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Gas Chromatographic Analysis of Trichloroethanol, Chloral Hydrate, Trichloroacetic Acid, and Trichloroethanol Glucuronide

By EDWARD R. GARRETT and HOWARD J. LAMBERT*

A sensitive and specific assay has been developed for trichloroethanol, chloral hydrate, trichloroacetic acid, and trichloroethanol glucuronide utilizing gas chromatography and electron capture detection. Trichloroethanol and chloral hydrate are extracted from alkalinized water or urine with ether, and a portion of the ether extract is assayed with chlorobutanol and chloroform as internal standards. Trichloroacetic acid and trichloroethanol glucuronide are not extracted under these conditions. The trichloroacetic acid is decarboxylated as the potassium salt and the resultant chloroform extracted into ether and assayed. The trichloroethanol glucuronide is enzymatically hydrolyzed and the hydrolysate assayed for the trichloroethanol formed. Sensitivities obtained were 0.5 mcg. of trichloroethanol and chloral hydrate (3.3 and 3.02 mμmoles, 10^{-9} moles, respectively), 1.0 mcg. of trichloroacetic acid (6.1 mμmoles) in 2.0 ml. of sample and 0.5 mcg. of trichloroethanol glucuronide (1.54 mμmoles) in 3.0 ml. of sample. Statistical analysis of assays on urinary mixtures of the four compounds demonstrates the reliability of the method.

THERE ARE no sensitive specific assays for the hypnotic drug trichloroethanol (TCE), its precursor chloral hydrate (CH), or its detoxification products trichloroacetic acid (TCA) and trichloroethanol glucuronide (TCE-G), in the available literature.

Extant methods (1–3) are mostly modifications of the colorimetric method of Fujiwara (4) and were not sufficiently sensitive or specific for a planned pharmacokinetic study of TCE in this laboratory. Friedman and Cooper (2) obtained a sensitivity of 20 mμmoles of TCA and CH and 100 mμmoles of TCE in mixtures but found that some tissue preparations interfered with chromophore production as much as 25% and lessened the reliability of the Fujiwara procedure. Leibman and Hindman (3) improved the assay sensitivity down to 5 mμmoles of TCA and CH and 15 mμmoles of TCE, but separation of the components prior to reaction would be required as the procedure was nonspecific. A gas chromatographic separation of CH, TCA, and chlorobutanol, but not TCE, with a sensitivity of 306, 302, and 281 mμmoles, respectively, has also been reported (5). This sensitivity of detection is not as good as the modified Fujiwara (2, 3).

The purpose of this investigation was to develop a specific procedure for the quantitative determination of TCE and its metabolites separately and in mixtures with gas-liquid chromatography and the electron capture detector. If possible, this method would have greater sensitivity than those previously reported.

EXPERIMENTAL

Materials.—The TCE (Calbiochem, Inc.) used in the preparation of calibration curves and synthetic mixtures in water and urine was redistilled at 152° before use.

Chloral hydrate U.S.P., chlorobutanol U.S.P., and TCA (reagent grade and 5% solution) were obtained from the Fisher Scientific Co. Ether (Baker) was redistilled over sodium before use. The purity of these components was verified chromatographically, and only one peak was observed.

The sodium salt of trichloroethanol glucuronide

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(hereafter referred to as TCE-G) was isolated from rabbit urine by the method of Seto and Schultze (6).

Assay solutions of TCA were prepared by diluting the 5% stock solution and the final concentration verified by titration. TCE, CH, and TCE-G were weighed to within 0.1 mg., transferred to a clean 100-ml. volumetric flask, and brought to volume with water or urine.

Apparatus.—An F&M model 700 gas chromatograph with either a flame ionization or electron capture detector and equipped with a Minneapolis Honeywell recorder and disc integrator was used.

Column.—The column for calibration curve determination and studies with mixtures was an 8 ft. × 1/4 in. stainless steel tube containing 20% Carbowax 20 M on 60–80 mesh Chromosorb W. Other columns investigated but not chosen due to incomplete peak resolution or tailing were a 4 ft. × 1/4 in. stainless steel, 10% diethylene glycol succinate; a 4 ft. × 1/4 in. glass, 5% Carbowax 20 M and a 4 ft. × 1/4 in. glass, 5% Carbowax 20 M plus 2% H_3PO_4, all on 80–100 Diatoport S.

