as described before.[28] The S-(allyl)-L-cysteine used in this reaction was synthesised as described elsewhere.[14]

Synthesis of pentadeutero-phenylmercapturic acid (D_5-PMA)

D_5-PMA was synthesised and identified as described before.[27]

HUMAN STUDY AND ANALYSIS

Study design and collection of urine samples

This study was performed during so called shut down periods in a production factory for AC. In this factory AC is produced in a completely closed system with little chance for human exposure. During a shut down period the installation is rinsed and subsequently opened for maintenance and inspection operations. Before the start of the experiments the volunteers participating in this biomonitoring study were informed about the aim, the procedure, and the potential outcome of the study. Male workers (n = 136, age: 20–55 years) involved in maintenance and inspection operations during shut down periods in three consecutive years (1991, 1992, and 1993) participated in the study. Information on occupational history, demographic characteristics, and various lifestyle factors was obtained by a self administered questionnaire and from plant records. In 1992 and 1993 information was also gathered about garlic consumption. None of the workers was involved in two or three consecutive years. Before inspection and maintenance operations began, the production installations were successively rinsed with alkali and solvent, and finally steam cleaned until no organochlorine compounds could be detected in the effluent. Depending on the job, various types of personal protection equipment were used according to instructions of an occupational hygienist. During all tasks potential exposures to airborne AC were assessed by personal air monitoring (PAM). Urinary spot samples were collected at the beginning and the end of each shift. The samples were immediately acidified to pH 1–2 by addition of 6 M HCl and stored at 4°C until analysis. ALMA was stable for up to one month under these conditions.

Personal air monitoring

Potential exposure to airborne AC was assessed throughout the workshifts by personal air monitoring with 3M organic vapour monitors (type 3500), which were attached on the right lapel by the occupational hygienist. If any respiratory protection equipment was used the badges were worn on the outside. Immediately after sampling the badges were capped and stored frozen at −20°C until analysis. Within one week the absorbed amounts of AC were desorbed with 1.5 ml carbon disulphide with continuous shaking for 30 minutes. The mean (SD) recovery of AC according to this procedure was 91% (3%); all values were corrected for AC recovery. Aliquots of 1 µl of the carbon disulphide solution were injected (split injection; split flow 35 ml/min) on a 60 m × 0.32 mm (internal diameter) DB-1 column (Durabond, J and W, Folsom, California, USA) with a film thickness of 1 µm and helium as carrier gas (50 kPa prepressure; column flow about 1.6 ml/min) on a HP 5890 series 2 gas chromatograph equipped with flame ionisation detection. Injector and detector temperature were 250°C. The oven was programmed from 40°C (seven minutes) to 250°C at a rate of 10°/minute. The final temperature was kept for five minutes. The calibration curve was linear from the detection limit of the method (0.1 mg/m^3, S/N = 10) up to at least 20 mg/m^3 (8h-TWA).

Determination of urinary creatinine concentrations

Creatinine concentrations were determined on a Cobas Mira autoanalyser by the Jaffé method.[29]

Isolation and determination of ALMA in urine

To 1 ml aliquots of the acidified urine samples 30.0 µl of a solution of 20.0 µM BEMA (1991, 1992) or D_5-PMA (1993) in 0.06 M HCl was added as the internal standard. The urinary mixture was extracted with 4 ml ethyl acetate by an automated shaker during three minutes. The organic phase was separated by centrifugation (three minutes, 800 g), transferred into a test tube, and evaporated to dryness by a gentle stream of nitrogen on a waterbath maintained at 45°C. The residue was methylated at room temperature with 2.0 ml of a 1.25 M solution of HCl gas in methanol as described previously.[26] After 30 minutes, the excess methanolic HCl was evaporated by a gentle stream of nitrogen on a waterbath controlled thermostatically at 45°C and the residue was dissolved in 1.0 ml dichloromethane. This solution was analysed by GC-MS with an HP 5890 series 2 GC (Hewlett Packard, Palo Alto, USA) equipped with a 5971A mass selective detector (ion source temperature 180°C, electron energy 70 eV) and a 7673 autosampler. Splitless injections of 1 µl were made (injector and inlet line temperature 250°C) and chromatographed on a 60 m × 0.22 mm (internal diameter) fused silica DB-1 column (film thickness 0.1 µm) (Durabond) with helium as carrier gas at 170 kPa (about 1 ml/min) and the following temperature programme: 35°C for one minute and then at 10°/minute to 300°C, which was maintained for 7.5 minutes. 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. For the measurement of ALMA the signals at m/z 158 and 217 (ions of ALMA methyl ester) and either 176 and 208 (ions of BEMA methyl ester) or 199 and 258 (ions of D_5-PMA methyl ester) were monitored. Calibration curves were constructed from control urine samples, spiked with synthetic ALMA.

