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AUTOMATIC HEADSPACE GAS CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS DETERMINATION OF TRICHLOROETHYLENE AND METABOLITES IN BLOOD AND URINE

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SUMMARY

Trichloroethylene, trichloroethanol and trichloroacetic acid (TCA) were quantitated in blood and urine by automated headspace gas chromatography using a fused-silica capillary column coated with 3-μm silicone SE-30 and an electron-capture detector. Total trichloroethanol was determined after enzymatic hydrolysis with β-glucuronidase and analysed together with trichloroethylene and TCA as chloroform, which are produced by decarboxylation. Analytical conditions were developed under which the thermal decomposition of TCA was optimal. The automated headspace gas chromatography is rapid and good precision is possible. Sample preparation is simple and the sensitivity of the procedure (0.02 μg/ml) makes it suitable to estimate occupational exposure to trichloroethylene and other halocarbons in humans.

INTRODUCTION

Trichloroethylene (TRI) has been used since 1910 for cleaning and degreasing. Recently a study has shown an highly significant association between exposure to chlorinated solvents and symptoms of psychological dysfunction[1]. As industrial exposure varies considerably a quantitative index of human exposure is of interest.

The major *in vivo* metabolites in blood and urine are trichloroacetic acid (TCA), trichloroethanol (TCE) and the glucuronic acid conjugate of trichloroethanol (TCE-β-glucuronide)[2–4].

Many papers have been published on the measurement of these metabolites. Spectrophotometric determination based on the Fujiwara reaction[5] is the most common method for determination of TCA. However, this reaction has insufficient specificity and sensitivity. Therefore, the spectrophotometric methods have been replaced by gas chromatographic (GC) methods[6–10]. In these methods a widely used procedure

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is solvent extraction of the sample[6,7] in order to obtain a concentration high enough to permit quantitation. These GC methods are elaborate and time consuming.

Analytical methods using headspace gas chromatography (HSGC) have been suggested[9–11]. Headspace analysis is attractive because the procedure is rapid, the column is not contaminated and a rapid quantitation is possible. Thus, the simultaneous determination of TRI, TCE, TCE-β-glucuronide and TCA has been reported[9–11]. In the paper by Breimer *et al.*[10] the volatile TRI and TCE were determined directly by the headspace technique and TCA as its methyl ester. The amount of TCE-β-glucuronide was calculated as the difference between acid-hydrolyzed TCE and free TCE.

The determination of TCA as the volatile chloroform formed by its thermal decarboxylation has been reported by Schoknecht *et al.*[11]. Decarboxylation of TCA makes it possible simultaneously to determine TRI and metabolites in biological materials.

However, the methods so far available are time consuming and difficult to reproduce. Therefore, in this paper we report a sensitive headspace GC method using automated sampling, capillary GC and electron-capture detection for simultaneous determination of TRI, TCE and TCA in blood and urine. The TCE-β-glucuronide in blood and urine was hydrolyzed to TCE by β-glucuronidase. The efficiency of the enzymatic hydrolysis was determined using synthesized TCE-β-glucuronide[12]. TCA was analysed as chloroform formed upon its thermal decarboxylation. The results obtained using this procedure were compared with those obtained using the Fujiwara spectrophotometric method. The method described is very suitable for routine determination of TRI and its metabolites in biological materials from workers exposed to TRI.

EXPERIMENTAL

Reagents and chemicals

TCE and 1,2-propanediol of synthetic quality were obtained from Merck (Darmstadt, F.R.G.) and used without further purification. TCA, TRI and 1-bromo-2-chloroethane, the internal standard, all analytical grade, were from Merck. β-Glucuronidase (*Helix promatia*, Type H-3, activity 109.000 Fischerman units/ml) was from Sigma (St. Louis, MO, U.S.A.). TCE-β-glucuronide was synthesized as described by Køppen *et al.*[12] and stored desiccated.

Preparation of standard and control samples

Individual standard stock solutions containing 3–10 g/l of TCE, TCA and TRI or mixtures of these were prepared in 1,2-propanediol and diluted to concentrations of 3–100, 2–65 and 1–30 μg/ml, respectively.

Working TCE, TCA and TRI standards, 0.2, 0.7, 1.7, 10.0 and 13.0 μg/ml, were made by diluting the above standard solution in blood or urine. These standards were stored at −20°C in 10-ml glass vials sealed with aluminium foil caps provided with a PTFE-lined rubber septum.

Samples

Blood samples from humans exposed to chlorinated solvents were obtained by

venipuncture and collected in a 10-ml glass vial containing heparin. The vials were immediately sealed with an aluminium cap and a PTFE-lined rubber septum. Blood and urine samples were stored at −20°C before analysis.