Flame Ionization Detector.—The temperatures used were: injection port, 120°; column, 100°; and detector, 290°. The flow rate of the helium carrier gas was 60 ml./min. Hydrogen and air flow rates 60 and 500 ml./min. (20 p.s.i.), respectively.

Electron Capture Detector.—The optimum temperatures were: injection port, 160°; column, 125°; and detector, 190°. Carrier gas (helium) flow rate was 60 ml./min. while the purge gas (90% argon–10% methane) was fixed at 140 ml./min.

Sample Assay

Trichloroethanol and Chloral Hydrate.—Five-microliter aliquots of aqueous solutions of TCE and CH were injected directly on-column when the flame ionization detector was used. For electron capture detection, 2 ml. of the solution was shaken with 2 ml. of diethyl ether in a 10-ml. rubber-sealed injectable vial and 5 μl. of the ether extract was assayed. The ether contained 1.6 mg./100 ml. of chlorobutanol and 0.004 mg./100 ml. of chloroform as internal standards.

Trichloroethanol Glucuronide.—One milliliter of water or urine containing TCE-G was added to 1.0 ml. of pH 4.5 acetate buffer (ionic strength = 0.1 *M*) containing 5 mg. of β-glucuronidase (beef liver, 360 Fishman units/mg., Calbiochem). The mixture was sealed and allowed to react at 37.5° overnight. Two milliliters of diethyl ether (plus chlorobutanol) was then added, the mixture was shaken, and a 5-μl. aliquot of the ether extract assayed for TCE using the electron capture detector. No analysis was run on TCE-G using flame ionization.

To determine the purity of the TCE-G isolated, 1 ml. of a 5.14 mg./100 ml. solution was hydrolyzed overnight at 80° with 1 ml. of concentrated hydrochloric acid. The TCE formed was extracted into 2 ml. of ether, a 5-μl. aliquot of the ether extract assayed, and the assay value compared with the theoretical yield. The stated enzyme concentration was optimum in that it gave results equivalent to acid hydrolysis after overnight reaction at pH 4.5, 37°.

The identity of the glycone obtained by enzymatic hydrolysis was verified by thin-layer chromatography. Twenty microliters of the hydrolysate were spotted on a Silica Gel GF_{254} (Brinkmann Instruments) plate of 0.4-mm. thickness along with reference spots of D-glucuronic acid, D-glucuronolactone, TCE, and TCE-G. The plates were then developed in 1-butanol–acetic acid–water (4:5:1) up to approximately 10 cm. The plates were then dried, sprayed with concentrated sulfuric acid, and heated until carbonization was complete. The hydrolysate was resolved into two spots with R_f values corresponding to D-glucuronic acid (0.50) and a small amount of unhydrolyzed TCE-G (0.81). No D-glucuronolactone (R_f = 0.71) was found. The volatility of TCE prohibited its visualization by this method.

Trichloroacetic Acid.—A chromatographic peak for TCA could not be resolved with the authors' columns. Alkaline decarboxylation produced chloroform which was extracted with ether and assayed with the electron capture detector. One milliliter of 0.1 *M* KOH was added to 1 ml. of water or urine containing TCA in a 10-ml. injectable vial. Two milliliters of diethyl ether (no internal standards present) was then added, the vial was sealed and heated at 100° for exactly 30 min. The vial was then cooled in an ice bath, allowed to reach room

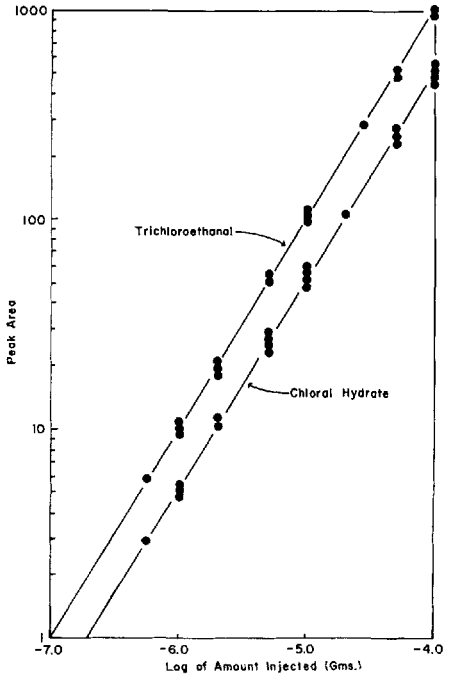

Fig. 1.—Calibration curves for 4-μl. aliquots of aqueous trichloroethanol and chloral hydrate solutions assayed with the flame ionization detector.