Table 2 Ambient air concentrations of allyl chloride (AC) during shut down periods in an AC production facility

		Personal air monitoring of ambient concentrations of AC†							
		Without respiratory protection				*With respiratory protection‡*			
Year	*n**	*n*	*Range*	*Mean (SD)*	*Median*	*n*	*Range*	*Mean (SD)*	*Median*
1991	46 (39)	33 (26)	<0.1–17	1.5 (3.6)	0.3	13 (13)	<0.1–23	2.2 (5.6)	0.3
1992	45 (34)§	10 (9)	0.2–12	3.3 (4.6)	1.1	16 (13)	0.2–195	18.3 (48.3)	2.7
1993	114 (63)	56 (35)	<0.1–14	1.5 (3.1)	0.1	58 (43)	<0.1–11	1.7 (3.1)	0.16

* n = number of air samples determined (number of people involved); † the detection limit of the method was 0.1 mg AC/m3 (8h-TWA); ‡ ambient concentrations of AC were determined outside of the respiratory protection equipment; § for 19 air samples no report was made on the use of respiratory protection.

Isolation and determination of ALMA and HPMA in urine

ALMA and HPMA were isolated from acidified urine samples as described elsewhere.[16] In short: to an aliquot of 1 ml urine 50 µl of a solution of 50 µM D_3-ALMA was added as the internal standard and the pH was adjusted to 1.2–1.7 by addition of 0.1–0.2 ml 2 N HCl. Organic components were extracted with solid phase extraction columns (RP18, Baker, Philipsburg, USA), which were pretreated with 3 ml methanol and 3 ml water (brought to pH 2 with HCl). The columns were dried by centrifugation (400 g for 10 minutes) and the organic components were subsequently eluted by 3 ml methanol. The methanol fraction was evaporated to dryness and the residue methylated as already described. After evaporation of methanol, the residue was dissolved in 0.5 ml ethyl acetate and 1 µl was used for GC-MS analysis, which was performed on an HP 5890 gas chromatograph equipped with a 5970 MSD (electron impact ionisation, electron energy = 70 eV), a split/splitless injector (used in splitless mode at injection). The oven was programmed from 50°C (one minute) to 250°C at a rate of 20°C/minute. A Cp Sil 5 CB column (length 50 m, internal diameter 0.25 mm, stationary phase thickness 0.2 µm, Chrompack, Bergen op Zoom, The Netherlands) was used. Helium was used as carrier gas (2.5 ml/min). The GC-MS was operated in selected ion monitoring (SIM) mode. Specific ions were measured for ALMA, D_3-ALMA, and HPMA (table 1).

Chemical ionisation GC-MS

To confirm the identity of ALMA, a Finnigan MAT 90 GC-MS system was used. A Cp Sil 19 CB column (50 m x 0.25 mm, film thickness 0.2 µm) was used. The temperature of the injection port and transfer line were 250°C. The oven was programmed from 70°C (0.5 minutes) to 250°C at a rate of 20°C/minute. The final temperature was kept constant for 15 minutes. Helium was used as carrier gas, with a column head pressure of 130 kPa, resulting in a column flow of about 2 ml/min. Positive chemical ionisation was performed with methanol as reagent gas (indicated source pressure 4×10^{-4} torr, source temperature 110°C, ionisation current 0.20 mA, and electron energy of 150 eV), signals were scanned between m/z 190 and 280 with a scan speed of 2.3 scans/s. For the synthetic products of ALMA and HPMA quasimolecular ions, $[M+H]^+$, were seen at m/z 218 and 236, respectively.

