Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Trichloroacetic Acid

MRID #: 417368-29

Matrix: Soil

Analysis: GC/ECD

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MRID No 41736829

Du Pont Study No. AMR-1253-88

Study Title

ANALYTICAL METHOD FOR THE DETERMINATION OF RESIDUES OF TRICHLOROACETIC ACID IN CROPS AND SOILS

A GLC-EC METHOD

Data Requirement

U. S. EPA Pesticide Assessment Guidelines
Subdivision O, 171-4

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Study Completed On

August 4, 1989

Performing Laboratory

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AMR-1253-88

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d) (1)(A), (B), or (C).

Company E. I. du Pont de Nemours and Company, Inc.

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(Typed Name)

August 9, 1989
(Date)

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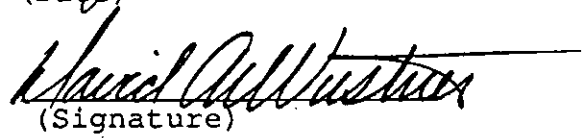
(Title)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GOOD LABORATORY PRACTICE STATEMENT

The Good Laboratory Practice (GLP) requirements specified in 40 CFR Part 160 were not applicable to residue chemistry requirements at the time of completion.

This study was conducted in the spirit of compliance with GLP regulations.

Sponsor: E. I. du Pont de Nemours and Company, Inc.

Submitter: E. I. du Pont de Nemours and Company, Inc.

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SUMMARY/INTRODUCTION

SCOPE

Trichloroacetic acid (TCA) is a possible soil metabolite of FORTRESS® INSECTICIDE. TCA may also occur as a by-product during the chlorination of water and as a metabolite of trichloroethylene and tetrachloroethylene in animals. The compound has been used as a herbicide in the U.S. and may still be used in some countries for this purpose. Hence, there are many references available for its uses, properties and analysis. A review of all available literature is beyond the scope and intention of this method. The analysis for the compound (at trace levels) is usually performed via conversion to an ester or thermal decarboxylation to chloroform, with subsequent quantitation by GLC-EC. The published reports are frequently concerned with water quality. The method contained in this report has been developed to provide a rugged method which is applicable to various crops and soil types without the need for diethyl ether and special alkylating agents which are frequently used in existing methodology. Further, since the following method does not incorporate conversion to chloroform, the time-consuming measures to insure chloroform-free glassware, reagents, and surrounding air are unnecessary.

PRINCIPLES OF METHOD

TCA is extracted from crops and soils using 95% methanol/5% water by maceration or sonication, respectively. The extract is slightly buffered and converted to methanol by sequential concentration/evaporation to eliminate water. TCA is converted to the methyl ester using methanol catalyzed with sulfuric acid at 60°C. The ester is partitioned into hexane following addition of water to the methanol reaction medium. The ester is then isolated from interferences using disposable clean-up columns (Si) and hexane as eluant. The ester is quantitated by GLC utilizing electron-capture detection. A standard solution of trichloroacetic acid is converted to the methyl ester using the same procedure as samples. The converted standard is diluted with hexane and used for GC standards, which simplifies calculation of results. The minimum quantifiable concentration using suggested sample weights and GC-standard concentrations is 0.01 ppm TCA in soils and crops.

MATERIAL IDENTIFICATION

ANALYTE	:	TRICHLOROACETIC ACID (TCA)
FORMULA	:	CCl_3 COOH
CAS NO.	:	76-03-9
M.W.	:	163.39
M.P.	:	54-56°C (760 mm Hg)
B.P.	:	196°C (760 mm Hg)
HAZARD	:	CORROSIVE; HIGH FOR INHALATION, SKIN, EYE, AND INGESTION
OTHER	:	HYGROSCOPIC, COLORLESS CRYSTALS, HIGH WATER SOLUBILITY

MATERIALS/METHODS

EQUIPMENT

a. Gas-Liquid Chromatograph, Hewlett-Packard, Model 5890, equipped with an electron-capture detector (or equivalent instrumentation).

b. GLC column, 30 M x 0.56 mm (ID), "MEGABORE", DB1, 1.5 micron film thickness, J&W SCIENTIFIC, INC., 3871 Security Park Drive, Rancho Cordova, CA 95670, cat. no. 1251032.