TABLE I.—DISTRIBUTION COEFFICIENTS FOR THREE NONPOLAR SOLVENTS AND AQUEOUS SOLUTIONS OF CHLORAL HYDRATE AND TRICHLOROETHANOL

	Pentane/H_2O	Benzene/H_2O	Diethyl Ether/H_2O
CH	0.003	0.03	0.191
TCE	0.34	3.5	22

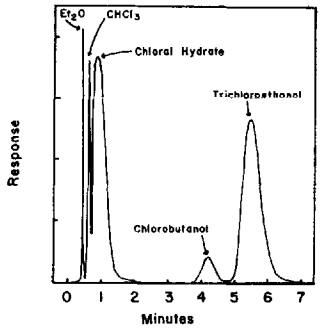

Fig. 2.—A typical chromatogram for a 5-μl. aliquot of a 1:1 ether extract of a solution of chloral hydrate and trichloroethanol (1.40 and 1.36 mg./100 ml., respectively). Pulse = 15. Sensitivity: 0.001 of maximum.

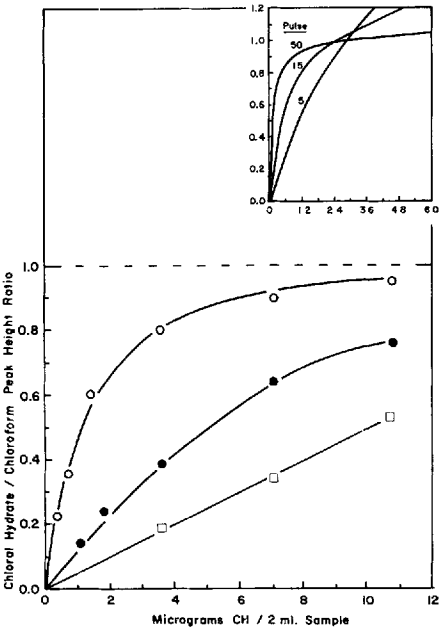

Fig. 3.—Calibration curves for chloral hydrate at three pulse intervals. Sensitivity: 0.001 of maximum. Insert shows the calibration curves for amounts of chloral hydrate up to 60 mcg./2 ml. sample. One microgram = 6.04 mμmoles. Key: ○, pulse = 50; ●, pulse = 15; □, pulse = 5.

temperature, and 5 μl. of the ether extract assayed, using the electron capture detector for the chloroform formed. No analysis was run for TCA using flame ionization.

Mixtures.—A 10-ml. sample of urine containing TCE, CH, TCA, and TCE-G was brought to pH 7.0 by dropwise addition of concentrated KOH. The volume change was negligible. Two milliliters of this urine was removed and assayed for TCE and CH. A 2.0-ml. sample was then extracted with six 10-ml. portions of ether to remove the TCE and CH, divided in half, and the 1-ml. portions assayed for TCA and TCE-G.

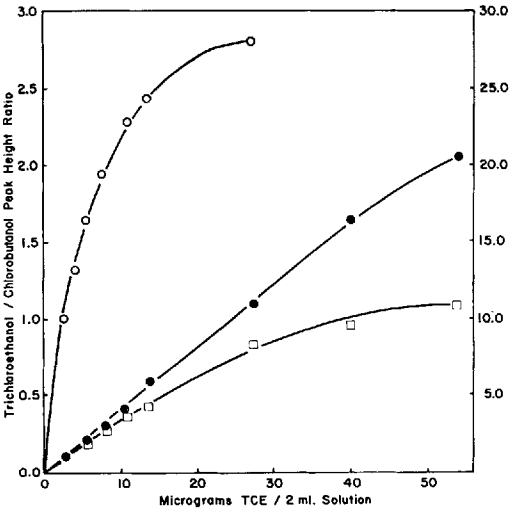

Fig. 4.—Calibration curves for trichloroethanol at three pulse intervals. Sensitivity: 0.001 of maximum. Pulse = 50, ordinate on left; pulse = 5 and 15, ordinate on right. One microgram = 6.6 mμmoles. Key: ○, pulse = 50; ●, pulse = 5; □, pulse = 15.

