

Methods for measuring trichloroethanol and trichloroacetic acid in blood and urine after exposure to trichloroethylene

by OLOF VESTERBERG, M.D., M.Sc., JADWIGA GORCZAK, M.Sc., and MUDITE KRASTS, M.Sc.¹

VESTERBERG, O., GORCZAK, J. and KRASTS, M. Methods for measuring trichloroethanol and trichloroacetic acid in blood and urine after exposure to trichloroethylene. *Scand. j. work environ. & health* 1 (1975) 243—248. A description is given of procedures for the analysis of trichloroethanol, conjugated trichloroethanol, and trichloroacetic acid (TCA) in blood and urine. The determination of TCA is effected by measuring its decarboxylation product chloroform. The methods depend on extraction with isooctane and subsequent analysis by gas chromatography.

Key words: gas chromatography, blood, urine, trichloroacetic acid, trichloroethanol, trichloroethylene exposure.

Trichloroethylene (TRI) is an organic solvent of extremely wide use, and one to which many workers are frequently exposed in varying intensities. Acute intoxication occurs frequently, but mainly from sniffing (7).

The metabolism, or biotransformation, of the substance has been studied (2, 5, 6, 8, 11). It seems that trichloroacetaldehyde (chloral) and chloralhydrate form rapidly (8). Oxidation yields trichloroacetic acid (TCA) (11), whereas reduction, probably mediated by the enzyme alcoholdehydrogenase, gives trichloroethanol (TCE) (8). TCE is then conjugated with glucuronic acid (TCE-G) before it is excreted in the urine (5). The compounds TCA and TCE-G are eliminated in the urine at different rates (14, 16).

Currently, the analysis of TCA is widely used as an index of TRI exposure; however, varying opinions have been expressed as to the level which corresponds to safe work conditions during exposure of this type (1, 3). Increasing popularity for this purpose has been gained by TCE or total trichlorocompounds, i.e., TCA plus TCE (10, 12).

Interpretation of the results of the analysis of TCA and TCE in urine is by no means simple, whereas it could be expected that the measurement of the concentrations of these compounds in blood would exclude some of the problems, such as the influence of kidney function. Although analyses of TCA and TCE in blood have earlier been effected (4, 9, 15), improvements and simplifications of the methods seemed desirable. The fact that TCA can produce chloroform (CHCl_3) (9) was taken as the basis for our method of measuring TCA in blood and urine. Although enzymes have been used for the hydrolyzation of the glucuronide of TCE, the methods involved call for long periods of incubation and may yield inconsistent amounts of free TCE, partly because of the probable presence of inhibitors such

¹ Chemical Division, Occupational Health Department, The National Board of Occupational Safety and Health, Stockholm, Sweden.

Reprint requests to: Dr. Olof Vesterberg, National Board of Occupational Safety and Health, Department of Occupational Health, Chemical Division, Fack, S-100 26 Stockholm, Sweden.

as glucaric acid in variable concentrations in urine (13). The enzyme methods were abandoned in this instance, and choice was made of the acid hydrolysis of glucuronide, principally in accordance with the method of Šedivec and Flek (17).

MATERIALS AND METHODS

The main principle for the analysis of TCA and TCE is dependent upon extraction into isooctane. The measurement of TCA is further dependent upon the decarboxylation of TCA to chloroform according to the method of Müller et al. (14) and with the modifications outlined later in this section. Isooctane was used not only for extraction but also for the elimination of water, which is deleterious to the electron-capture (EC) detector.

The aim of the gas chromatographic analysis was that of finding a stationary phase at which TCE and chloroform could be measured and ethanol, acetone, and trichloroethylene, along with isooctane, could be separated. The retention time was set to ensure that it would not be too short (because of the possible increase in the risk of false peaks) or too long (too long a time for each analysis would be impractical). Several columns were tried, and the two best were 20 % Didecylphthalate on Chromosorb W, DMCS 100—120 m, length 400 cm, and 15 % SE 30 on Chromosorb W, A.W. 100—120 m, length 210 cm.