CALCULATIONS

From the amounts of AC absorbed on the organic vapour monitors the 8h-TWA air concentrations of AC were calculated for the various workshifts. By plotting the urinary ALMA concentration, adjusted for creatinine excretion, and the 8h-TWA air concentrations of AC versus time, urinary excretion-time profiles were constructed for every worker. From the values before and after the shift of all workers involved, the increase in ALMA concentration during the shift was calculated and plotted against the average respiratory air concentrations of AC to obtain the correlation between air concentration and urinary ALMA excretion.

Results

PERSONAL AIR MONITORING OF POTENTIAL EXPOSURE TO AC

In total 205 workshifts were evaluated (table 2). During 99 workshifts no respiratory protection equipment was used. In 86 of these shifts the air concentrations of AC were below the Dutch 8h-TWA occupational exposure limit (OEL) of 3 mg AC/m^3,[2] but in 13 workshifts the potential exposure to AC exceeded the Dutch 8h-TWA OEL (range 3.3 to 17 mg AC/m^3). The median air concentrations of AC for workers not using personal respiratory protection equipment were found between 0.16 and 2.7 mg AC/m^3 (table 2). In two cases (1992) high air concentrations of AC were measured outside the respiratory equipment.

IDENTIFICATION OF URINARY METABOLITES OF AC

ALMA was identified in ethyl acetate extracts of acidified urine samples of maintenance workers with the aid of chemical ionisation and electron impact GC-MS. With chemical ionisation GC-MS, the urinary excretion of ALMA was identified by a quasimolecular ion at m/z 218 (data not shown), and with electron impact GC-MS by selective ion chromatograms of the ions at m/z 144, 158, and 176 from ALMA and at m/z 147, 158, and 179 from the trideuteroacetyl-analogue of ALMA (fig 1). HPMA was isolated from acidified urine samples by solid phase extraction. Attempts to identify the excretion of HPMA with chemical ionisation GC-MS were unsuccessful. However, the excretion of HPMA was identified in a few urine samples, by SIM-GC-MS (electron impact) of the ions at m/z 144, 158, and 176 (data not shown). The HPMA excretion was not always related to exposure to AC. Due to the relatively low response of HPMA on GC-MS under electron