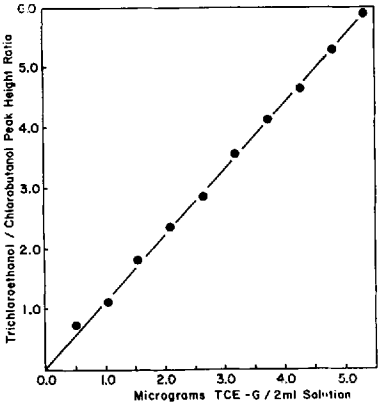

Fig. 5.—Calibration curve for trichloroethanol glucuronide based on the assay of trichloroethanol obtained by enzymatic hydrolysis. Pulse = 15, sensitivity: 0.001 of maximum. One microgram = 3.08 mμmoles.

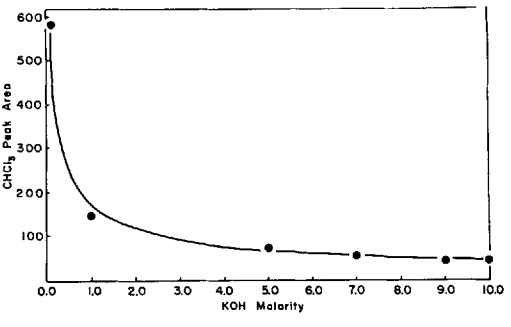

Fig. 6.—Effect of potassium hydroxide concentration on the amount of ether-extracted chloroform after trichloroacetic acid decarboxylation.

RESULTS AND DISCUSSION

Flame Ionization Detector.—The calibration curves for TCE and CH obtained by injecting 4-μl. aqueous aliquots are seen as Fig. 1. The sensitivity of flame ionization detection was only slightly greater than that obtained with the Friedman-Cooper assay. Therefore, this was not the method of choice.

Electron Capture Detector—Chloral Hydrate and Trichloroethanol.—Since direct injection of aqueous solutions gave poor peak reproducibility with electron capture detection, extraction with a nonpolar solvent was used. The distribution coefficients for several solvents are located in Table I. Figure 2 is a typical chromatogram of ether-extracted CH and TCE, with chloroform and chlorobutanol as internal standards. Better resolution of the CH and chloroform peaks could be obtained by decreasing either the carrier gas flow rate or the column temperature, but only at the expense of prolonging the retention time and increasing the width of the TCE and chlorobutanol peaks.

The potential across the detector cell was applied by the square wave pulse technique (7). The intervals available between $^3/_4$ μsec. voltage pulses were 5, 15, 50, and 150 μsec. An increased linear relationship between response and concentration was obtained by using shorter pulse intervals when high concentrations of CH or TCE were used. This is demonstrated in Figs. 3 and 4, the calibration curves for CH and TCE, respectively. However, greater sensitivity was available when the longer pulse intervals were used. These pulse intervals were more advantageous at lower drug concentrations. Thus, the pulse interval used for assay would depend on the concentration of drug which is physiologically encountered.

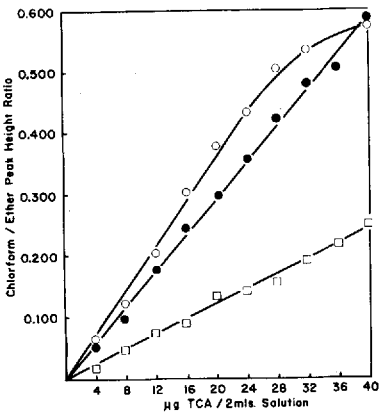

Fig. 7.—Calibration curve for trichloroacetic acid based on the assay of ether-extracted chloroform after alkaline (0.1 *M* KOH) decarboxylation. Sensitivity: 0.001 of maximum. One microgram = 6.1 mμmoles. Key: ○, pulse = 50; ●, pulse = 15; □, pulse = 5.

The assay parameter used for TCE and CH is peak height ratio (compound/internal standard). Although peak area ratios are conventionally used for compounds exhibiting broad peaks, peak height ratios gave less error when calibration curves using the two parameters were compared.

Trichloroethanol Glucuronide.—The acid hydrolysis yielded a TCE assay with peak height ratio of 6.0 at pulse = 15. Since this region of the pulse = 15 calibration curve (Fig. 4) showed a slight negative deviation from linearity, a second assay was run at pulse = 5, and a peak height ratio of 9.0 was obtained. These values corresponded to concentrations of 19.2 and 22.4 mcg. of TCE in 2 ml. of solution (87.2 and 100.3% of calculated theoretical yield) when compared with the calibration curves for TCE. Enzymatic hydrolysis at 37°, pH 4.5 with varying amounts of enzyme gave the same yield with 5 mg. of β-glucuronidase/2 ml.; 1.0 mg. of enzyme gave only 65.8% of theoretical yield.