Apparatus

The gas chromatograph employed was a Perkin—Elmer model F-11 equipped with an EC detector unit. The radioactive source for the chromatograph was ^{63}Ni 1 mCi. Its stainless steel column had an inner diameter of 1.9 mm, was 210 cm long, and was packed with 15 % SE 30 on Chromosorb W, A.W. 100—120 m. For the analysis of chloroform the temperatures for the inlet, column, and detector were 150°, 80°, and 200° C, respectively; for the analysis of TCE the corresponding temperatures were 175°, 125°, and 200° C, respectively. The nitrogen flow was 30 ml/min.

A 10 mV Servogor graphic recorder was used.

Reagents

The following reagents were used: trichloroethanol for synthesis (Merck—Schuchardt); trichloroacetic acid, p.a. (Merck, Darmstadt); isooctane, certified A.C.S. (Fisher Scientific Company); sulfuric acid, 98.08 % analytical reagent (Analar, Poole, England); and acetone, p.a. (Merck, Darmstadt).

The ACD² solution was a water solution of 4.8 g of citric acid, 13.2 g of sodium-citrate, and 14.7 g of glucose per 1,000 ml. This solution was used as an anticoagulant and as a preservative for blood.

Standards

Bottles for standards. Blood samples and standards in isooctane were stored in glass bottles (25 ml); the screw caps were lined with rubber gaskets and aluminum laminate (polyester-Al-polyethylene, polyester inside) or other inert foil to prevent contact between the organic gas phase and the rubber.

Standards in isooctane. These solutions were used for calibrating the detector response at least once every alternate hour.

Standards of TCE in isooctane. For the analysis of TCE, standards were prepared by adding TCE in μl amounts to isooctane (10 ml). These standards could be stored for several months at room temperature in the previously described glass bottles.

Standards of TCA in isooctane. For the analysis of TCA an estimate was made of its decarboxylation product, chloroform. Stock solutions were made with 500 $\mu\text{g/l}$ of chloroform in isooctane, after which suitable concentrations were prepared by dilution. The standard solutions could be stored for several weeks at room temperature.

Blood and blood preparations. In view of the difficulties involved in acquiring a continuous supply of fresh blood, blood from 4 to 6 weeks old, and supplied by a blood bank, was utilized for the preparation of standards, and for the determination of suitable conditions for the analyti-

² ACD refers to acidum citricum, dextrose.

cal procedures. The blood consisted of a mixture of four parts human blood and one part ACD solution. In the blood analysis glass test tubes with glass stoppers were used. For each analysis 0.5 ml of the blood standard was used. In the analysis of original samples, 0.10 ml of the ACD solution was added, and the tubes were weighed. Seven drops (approximately 0.4 ml) of artery or capillary blood were then added and mixed in immediately, after which the tubes were reweighed.

Standards of TCA in blood. Known amounts of TCA were dissolved in 10.0 ml of water and injected with a microsyringe into 20.0 ml of ACD blood. After the addition of the standard substances, the vessels were rolled for 20 min for equilibration. The blood standards were stored at $+4^{\circ}\text{C}$. The period of storage was limited by blood coagulation, which occurred after about 1 or 2 weeks.

Standards of TCE in blood. A stock solution was made of TCE in ethanol (99 %) with a content of $0.50\text{ }\mu\text{l/ml}$. Micro-liter amounts of the stock solution were injected into 20.0 ml of ACD blood, which was rolled and stored as was previously described.

Standards of TCA and TCE in urine. Standards for TCA and TCE in urine were prepared and stored in basically the same way as the blood standards.