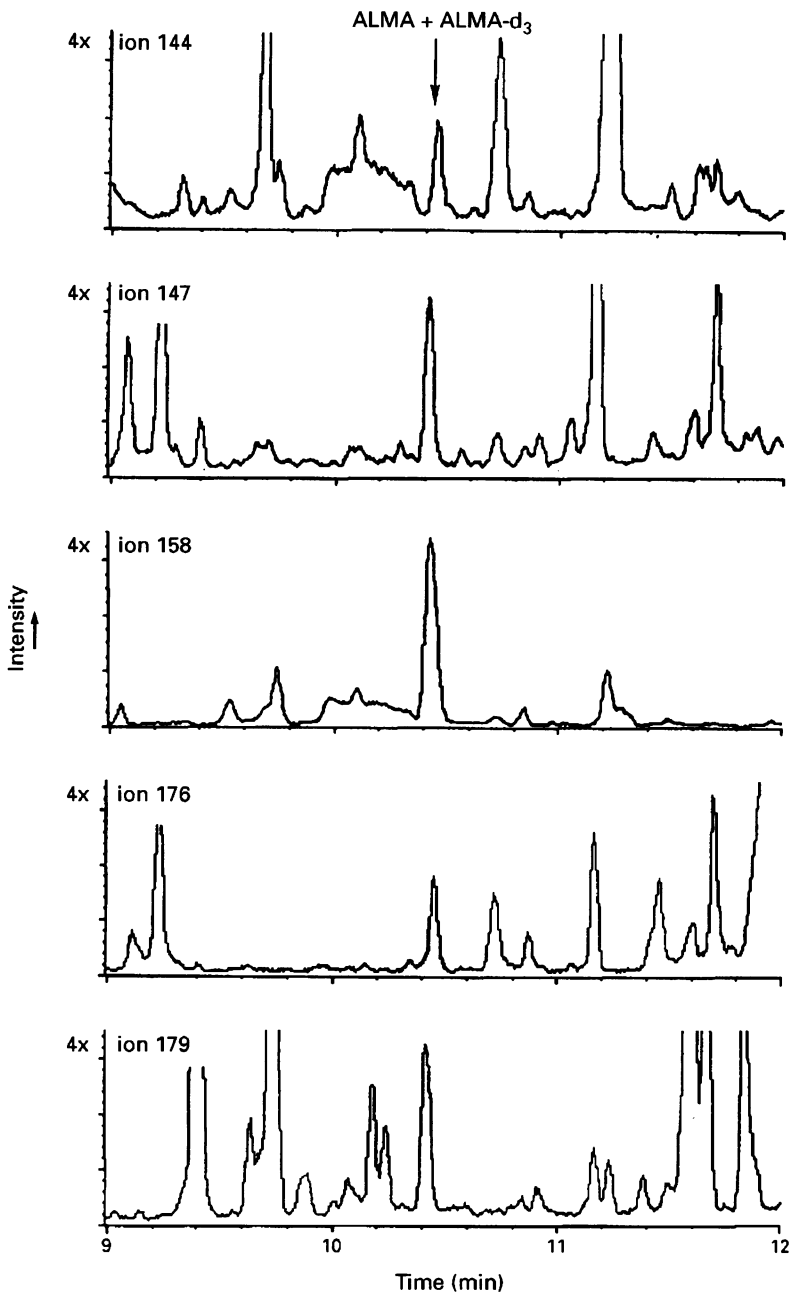

Figure 1 Identification of ALMA in a urine sample collected from worker C (1993), occupationally exposed to ambient 1.4 mg/m³ (8h-TWA) AC. Worker C did not use personal respiratory protection equipment during this workshift. The urinary excretion of ALMA was confirmed by selected ion monitoring GC-MS (electron impact) of the ions at m/z 144, 158, and 176 from ALMA and the ions at m/z 147, 158, and 179 from the trideuteroacetyl-analogue of ALMA.

impact conditions and due to poor reproducibility, we were not able to measure the small amounts of HPMA excreted in urine.

MEASUREMENT OF URINARY ALMA EXCRETION

The recovery of ALMA upon extraction with ethyl acetate from acidified urine samples was invariably ≥90%. Calibration curves constructed with spiked urine samples and determined with SIM-GC-MS (electron impact) were linear ($r > 0.98$) from 25 to 5000 µg/l urine, when the ions at m/z 158 and 176 were used for ALMA and the internal standard (BEMA), respectively. The limit of detection of ALMA in urine was 10 µg/l for identification (signal to noise ratio (S/N) = 3) and 25 µg/l for measurement purposes (S/N = 10). Urinary ALMA concentrations were corrected for differences in urine production by creatinine excretion. The urinary ALMA concentrations found were between the limit of detection (25 µg/l) and 3550 µg/l.

In this report, the shutdown period in 1993 is evaluated in more detail, because full record was made on the lifestyle factors smoking and garlic consumption in 1993. During this shut down period data from 63 workers and 114 workshift periods were collected and evaluated. Table 3 shows data on potential AC exposure, ALMA excretion, and lifestyle factors from a representative part of this population. Twelve of the workers reported the consumption of garlic on a regular basis, whereas 20 and 31 workers were irregular and non-consumers of garlic. Twenty eight workers reported that they smoked. The ALMA excretion before the shift ranged from the detection limit of the assay to 2354 µg/g creatinine (mean (SD), median, 193 (335), 72). The ALMA excretion in samples after the shift ranged from 4 to 2111 µg/g creatinine (mean (SD), median 254 (351), 133). The urinary excretion of ALMA upon the consumption of garlic is evident from the first samples before the shift. The mean (SD), median (range) ALMA excretion in these samples from irregular and non-consumers of garlic was 82.5 (121), 38 (not detectable–546) and 75.4 (90.0), 34 (7–356) µg/g creatinine, respectively. Regular garlic consumers excreted significant (Wilcoxon rank sum test, $P < 0.05$) higher amounts of ALMA in the first samples before the shift: mean (SD), median (range) 411 (687), 70 (20–2354) µg/g creatinine. Garlic consumption could occasionally lead to high ALMA excretion in other samples, as illustrated by the urine samples collected from workers F and H (table 3). Smoking did not influence the urinary excretion of ALMA upon exposure to AC.