The calibration curve for TCE-G is in Fig. 5. A pulse interval of 15 μsec. was used because it gave a linear response for physiological concentrations of TCE-G.

Trichloroacetic Acid.—The effect of KOH concentration on the assay was checked by decarboxylating a 1-ml. sample of 5 mg./100 ml. of TCA with 1.0 ml. of base ranging in concentration from 0.1 to 10 *M* and assaying after 30 min. for chloroform. The results of this experiment are depicted in Fig. 6.

Coulometric titration with a Cotlove chloridometer (Laboratory Glass and Instrument Corp.) demonstrated an increase in ionic chloride concomitant with the decrease of assayable chloroform from decarboxylation as the concentration of alkali was increased. For KOH concentrations of 1.0, 3.0, 5.0, and 10.0 *M*, the ionic chloride concentrations (and $CHCl_3$ peak heights) were 0.0296 (3.0), 0.0866 (1.20), 0.1104 (0.65), and 0.1626 meq./L.

TABLE II.—ASSAY DATA FOR LEVELS OF CHLORAL HYDRATE IN URINARY MIXTURES

TCE Level, mg./100 ml.	TCA Level, mg./100 ml.	CH/$CHCl_3$ Peak Ht. Ratio: CH, 0.14 mg./100 ml. — Day 1	CH, 0.14 mg./100 ml. — Day 2	CH, 1.40 mg./100 ml. — Day 1	CH, 1.40 mg./100 ml. — Day 2
0	0	.270, .270	.270, .283	1.025, 1.025	1.010, 1.025
0	5.00	.259, .270	.289, .260	1.025, 1.020	1.010, 1.010
0	3.75	.250, .270	.270, .289	1.010, 1.020	1.025, 1.020
0	1.25	.293, .257	.274, .260	1.010, 1.025	1.030, 1.020
1.36	0	.261, .276	.279, .282	1.020, 1.010	1.020, 1.030
1.36	5.00	.256, .243	.240, .259	1.010, 1.010	1.010, 1.020
1.36	3.75	.262, .268	.282, .260	1.010, 1.020	1.020, 1.020
1.36	1.25	.270, .261	.260, .278	1.020, 1.010	1.010, 1.010
0.136	0	.226, .236	.261, .253	1.010, 1.005	1.010, 1.005
0.136	5.00	.240, .240	.258, .253	1.003, 1.016	1.008, 1.004
0.136	3.75	.240, .247	.261, .265	1.020, 1.005	1.005, 1.002
0.136	1.25	.245, .251	.269, .269	1.020, 1.004	1.005, 1.004

(0.36), respectively. These data can explain the decrease in assayable chloroform with increased concentrations of alkali.

The first-order rate constants at the lowest KOH concentration (0.1 M) were checked at three different initial TCA concentrations. These rate constants were reasonably independent of the TCA concentrations, given in the parentheses, and were 15.0 (0.75×10^{-3} M), 13.8 (1.5×10^{-3} M), and 14.3 $\times 10^{-4}$ sec.$^{-1}$ (3.0×10^{-4} M). These values were in good agreement with the 13.2 $\times 10^{-4}$ sec.$^{-1}$ reported by Fairclough (8).

The 0.1 M KOH was used in the decarboxylation procedure since the greatest yield of chloroform was obtained in contrast to higher concentrations of alkali (Fig. 6).

The calibration curve for TCA (chloroform/ether peak height ratio *versus* concentration of TCA) is in Fig. 7. The use of the extraction solvent as an internal standard is deemed justifiable since the

TABLE III.—ANALYSIS OF VARIANCE FOR THE ASSAY[a] OF LOW LEVEL OF CHLORAL HYDRATE IN URINARY MIXTURES

Sources of Variation	d.f.	S.S.	M.S.	Components of Variance
Days (D)	1	0.0014	0.0014	$\sigma_S^2 + 2\sigma_E^2 + 24\sigma_D^2$
TCE (A)	2	0.0033	0.00165	$\sigma_S^2 + 2\sigma_E^2 + 4\sigma_{AB}^2 + 16\sigma_A^2$
TCA (B)	3	0.007	0.00023	$\sigma_S^2 + 2\sigma_E^2 + 4\sigma_{AB}^2 + 12\sigma_B^2$
TCE × TCA (AB)	6	0.0013	0.00021	$\sigma_S^2 + 2\sigma_E^2 + 4\sigma_{AB}^2$
Expt. error (E)	11	0.0011	0.0001	$\sigma_S^2 + 2\sigma_E^2$
Sampling error (S)	24	0.0026	0.0001	σ_S^2

[a] Assay data for chloral hydrate presented as CH/$CHCl_3$ peak height ratios (Table II).