Estimation of TCA in blood

The decarboxylation of TCA was effected after the addition of an ethanol-acetone (1 : 7 v/v) mixture to the sample to which 1.0 ml of isooctane had already been added for the estimation of free TCE. The solutions were mixed with a Whirlimixer, and then kept at room temperature for 26 h. Of the isooctane phase, $1.0\text{ }\mu\text{l}$ was injected into the gas chromatograph and analyzed for chloroform. Analysis is also practicable during a more extended period, but it is essential that the reagent solution be added to both the sample and the blood standard to ensure equal reaction times. Fig. 1 illustrates the influence of different amounts of the ethanol-acetone solution, along with the reaction time required to reach equilibrium. As a plateau was obtained after 26 h with

2.5 ml of the ethanol-acetone solution added to 0.50 ml of ACD blood, these conditions were adhered to for the subsequent analysis of samples. As a consequence of the diluting effect, larger amounts of ethanol-acetone were not taken into account.

Estimation of free TCE in blood

The estimation of the glucuronide of TCE had to be done in a separate sample, as the difference between total TCE and free TCE. With regard to original blood samples, the analysis of free TCE and TCA could be performed with the same blood sample. Free TCE was estimated first by the addition of 1.0 ml of isooctane to 0.5 ml of the ACD blood mixture. (For original samples about 0.4 ml of blood was used; in each case the exact amount was determined by weighing.) After vigorous mixing for 30 s with a Whirlimixer, the test tubes were allowed to stand for some minutes so that the phases could separate. Centrifugation was sometimes necessary. When the upper phase was clear, samples of $1.0\text{ }\mu\text{l}$ were drawn from it for injection into the gas chromatograph.

Estimation of total TCE in blood

An amount of 0.25 ml of concentrated sulfuric acid was added to 0.50 ml of the

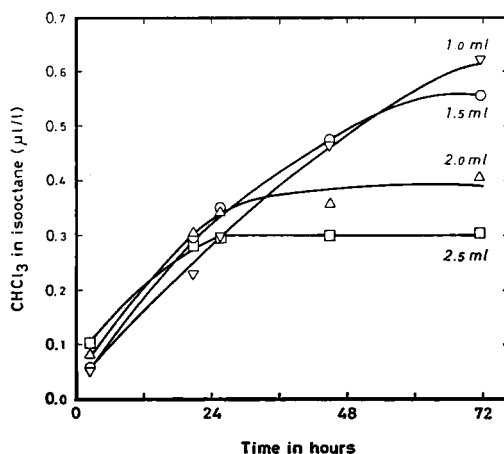


Fig. 1. The rate of the decarboxylation of trichloroacetic acid (TCA) in blood when varying amounts of the reagent solution and $5\text{ }\mu\text{g}$ of TCA per ml of blood were used. Chloroform (CHCl_3) was extracted with 0.5 ml of isooctane.

blood sample (containing 0.1 ml of the ACD solution) for the hydrolysis of TCE glucuronide, which was accomplished by placing the glass tubes with stoppers in a boiling water bath for 30 min. [According to Sedivec and Flek (17) the optimal conditions for the hydrolysis of TCE glucuronide are 6–7.5 M of H_2SO_4 and 30 min of heating at 100° C.] After the hydrolyzed sample had cooled, 2.0 ml of isooctane was added and mixed in, and 1.0 μ l of the isooctane phase was injected into the gas chromatograph. Calculations and corrections were made as described in the results section.

Estimation of TCA and TCE in urine

TCA and TCE in urine were analyzed in basically the same way as that employed for the blood analyses. Volumes of urine were measured by pipetting 0.50 ml for both the standards and the original samples. When high concentrations of TCA and TCE were expected, the urine samples and the standards were diluted with water. If the isooctane phase is diluted, it is recommended that the standards contain a level comparable to that of the substances to be analyzed.