For every worker a time course for ALMA excretion in relation to airborne AC was constructed. Figure 2 shows three of them. In workers C and E (fig 2A and B), both non-smokers and not garlic consuming, repeated exposures to airborne AC resulted in increases of the urinary ALMA excretion over the workshifts. In worker E, the occupational hygienist noted dermal exposure during one workshift. The additional dermal exposure resulted in a relatively high urinary excretion of ALMA compared with the air concentrations of AC. Dermal exposure was also identified in the same way, in two more cases (not shown). Worker F (fig 2C), who consumed garlic and smoked, excreted relatively high amounts of ALMA. Garlic consumption may well have contributed to the urinary ALMA excretion in this worker. However, during the first workshift no occupational exposure to AC was evident from personal air monitoring data, and consequently the ALMA excretion in urine decreased during the shift as was expected. During the second shift, however, a respiratory exposure to AC was measured with personal air monitoring and this resulted in an increase in urinary ALMA excretion during this shift. The relatively high ALMA excretion at the begin-

Table 3 Inhalatory potential exposure to allyl chloride (AC) (8h-TWA), lifestyle factors and urinary excretion of ALMA in some of the workers (n = 16) involved in the shutdown period of the AC production factory in 1993*

Worker	Life-style factor		Shift n	AC mg/m³ (8h-TWA)	Respiratory protection	ALMA (µg/g creatinine)	
	Garlic	Smoking				Before shift	After shift
A	−†	−‡	1	0.19	+¶	28	211
			5	0.0§	−	113	60
B	−	−	1	0.26	+	43	91
			2	0.0	+	1246	253
C	−	−	1	2.90	−	7	227
			2	1.60	−	232	295
			3	1.80	−	202	587
			4	1.40	−	36	393
D	−	+	1	7.70	−	20	821
E	−	−	1	0.34	+	13	72
			2	1.80	+	59	1265 ‖
			3	0.26	+	411	396
			4	3.80	+	122	641
F	+	+	1	0.0	?	2354	1124
			2	0.14	?	598	818
G	±	+	1	14.0	−	25	905
H	+	−	1	0.0	?	753	617
I	±	−	1	0.0	+	154	304
			2	0.15	+	82	45
J	+	−	1	0.0	+	73	55
K	−	−	1	4.9	+	51	45
L	+	+	1	0.0	−	28	31
			2	0.0	−	104	70
M	+	−	1	0.31	+	136	132
			7	0.0	−	14	9
N	−	−	1	5.9	−	18	541
			3	2.3	−	104	349
O	−	−	1	1.0	−	153	294
			2	0.0	−	202	151
P	±	+	1	14	−	28	686

* 8h-TWA = eight hour time weighted average; † garlic consumption − = no, ± = irregular, + = regular garlic consumer; ‡ smoking habits − = non-smoker, + = smoker; § the detection limit of the method was 0.1 mg AC/m³ air (8h-TWA); ¶ respiratory protection − = without, + = with, ? = unknown; ‖ = dermal exposure was noted.

ning of the second shift of worker B is enigmatic (table 3). This high excretion was found despite low exposures, and no reported garlic consumption nor potential dermal exposure, although both factors could have contributed to this result.

In 1992, two high air concentrations of AC were measured outside the respiratory protection equipment. These potential exposures did not lead to high ALMA excretion (<352 µg/g creatinine), indicating low internal doses of AC.