c. Solvent evaporator, Meyer N-EVAP (ORGANOMATION, INC., South Berlin, MA 01549), p/n 11155-T. The efficiency of this apparatus can be significantly increased for this procedure by: (1) removing the bottom spring which supports the sample rack--this will allow the samples to go deeper into the water bath, and (2) replacing the air delivery tubes with larger diameter tubes (1/8-inch thinwall tubing is ideal), to increase air flow but not velocity.

d. Multiport solvent valve, Baker MISER (J. T. BAKER CHEMICAL CO., Phillipsburg, NJ), p/n 7220-0. *Optional for all analyses*

e. Sample homogenizer, Tekmar TISSUMIZER, probe and speed controller, Model SDT-1810, (THOMAS SCIENTIFIC, Swedesboro, NJ), p/nos. 3411-C10, 3411-C40, and 4311-C60. *Not required for soil, flour, and starch analyses*

f. Centrifuge, with capacity for 250-mL centrifuge bottles, SORVALL Model RC5C (DU PONT COMPANY, Clinical and Instrument Systems Division, Newtown, CT 06470). *Required for soils, grains, and other oily or starchy commodities*

g. Ultrasonic bath, 0.75 gallon capacity, Cole-Parmer, Model 8851 (COLE-PARMER INSTRUMENT CO., 7425 North Oak Park Ave, Chicago, IL 60648), p/n J-8851-00. *Required for soil, flour, and starch analyses only*

h. Sieve and receiver, No. 8, US Standard, 8-inch diameter, 2.36 mm (THOMAS SCIENTIFIC, Swedesboro, NJ 08085-0099), p/nos. 8323-R16 and 8328-S60. *Required for soil analyses only*

i. Centrifuge tubes, graduated, 15- and 40-mL capacity, FISHER SCIENTIFIC, cat. nos. 05-538-35A and 05-538-38B, respectively. These tubes must be silanized prior to use. Fill each tube with silanizing solution (Reagents and Standards sec.(j)), stopper and allow to sit for 15 minutes. Save solution for re-use. Rinse tubes with toluene, methanol, and acetone, in that order.

REAGENTS AND STANDARDS

a. Hexane, OmniSolv®, HR-GC Grade, EM SCIENCE, 111 Woodcrest Road, Cherry Hill, NJ 08034-0395, cat. no. HX0297. Hexane should be analyzed by GLC-EC. If peaks occur in the area of TCA-methyl ester, the hexane should be purified using silica. Use one disposable Si column (see item (1) below) per 100 mL of hexane and the Baker MISER with a large syringe (50-mL or larger). Do this for hexane used for preparation of GLC standards and sample dilutions (GLC stage) only.

b. Methanol, OmniSolv®, Pesticide Residue Quality, EM SCIENCE, 111 Woodcrest Road, Cherry Hill, NJ 08034-0395, cat. no. MX0484.

c. Toluene GR, EM SCIENCE, 111 Woodcrest Road, Cherry Hill, NJ 08034-0395, cat. no. TX0735. *Required for silanizing glassware only*

d. 10% sodium acetate solution. Dissolve 10 grams of sodium acetate in 90 mL of distilled water. Sodium acetate GR crystals, EM SCIENCE, 111 Woodcrest Road, Cherry Hill, NJ 08034-0395, cat. no. SX0255. Make fresh solution monthly.

e. 10% sodium chloride solution. Dissolve 10 grams of sodium chloride in 90 mL of distilled water. Sodium chloride, crystals, BAKER ANALYZED reagent, J. T. BAKER CHEMICAL CO., cat. no. 3624-1.

f. Sulfuric acid GR, 95-98%, EM SCIENCE, 111 Woodcrest Road, Cherry Hill, NJ 08034-0395, cat. no. SX1244-3 (500 mL). *VERY CORROSIVE MATERIAL, use extreme care in handling*

g. Trichloroacetic acid GR, 99%, EM SCIENCE, 111 Woodcrest Road, Cherry Hill, NJ 08034-0395, cat. no. TX1045-3 (125 mL). *VERY CORROSIVE MATERIAL, use extreme care in handling*

h. TCA stock solution. Weigh a 100-mL volumetric flask and stopper on an analytical balance to ±0.1 mg. Working in a fume-hood, transfer 0.1-0.2 grams of trichloroacetic acid to the flask and stopper immediately (use TEFLON® stopper if available). Re-weigh the flask to determine weight of TCA, add distilled water to the 100-mL mark and mix well. Determine concentration of TCA based on weight and purity (should be in the range of 1000-2000 µg/mL). While the useful shelf-life of this standard has not been determined, it appears to be stable for several months (ambient).