Gas chromatography

A Carlo Erba 4200 gas chromatograph with a nickel-63 electron-capture detector and Shimatzu electronic integrator was used. Automatic headspace sampling was performed using the Carlo Erba HS 250 sampling unit. To provide a constant incubation time prior to the analysis the headspace sampler was equipped with a sampling pipe containing the headspace vials. It was cooled by air and controlled by the temperature programme of the gas chromatograph. Following injection a headspace vial was dropped into the water bath and the equilibration time was therefore controlled by the number of injections between the sample being analysed and the headspace vial being released from the sampling pipe. In consequence, each vial was automatically equilibrated exactly 90 min at 90°C prior to the headspace analysis.

The syringe temperature was 110°C, syringe cleaning stroke 7.

GC was carried out on a 25 m × 0.31 mm fused-silica capillary column coated with a permanently bonded non-polar SE-30 liquid phase (film thickness 3 μm). The oven temperature programme was as follows: isothermal at 70°C for 1 min, then raised at 20°C/min to 140°C and finally held at 140°C for 2 min. The injector block was maintained at 170°C and injection was performed in the split mode (1:5) and headspace gas volume was injected was 0.5 ml.

The nickel-63 pulsed electron-capture detector (Carlo Erba) was used in the constant-current pulse-modulated mode with a pulse voltage of 50 mV, 0.1-μs pulse and a reference current of 10 mA. Detector temperature: 250°C. Helium was used as the carrier gas at a flow-rate of 2 ml/min and the purge gas was argon–methane (90:10).

Sample treatment

For simultaneous determination of TRI, total TCE and TCA, 0.5 ml of blood or urine were diluted in 0.5 ml of a solution of β-glucuronidase containing 400 U/ml (0.5 *M* acetate buffers, pH 4.5).

The incubation was performed at 37°C for 24 h. A 0.5-ml volume of the enzyme digest was pipetted into a 10-ml glass vial containing 1 ml of saturated diammonium sulphate solution, 0.2 ml of the internal standard 1-bromo-2-chloroethane (25 μg/ml) and 0.2 ml of concentrated sulphuric acid. The glass vial was closed with a PTFE-lined rubber septum and incubated at 90°C for 90 min before HSGC analysis.

TCA was determined in urine samples from workers exposed to organic halides, using the method of Fujiwara[5]. The formation of a gel-like substance in the pyridine layer was disrupted by centrifugation at 500 *g* for 10 min.

RESULTS

Evaluation of the analytical procedure

Fig. 1 shows a typical chromatogram obtained from blood analysis. The calibration graphs for the of TRI, TCE and TCA were linear over the range 0–30 μg/ml, but they did not pass through the origin. The sensitivity of the method is limited by

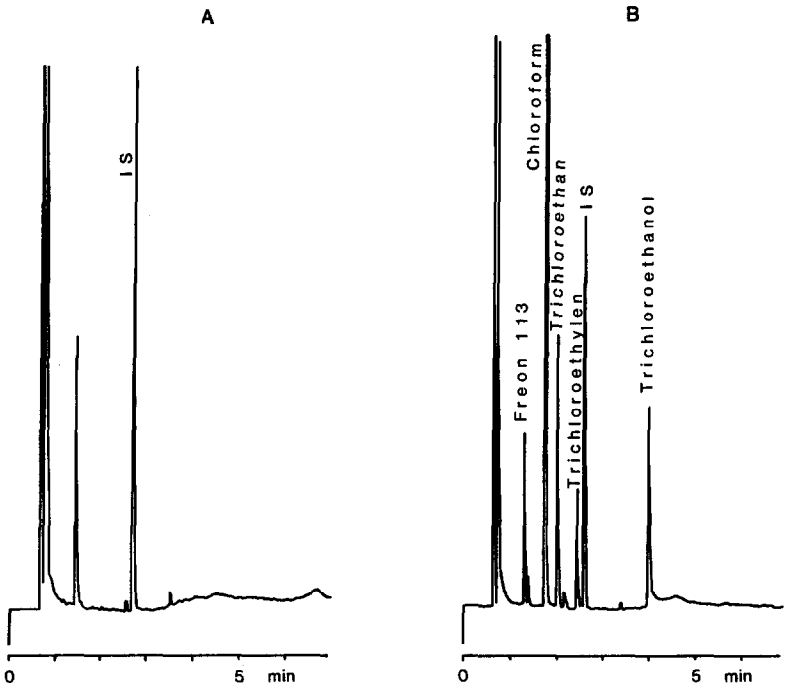

Fig. 1. Typical chromatograms of human blood analysed as described in the text. (A) Normal blood; (B) blood to which Freon, TRI, TCE and TCA were added. IS = Internal standard.

the blank values for urine and blood and the standard deviation of the y-intercept of the calibration curve, and not by the detection limit of the nickel-63 electron-capture detector.

The determination of blank blood and urine samples from eight non-exposed subjects gave values of 0.005 μg/ml. A detection limit (mean ± 3 S.D.) of *ca.* 0.02 μg/ml was thus estimated. The detection limit calculated from the standard deviation of the y-intercepts of eight calibration curves was about 0.01 μg/ml. The precision and accuracy of the method have been investigated within as well as between

TABLE I

BETWEEN-RUN COEFFICIENTS OF VARIATION AND ACCURACY FOR TRICHLOROETHYLENE, TRICHLOROETHANOL AND TRICHLOROACETIC ACID IN HUMAN BLOOD

Compound	*Concentration, mean ± S.D.* (μg/ml)*	*Coefficient of variation (%)*	*Accuracy, mean (%)*
TRI	1.24 ± 0.08	6.5	95
TCE	0.74 ± 0.08	10.8	102
TCA	0.69 ± 0.09	13.0	94

* Mean values from eight duplicate samples at each concentration.