Figure 3 shows a correlation between the respiratory 8h-TWA exposures to AC and the values of ALMA in urine samples collected at the end of the shift minus the values before the shift of workers who did not use respiratory protection equipment. In some of the cases, the difference in ALMA excretion between values after and before the shift resulted in a negative increase, because relatively high excretion of ALMA at the beginning of the workshift was noted. Apparently garlic consumption or potential exposures during the preceding day caused this result and therefore these data were not used in the correlation. Only one data point per worker was used in the correlation. However, not all workers are involved due to missing samples or obvious other potential exposures—for example, dermal). The correlation is independent of such lifestyle factors as smoking or garlic consumption. The increases of the urinary ALMA concentrations during the shifts correlated well with the corresponding 8h-TWA air concentrations of AC (y = 27 + 107 x, r = 0.816, P=0.0001, n = 39). From

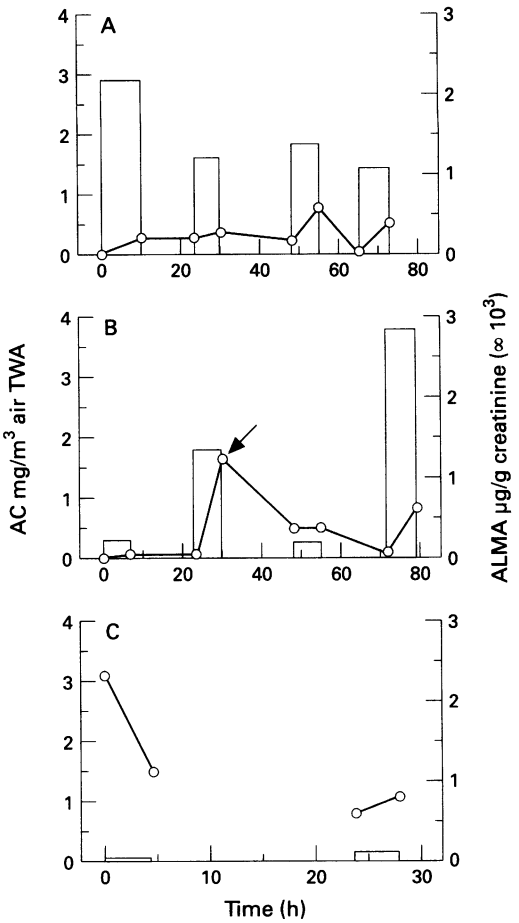

Figure 2 Time courses for urinary ALMA excretion (circles) relative to airborne AC (blocks) in three different workers during one working week. (A) Worker C: a typical example of a clear relation between the workshifts and the urinary excretion of ALMA. (B) Worker E: during the second day that dermal exposure to AC occurred (indicated by arrow). (C) Worker F: relative high urinary concentrations of ALMA possibly resulting from garlic consumption. Despite garlic consumption, the potential exposure to airborne AC still resulted in an increase in urinary ALMA concentrations during the second workshift.

fig 3, it is estimated that exposure to airborne concentrations of AC equal to the OEL (1 ppm or 3 mg/m³ air; 8h-TWA) would lead to a mean urinary ALMA excretion of 352 µg ALMA/g creatinine. The 95% confidence intervals (95% CIs) for the regression line give 242–462 µg ALMA/g creatinine at the level of the OEL. As expected, no relation was found between air concentrations of AC and the increase of ALMA excretion during the workshifts when respiratory protection equipment was used.

Discussion

During three annual shut down periods in an AC production factory this biomonitoring study was undertaken in maintenance workers to elucidate whether urinary mercapturic acid excretion might be used as a biomarker of exposure to AC. During the shut down periods, the production was stopped, installations were cleaned, and maintenance was performed. Depending on the job type, workers wore different kinds of protective clothing and respiratory protective equipment. Potential exposure to AC was determined in breathing zone air by personal air monitoring with monitors for organic vapours. In most cases