RESULTS

The determination of TCE in the isooctane phase, in relation to the standard of TCE in isooctane, was applied in calculating the concentration of free TCE in blood. The partition coefficient of TCE between isooctane and blood was estimated as follows. Analyses were performed of a series of TCE concentrations, 0.2, 0.5, 1.0 and 2.0 nl/ml, in blood from different persons. The coefficients of variation for the results were 5.4, 1.9, 2.2, and 2.1 %, respectively, for seven analyses at each level of concentration. Regression analysis gave the equation $Y = 0.00008 + 0.150 X$, in which X was the concentration of TCE added to the blood and Y was the concentration of TCE found in the isooctane phase. The results corresponded to a partition coefficient of 0.214, with a coefficient of variation of 2.9 %. As varying volumes of blood had been sampled, detailed consideration had to be given to the parti-

tion coefficient for TCE between blood and isooctane. This was done by the determination of the recovery of TCE in the isooctane phase at different concentrations of TCE (range 0.2–2.0 nl TCE/ml of blood) as a function of varying amounts of ACD blood. Within the range 0.3–0.6 ml of ACD blood, the variation in the partition coefficient was so small that it could be ignored. Other volumes produced more variation, the influence of which should be regarded closely.

The possible influence of variable temperature upon the partition coefficient was also taken into account; in this study the temperature was between 18° and 25° C. The coefficient of variation obtained provided an indication that the influence of temperature was quite small for the range of temperatures applied. At a density of 1.06 the amount of the standard blood used, 0.50 ml, was equivalent to 0.42 g of whole blood. When samples of seven drops of blood were utilized, for practical reasons, the variation in the amount was between 0.35 and 0.55 g. This variability made it necessary for the amount of blood in each sample to be determined by weighing. The concentration of TCE in blood was then derived with the following equation:

$$C_i \cdot V_i + C_b \cdot V_b = C_{wb} \cdot V_{wb},$$

where C_i = concentration of TCE in isooctane, nl/ml (determined gas chromatographically), C_b = concentration of TCE in ACD blood, nl/ml, C_{wb} = concentration of TCE in whole blood, nl/ml, V_i = volume of isooctane = 1.0 ml, V_b = volume of ACD blood in ml, V_{wb} = volume of whole blood in ml.

If the partition coefficient is $C_i/C_b = 0.214$ and g_{wb} = the weight of the blood sample with a density of 1.06, the preceding equation can be written as:

$$C_i + C_i (g_{wb}/1.06 + 0.1)/0.214 = C_{wb} \cdot g_{wb}/1.06.$$

If the result is to be expressed in μ g TCE/ml, the value of the density for TCE, which is 1.55, must be introduced and the formula becomes:

$$C_{wb} = C_i \left[\frac{2.412}{g_{wb}} + 7.243 \right] \mu\text{g/ml}.$$

As experimental exposure meant that the amount of TCE glucuronide was very low in some samples (Vesterberg et al., to be published), it was assumed that C_{wb} corresponded to total TCE in blood. However, in a study of some occupationally exposed workers the proportion of TCE-G was found to be rather high in some cases.

If the concentration of TCE-G in the blood is high, a decision must be made as to whether interest is attached to its analysis. From a toxicological standpoint it has been claimed that free TCE is of significance, whereas TCE-G is probably of much less importance (14). The possible influence of TCE-G upon the analysis of free TCE was not investigated.

Fig. 2 illustrates the recovery, by extraction, of added TCE in the analysis of TCE in blood, urine, and water. As recovery from blood was found to be lower than that from water or urine, water cannot be used for the preparation of standards intended for the evaluation of blood samples.

The concentration of TCA in blood was read from a standard curve prepared from ACD blood with known additions of TCA; the standard was treated in the same way was the blood samples, and the result was corrected for the amount of blood obtained by weighing and by applying the factor 1.25 for dilution with the ACD solution. The reproducibility was satisfactory (fig. 3).