TABLE IV.—SUMMARY OF SIGNIFICANT AND NONSIGNIFICANT EFFECTS (F TEST, 5% LEVEL) FOR ASSAYS OF CHLORAL HYDRATE, TRICHLOROETHANOL, AND TRICHLOROACETIC ACID IN MIXTURES

Drug	Level Tested	Significant Effects and F Values	Nonsignificant Effects
CH	0.14 mg./100 ml.	Days, 14.0[a] TCE levels, 8.25[b]	TCA, levels, TCE × TCA, exptl. error
CH	1.40 mg./100 ml.	TCE levels, 22.1[b]	TCA levels, TCE levels, TCE × TCA, exptl. error, days
TCE	0.136 mg./100 ml.	None	CH levels, TCA levels, CH × TCA, exptl. error, days
TCE	1.36 mg./100 ml.	None	CH levels, TCA levels, CH × TCA, exptl. error, days
TCA	1.25 mg./100 ml.	None	Treatments,[c] days, exptl. error
TCA	3.75 mg./100 ml.	Treatments,[c] 4.0[d]	Days, exptl. error
TCA	5.00 mg./100 ml.	Treatments,[c] 3.33[d]	Days, exptl. error

[a] Tabulated F (.05) = 4.84 for 1 and 11 d.f. [b] Tabulated F (.05) = 5.14 for 2 and 6 d.f. [c] A missing piece of data for all levels of TCA tested eliminated the isolation of the interactions for TCE levels, CH levels, and TCE × CH from the treatment sum of squares. The effect noted, therefore, is a pooled effect of all three of the above. [d] Tabulated F (.05) = 3.29 for 7 and 9 d.f.

TABLE V.—ISOLATED COMPONENTS OF VARIANCE AND PER CENT STANDARD DEVIATION FOR ASSAYS OF CHLORAL HYDRATE, TRICHLOROETHANOL, AND TRICHLOROACETIC ACID

Drug	Level Tested	Components of Variance × 10^6	$\sigma_{x_{ij}} \times 10^3$[a]	$\bar{\bar{X}}$[b]	S.D., %[c]
CH	0.14 mg./100 ml.	σ^2 days = 54.2 σ^2 TCE = 90.6 σ^2 TCE × TCA = 25.0 σ^2 sampling = 100	16.4	0.262	6.25
CH	1.4 mg./100 ml.	σ^2 TCE = 28.5 σ^2 error = 3.90 σ^2 sampling = 45.8	8.84	1.014	0.87
TCE	0.136 mg./100 ml.	σ^2 days = 5.4 σ^2 CH = 46 σ^2 CH × TCA = 84 σ^2 sampling = 210.0	18.6	0.885	2.1
TCE	1.36 mg./100 ml.	σ^2 days = 5340 σ^2 TCA = 650 σ^2 error = 5760 σ^2 sampling = 25890	194.0	6.326	3.1
TCA	1.25 mg./100 ml.	σ^2 error = 1.5 σ^2 sampling = 42.0	8.75	0.337	2.6
TCA	3.75 mg./100 ml.	σ^2 treatments = 18.8 σ^2 treatments = 79.5 σ^2 sampling = 105	13.6	0.574	2.36
TCA	5.0 mg./100 ml.	σ^2 error = 5.8 σ^2 treatments = 7.0 σ^2 sampling = 12.5	5.04	0.762	0.66

[a] $\sigma_{x_{ij}}$, the estimated standard deviation of a single assay. The square root of the sum of the isolated components of variance (σx^2_{ij}). [b] $\bar{\bar{X}}$, the mean assay value, over all treatments, for a given level of a drug. Presented for TCE as the TCE/chlorobutanol peak height ratio, for CH as the CH/$CHCl_3$ peak height ratio, and for TCA as the $CHCl_3$/ether peak height ratio. [c] Per cent standard deviation = $\sigma_{x_{ij}} \times 100/\bar{\bar{X}}$.