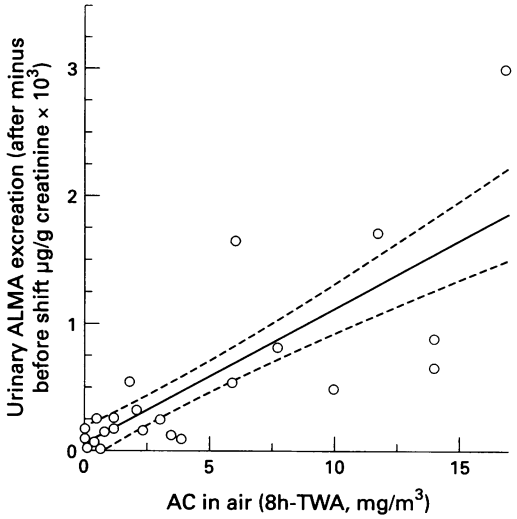

Figure 3 Correlation between 8h-TWA respiratory exposure of maintenance workers to AC and urinary ALMA excretion (value after minus value before the shift). Only workers who did not use any respiratory protection equipment were included (n = 39). The 95% CIs for the regression line are shown by the dotted lines.

where no respiratory protection was applied (n = 86) the potential exposure to AC was below the 8h-TWA Dutch occupational exposure limit (OEL) of 3 mg AC/m³.[2] However, during a limited number of workshifts (n = 13) the potential exposure exceeded the OEL (ranging from 3.3 to 17 mg AC/m³). In other production units where AC is produced or used in the production of epichlorohydrin, comparable or higher air concentrations of AC were reported: 3–350 or 0.016–19 mg AC/m³ or < 0.3–11.2 mg AC/m³ 8h-TWA.[1]

ALMA is a known urinary mercapturic acid metabolite of AC in the rat.[14 15] However, different percentages of the AC dose have been reported to be excreted in urine. In the rat, excretion percentages ranged from 1.7%[14] to 30% of the AC-dose.[16] The route of administration, the dose level, the strain of rats, and analytical aspects may have contributed to these differences. The excretion of ALMA was identified by electron impact and chemical ionisation mass spectrometry, in urine samples of maintenance workers involved in shut down periods in the AC production factory in the present study. The excreted amounts of ALMA as measured with electron impact SIM-GC-MS were in a range from the detection limit (25 µg/l) to 3550 µg/l urine. Corrected for creatinine excretion, the increases in the urinary excretion of ALMA found during the various work shifts correlated well with the measured potential exposure to AC ($r = 0.816$, $P=0.0001$, n = 39; fig 3). In some samples the values of ALMA excretions collected at the end of the shift minus values before the shift were negative due to relatively high excretion of ALMA at the beginning of the workshifts. Apparently garlic consumption or potential exposures during the preceding day caused this result and therefore these data were not used in the correlation. As expected, no relation was found between air concentrations of AC and the increase of ALMA excretion during the workshifts when respiratory protection equipment was used. This indicates that, in general, the protection equipment when applied was effective in preventing internal exposure to AC. With Z- and E-3-chloropropenylmercapturic acid, previously used as biomarkers of human exposure for the nematocides Z- and E-1,3-dichloropropene, similar correlations were found.[25] From the correlation found between the urinary ALMA excretions during the workshifts and the concomitant air concentrations of AC, it was calculated that exposure to air concentrations of AC at the level of the present OEL (3 mg/m³)[2] would lead to a mean urinary ALMA excretion of 352 µg ALMA/g creatinine (95% CIs: 242–462 µg ALMA/g creatinine). Based on this correlation, we propose a biological exposure index (BEI) for human exposure to AC as an increase of 352 µg ALMA/g creatinine during an eight hour workshift.

In the present study some unexpectedly high excretions of ALMA in urine were found. In some cases, it was evident that dermal exposure contributed to the higher urinary excretion of ALMA. However, in other cases of unexpectedly high excretions of ALMA in urine, no clear alternative occupational exposures to AC were found. These cases could also not be related to reported smoking habits. Most likely garlic consumption is a major course of the incidentally high ALMA excretions. The excretion of ALMA after garlic consumption was identified before[17] and in a study with human volunteers, garlic consumption has been shown to lead to urinary ALMA concentrations up to 2000 µg/l urine.[30] In the urine of several maintenance workers occupationally exposed to AC and not consuming garlic, urinary ALMA concentrations up to 3550 µg/l were found. Additionally, regular garlic consumers showed higher ALMA concentrations in the first samples before the shift than did irregular and non-consumers of garlic. Garlic consumption apparently has to be considered as a potential confounder for urinary ALMA excretion. However, despite this potential confounding factor the increases in ALMA concentrations during workshifts correlated well with the potential exposure of the workers to AC. The phenomenon of confounding factors has been well described for biomarkers of exposure. For instance, phenylmercapturic acid, a biomarker for occupational exposure to benzene, was also found to be excreted in a group of smokers and non-smokers not occupationally exposed to benzene.[31] Nevertheless, phenylmercapturic acid was successfully used as a biomarker of occupational exposure to benzene.[26 27] Another example is N-(2-hydroxyethyl) valine in haemoglobin. This protein adduct derived compound has been used as a biomarker of exposure to ethylene oxide and has also been detected in smokers and non-smokers not occupationally exposed to ethylene oxide.[32 33]