TABLE II

BETWEEN-RUN COEFFICIENTS OF VARIATION AND ACCURACY FOR TRICHLOROETHANOL AND TRICHLOROACETIC ACID IN HUMAN URINE

Compound	*Concentration, mean ± S.D.* (μg/ml)*	*Coefficient of variation (%)*	*Accuracy, mean (%)*
TCE	1.08 ± 0.07	6.5	96
TCA	0.78 ± 0.08	10.3	98

* Mean values from eight duplicate samples at each concentration.

runs, using spiked urine and blood samples over an 8-week period (Tables I and II). Urine samples from workers exposed to chlorinated solvents were analysed using the headspace GC method and the Fujiwara spectrophotometric method (Table III).

DISCUSSION

Several authors have mentioned the thermal decarboxylation of TCA to chloroform[9,13]. The optimum conditions were chosen as heating for 90 min at 90°C in agreement with the findings of Køppen *et al.*[12]. The conversion of TCA into chloroform is reproducible if the incubation time prior to the headspace analysis is 90 ± 5 min. Therefore, it is necessary to modify the automatic sampling unit.

The concentration of chloroform, TRI and TCE in the headspace gas depends on the composition of the blood and urine samples. To improve the precision and sensitivity, saturated ammonium sulphate has been used to increase the ionic strength. The presence of ammonium sulphate increases the response of chloroform, TRI and TCE by a factor of 10–15.

TABLE III

CONCENTRATIONS OF TCE AND TCA (μg/ml) IN URINE FROM INDUSTRIAL WORKERS

Comparison of two methods for analysis of TCA.

Sample No.	*TCE concentration (A) HSGC*	*TCA concentration (B) HSGC*	*TCA concentration (C) Fujiwara*	*Ratio A/B*	*Ratio B/C*
1	4.37	5.03	4.07	0.87	1.23
2	4.93	1.47	2.32	3.35	0.63
3	5.79	1.12	1.54	5.17	0.73
4	0.53	0.64	0.62	0.88	1.03*
5	4.07	2.13	2.34	1.91	0.91*
6	6.05	4.36	3.60	1.39	1.21*
7	19.92	9.31	25.10	2.19	0.37
8	3.26	0.76	1.34	4.29	0.57
9	13.37	24.44	21.16	33.28	1.16*
Mean	6.93	5.47	6.73	0.87	0.87

* Only TRI exposure.

Ziglio *et al.*[14] showed that for chloroform better precision could be achieved by using a magnesium sulphate saturated medium. However, we prefer ammonium sulphate because it enhances the response of the volatiles.

To determine the total TCE in blood and urine the samples must be hydrolysed before analysis. Then conjugated TCE is calculated as the difference between the total and free TCE. Total TCE is usually determined after hydrolysis of the biological matrix using the enzyme β-glucuronidase[6,11], hydrochloric acid or sulphuric acid[10]. Buchet *et al.*[15] compared enzymatic and acidic hydrolysis of the conjugate in urine samples. They found that 12.5 *M* hydrochloric acid at 110°C gives complete hydrolysis in 1 h, whereas β-glucuronidase (pH 4.5, 37°C) incubation with for 20 h was necessary. However, evaluation of the optimum hydrolysis was difficult, because TCE-β-glucuronide was not available.

Køppen *et al.*[12] synthesized TCE-β-glucuronide, and by preparing urine and blood standards of TCE-β-glucuronide we have compared the enzymatic and strong acid hydrolysis. In urine samples only 5% of TCE-β-glucuronide was hydrolysed in the presence of 2 *M* sulphuric acid. The use of β-glucuronidase for 24 h at 37°C at pH 4.5 resulted in complete hydrolysis. Consequently, to measure the total TCE, all the urine and blood samples in our studies were analysed after hydrolysis by the enzyme β-glucuronidase.

The urine TCA results of the photometric method (Table III) were compared with the urine TCA results of the HSGC method. It was found, that the results were equal in urine samples from working places with only TRI exposure. Since the photometric method is non-specific, other compounds possessing trihalogen-substituted carbon atoms being able to undergo the Fujiwara reaction[16], comparison of the results obtained by the two methods is expected to reveal differences.

The results in Table III show that the ratio of TCE to TCA varies. This indicates that the determination of the total trichloro compounds gives a better index of trichloroethylene exposure than does the determination of TCA in urine alone.

The present method has been in constant use in this laboratory in connection with occupational health studies. The sensitivity is adequate and the precision and accuracy good. It may provide a useful guide to more rational and safe evaluation of human exposure to trichloroethylene and other halocarbons.

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