TABLE VI.—STATISTICAL ANALYSIS OF ASSAY DATA FOR MIXTURES OF TRICHLOROACETIC ACID AND TRICHLOROETHANOL GLUCURONIDE IN DOG URINE

Drug Assayed	Components of Variance × 10^6	$\sigma_{x_{ij}} \times 10^3$[c]	$\bar{\bar{X}}$[d]	S.D., %[e]
TCA[a]	σ^2 TCE-G = 8.0			
	σ^2 error = 50	7.6	0.99	0.76
TCE-G[b]	σ^2 TCA = 1870			
	σ^2 error = 10430	111.0	4.11	2.70

[a] TCA concentration = 7.0 mg./100 ml.; TCE-G concentrations = 5.14, 2.57, and 0.514 mg./100 ml. [b] TCE-G concentration = 5.14 mg./100 ml.; TCA concentrations = 7.0, 3.5, and 0.7 mg./100 ml. [c] $\sigma_{x_{ij}}$, the estimated standard deviation of a single assay. The square root of the sum of the isolated components of variance ($\sigma^2_{x_{ij}}$). [d] $\bar{\bar{X}}$, the mean assay value over all treatments. Presented for TCA as $CHCl_3$/ether peak height ratio and for TCE-G as the TCE/chlorobutanol peak height ratio. [e] Per cent standard deviation = $\sigma_{x_{ij}} \times 100/\bar{\bar{X}}$.

assay conditions (sealed vials) prohibit volatilization and the ether peak height is proportional to the volume injected.

Statistical Evaluation.—Three levels of TCE (0, 0.136, and 1.36 mg./100 ml.), three levels of CH (0, 0.140, and 1.40, mg./100 ml.), and four levels of TCA (0, 1.25, 3.75, and 5.0 mg./100 ml.) were combined in all possible mixtures (a total of 36) in dog urine and assayed randomly with two assays of each component per sample on each of two days. A typical series of results, those for 0.140 and 1.40 mg./100 ml. CH, are listed in Table II. Analyses of variance (9) of the assay data were obtained for each concentration of drug in the presence of the various concentrations of the other drugs. Such analyses were run on the raw assay data; for TCE, as TCE/chlorobutanol peak height ratios and for TCA, as chloroform/ether peak height ratios. A typical analysis of variance, for 0.140 mg./100 ml. CH in mixtures of various concentrations of TCE and TCA, is given in Table III. This analysis is based on data for this CH level given in Table II.

On each of the analyses of variance performed the presence of significant factors was determined by the F test at the 5% level (Table IV). The isolated components of variance are listed in Table V along with $\sigma_{x_{ij}}$, the standard deviation of a single assay (which is the square root of the sum of the isolated components of variance, $\sigma^2_{x_{ij}}$), the mean assay value over-all treatments $\bar{\bar{X}}$, and the per cent standard deviation of the assay ($\sigma_{x_{ij}} \times 100/\bar{\bar{X}}$). From this table it was observed that the major source of variation for all components assayed was due to sampling error. Experimental error was either absent or much smaller than that for sampling.

The variance due to the presence of TCE in assays for CH was postulated to be a result of formation of a soluble chloral alcoholate (10) which was then irregularly distributed between ether and urine. This could be corrected by running CH calibration curves in the presence of varying concentrations of TCE.

If one excludes the variation due to days noted in the assay of 0.140 mg./100 ml. CH by assuming a weighing error during sample preparation, the per cent standard deviation for the remaining drug concentrations is never greater than 3.1%. Hence, the assay is both reliable and reproducible.

Two similar experiments, the first with 7.0 mg / 100 ml. TCA solution in the presence of 5.14, 2.57, and 0.514 mg./100 ml. TCE-G and the second with 5.14 mg./100 ml. TCE-G in the presence of 7.0, 3.5, and 0.70 mg./100 ml. TCA, were also run. Analyses of variance were carried out on the assay data and the results are found in Table VI. No significant effect was found for either the presence of TCA in TCE-G assays or the presence of TCE-G in TCA analyses.

Finally, to determine possible assay variation when different experimental animals were used, analyses of variance were run on assay data for TCE, CH, and TCA in aqueous solution and in the urine of three different dogs (four urine samples per dog per day on two different days). The design had unequal numbers of replicates which prohibited isolation of components of variance. No significant difference was noted among different urines using the F test (5% level).

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