For the purpose of risk assessment of human exposure to selected chemicals, information about the internal dose is vital.[34 35] Historical records, environmental monitoring, and biological monitoring are used by occupational hygienists to assess exposures.[35] With historical

records and environmental monitoring the potential exposure of workers to a certain chemical may be identified. By performing biological monitoring, however, information on a cumulative internal dose may be obtained or the effectiveness of respiratory protection may be assessed.[36] Recently, several criteria for biomarkers of exposure were formulated—for example, relation with dose, sensitivity, selectivity, and relation with adverse health effects.[34] In the present study, a clear relation was found between the potential exposure to AC and the biomarker ALMA in urine of workers. The present method of analysing ALMA in urine was sensitive enough to determine the excretion of ALMA upon exposure to AC at or below the 8h-TWA OEL. Although ALMA is apparently a good biomarker for the assessment of the internal dose of AC, the consumption of garlic is a potential confounder. Some other electrophilic chemicals, such as the other allyl halides,[14] allyl nitrate, allyl sulphate, and allyl phosphate,[13] may lead to the urinary excretion of ALMA as well. However, none of these chemicals were used in the AC production factory in which the use of ALMA as biomarker was evaluated. AC is often used in the production of epichlorohydrin.[1] ALMA was not identified as a metabolite of this compound in the urine of rats treated with epichlorohydrin[37] and no influence of potential exposure to epichlorohydrin could be identified on the urinary excretion of ALMA in workers exposed to AC.

By concomitant determination of more urinary metabolites of AC it would, in principle, be possible to construct a metabolite profile. Such a profile may possibly be used to assess the relative contribution of exposure to AC and of other sources of ALMA to the urinary ALMA excretion. To test this hypothesis attempts were made to determine the urinary excretion of HPMA in this study as well. The urinary excretion of α-CH or CHPMA were not selected for this purpose, because epichlorohydrin was used in a neighbouring production factory and the urinary excretion of α-CH and CHPMA was described in rats treated with epichlorohydrin.[37] The excretion of HPMA was identified in traces in urine samples of a few workers. However, in some of these cases there was no clear previous exposure to AC. The excretion of HPMA has also been identified in rats treated with acrolein.[13 15] Acrolein is a known constituent of cigarette smoke, which has been found in the so called main stream[18] and side stream smoke.[19] Therefore, smoking may be a source of urinary HPMA in those cases where no exposure to AC was reported. We were not able to measure urinary HPMA concentrations accurately, due to a relatively low response and poor reproducibility of the analytical method applied.

Conclusions

The mercapturic acid ALMA was unequivocally identified in urine samples of maintenance workers with potential inhalatory exposure to AC, and the analytical method, which was based on SIM-GC-MS, was selective and sensitive enough to measure the urinary excretion of ALMA in workers exposed to ambient air concentrations of AC around and below the current 8h-TWA-OEL of 3 mg AC/m^3.[2] Apart from inhalatory exposure to AC, urinary ALMA excretion may also be used to identify dermal exposure to AC. Garlic consumption, but not smoking, is a potential confounding factor for ALMA as a biomarker of human exposure to AC. The increases in ALMA excretion in urine during workshifts correlated well with the potential exposure of workers to AC. Based on this correlation an increase of 352 μg ALMA/g creatinine during an eight hour workshift is proposed as a biological exposure index (BEI).

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