The finally adopted procedure, utilizing 0.4 ml of blood and 1.0 ml of isooctane with the injection of 1 μ l of solvent, had a detection limit of 0.03 μ g per ml of blood for TCE. The detection limit was 0.2 μ g/ml for TCA with the same amounts of blood and isooctane and with 2.5 ml of the ethanol-acetone solution (1:7 v/v) with the injection of 1 μ l of the isooctane phase. The retention time was 1.5 min for both chloroform and TCE.

DISCUSSION

The head-space procedure, which is known to be very sensitive for some volatile organic substances, seems to be achieving increased popularity. Those who have applied this procedure for TCE and TCA include Breimer et al. (4). In our attempt to employ this method for the analysis

of TCE, however, the results displayed great variability and a low degree of reproducibility, perhaps attributable to different compositions of lipids and lipoprotein in different blood samples, which might give TCE variable degrees of

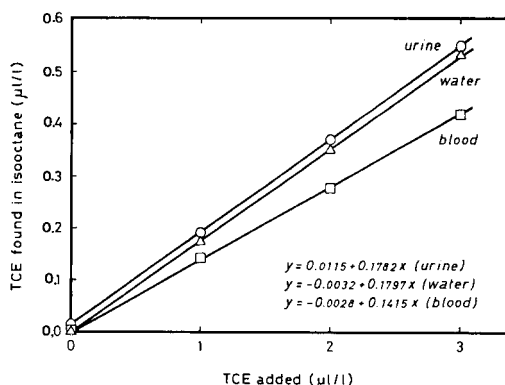


Fig. 2. The recovery of trichloroethanol (TCE) upon extraction with isooctane as a function of TCE added to 0.5 ml of urine, water, and blood.

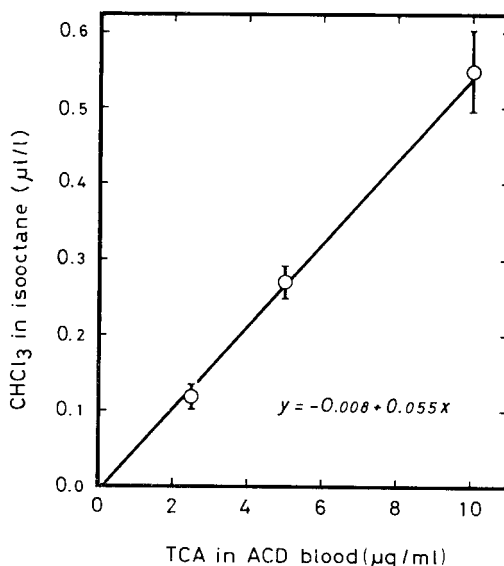


Fig. 3. The concentration of chloroform (CHCl_3) in the isooctane phase as a function of the concentration of trichloroacetic acid (TCA) in blood containing the ACD solution. Analyses for some of the concentration levels were made on different days with blood from different persons. For the levels of 2.5, 5.0, and 10.0 μ g/ml of added TCA, with 10 determinations at each level, the corresponding CHCl_3 concentrations in μ l/l isooctane were 0.12, 0.27, and 0.55, the coefficients of variation being 10.0, 6.4, and 10.0 % respectively.

affinity for blood. Extraction with a solvent such as isooctane could be expected to have several advantages. First solvent extraction allows TCE and TCA to be determined in the same sample. Second, as the extracts can be stored, it is not imperative that analyses be made directly after the blood samples have been drawn. Third water, which is deleterious to the EC detector, is almost eliminated from the injected sample.

As far as TCA is concerned, it seems difficult to design a reliable head-space procedure without esterification. The head-space procedure seems to give a low sensitivity for TCA; moreover esterification has some drawbacks from a general point of view. Since the analysis procedures described in this paper for TCE and TCA have high degrees of sensitivity at levels that indicate safety for occupationally exposed workers, the volume of the blood samples can be reduced.

ACKNOWLEDGMENTS

Thanks are due to Prof. G. Lindstedt for his critical comments of the manuscript, and to Ms. P. Boston for her review of the English text.

REFERENCES

- ANDERSSON, A. Gesundheitliche Gefahren in der Industrie bei Exposition für Trichloräthylen. *Acta med. scand.* 157 (1957): suppl. 323, T 47.
- BARDODEJ, Z. and VYSKOCIL, J. The problem of trichloroethylene in occupational medicine. *Arch. ind. health* 13 (1956) 581—592.
- BOILLAT, M.-A. L'intérêt de la détermination de l'acide trichloroacétique dans les urines pour le dépistage des intoxications par le trichloréthylène et le perchloréthylène: Etude de quelques entreprises. *Z. Präventivmed.* 15 (1970) 447—456.
- BREIMER, D. D., KETELAARS, H. C. J. and ROSSUM, J. M. VAN. Gas chromatographic determination of chloral hydrate trichloroethanol and trichloroacetic acid in blood and in urine employing head-space analysis. *J. chromatogr.* 88 (1974) 55—63.
- BUTLER, T. C. Metabolic transformations of trichloroethylene. *J. pharmacol.* (Kyoto) 97 (1949) 84—92.
- BYINGTON, K. and LEIBMAN, K. Metabolism of trichloroethylene in liver microsomes: II. Identification of the reaction product as chloral hydrate. *Mol. pharmacol.* 1 (1965) 247—254.
- EDH, M., SELERUD, A. and SJÖBERG, C. Deaths and sniffing: A report on 63 cases. *Läkartidningen* 70 (1973) 3949—3959.
- FRIEDMAN, P. and COOPER, J. The role of alcohol dehydrogenase in the metabolism of chloral hydrate. *J. pharmacol.* (Kyoto) 129 (1960) 373—376.
- GARRETT, E. R. and LAMBERT, H. J. Gas chromatographic analysis of trichloroethanol, chloral hydrate, trichloroacetic acid, and trichloroethanol glucuronide. *J. pharm. sci.* 55 (1966) 812—817.
- IKEDA, M., OHTSUJU, H., IMAMURA, T. and KOMOIKE, Y. Urinary excretion of total trichloro-compounds, trichloroethanol and trichloroacetic acid as a measure of exposure to trichloroethylene and tetrachloroethylene. *Br. j. ind. med.* 29 (1972) 328—333.
- LEIBMAN, K. and MC ALLISTER, W. Metabolism of trichloroethylene in liver microsomes: III. Induction of the enzymic activity and its effect on excretion of metabolites. *J. pharmacol. exp. therap.* 157 (1967) 574—580.
- LOWRY, L., VANDERVORT, R. and POLAKOFF, P. Biological indicators of occupational exposure to trichloroethylene. *J. occup. med.* 16 (1974) 98—101.
- MARSH, C. A. Metabolism of D-glucuronolactone in mammalian systems. *Biochem. j.* 86 (1963) 77—86.
- MÜLLER, G., SPASSOVSKI, M. and HENSCHLER, D. Trichloroethylene exposure and trichloroethylene metabolites in urine and blood. *Arch. toxicol.* (Berl.) 29 (1972) 335—340.
- OGATA, M. and SAEKI, T. Measurement of chloral hydrate, trichloroethanol, trichloroacetic acid and monochloroacetic acid in the serum and the urine by gas chromatography. *Int. Arch. Arbeitsmed.* 33 (1974) 49—58.
- OGATA, M., TAKATSUKA, Y. and TOMOKUNI, K. Excretion of organic chlorine compounds in the urine of persons exposed to vapours of trichloroethylene and tetrachloroethylene. *Br. ind. med.* 28 (1971) 386—391.
- ŠEDIVÉČ, V. and FLEK, J. Bestimmung toxischer Substanzen und ihrer Metaboliten in biologischen flüssigkeiten Mittels der Gaschromatographie I. Trichloroäthanol im Urin. *Collection Czechoslov. Chem. commun.* 34 (1969) 1533—1542.

Received for publication: 1975-06-03