i. TCA spiking standards. Make serial dilutions of the stock solution with distilled water which result in 100-mL volumes of 2.5 and 0.25 µg/mL TCA. Use 1.0 or 2.0 mL of these standards for spiking levels in the range of 0.01 to 0.2 ppm TCA, depending on the weight of sample taken for analysis. These standards appear to be stable for several months at room temperature.

j. Silanizing solution. Working in a fume hood, combine 60 mL of dichlorodimethyl silane and 60 mL of chlorotrimethyl silane with 2 liters of toluene in a brown glass bottle. These volumes may be scaled up or down as necessary. Dichlorodimethyl silane and chlorotrimethyl silane are available from EASTMAN KODAK CO., Rochester, NY 14650, cat. nos. 9650 and 8710, respectively.

k. Extraction solvent (95/5). Add 1.9 liters of methanol to a 2-liter graduated mixing cylinder and add distilled water to the 2.0-liter mark. Cap and tumble until mixed. This solvent mixture (95% methanol/5% water v/v) will be used for extraction of TCA from all samples. Prepare sufficient amount to allow approximately 300 mL per sample. The extraction solvent will be referred to as "95/5" throughout the remainder of this method.

l. Disposable clean-up columns, Si, 500 mg/2.8 mL, ANALYTICHEM INTERNATIONAL, 24201 Frampton Ave, Harbor City, CA 90710, p/n 601303.

ANALYTICAL PROCEDURE

1. Sample Preparation

a. Crops

Sample preparation will vary with crop type, sample size, and available equipment. In general, a 25-gram subsample will be taken for actual analysis which must be representative of entire sample received. Most samples will require thorough chopping with a knife or, preferably, an industrial food processor, followed by thorough manual mixing with a large spoon or spatula in order to form a homogenate. Watery crops require special care since they tend to separate into juice, peels, seeds, pulp, fibers, etc. Samples of this type must be mixed intermittently during the course of any subsampling to insure that a representative sample is indeed obtained.

If watery crop sample homogenates are to be frozen prior to subsampling, the entire sample homogenate must be thawed and remixed prior to any subsampling, since the samples probably separated into zones according to density. Do not attempt to subsample from partially thawed homogenates by sampling from a liquid layer or chipping off pieces of icy sample.

b. Soil

Fit the 8-inch, No. 8 sieve into the receiver and add soil slowly through the sieve until the receiver is full or until the entire sample has been added. Discard rocks and plant debris remaining on the sieve. If the soil is lumpy or wet, add small amounts of soil to the sieve and work it through the sieve using a metal or plastic spatula. Discard extraneous materials between each aliquot of soil. When the entire soil sample has been passed through the sieve, blend it into a homogeneous mixture by rolling and tumbling in a large, wide-mouth jar or plastic bag with sufficient headspace (usually 2-3 minutes is sufficient).

c. Muddy Soil

If the samples are too wet to be manageable some drying may be required prior to sieving. Spread the samples out on aluminum foil to minimize the time required for drying. Overnight is usually sufficient for adequate drying. If the drying time is significant relative to sample treatment interval (for instance a 0-time interval), a sample should be spiked prior to drying. Weigh out a 50-gm subsample of the untreated soil on a piece of foil and spike with 1.0 mL of the 2.5 µg/mL TCA standard, distributing the standard over the surface area. Allow this sample to dry for the same time (and temperature) as the other samples but do not pass it through the sieve. After drying, pour the spiked sample into a 250-mL polyethylene centrifuge bottle for analysis with other samples. This 0.05 ppm spiked sample should be analyzed along with the other samples to monitor possible effects of the drying step.

d. Soil Moisture Determination

When samples have been sieved and thoroughly mixed, transfer to suitable, labeled containers such as wide-mouth jars. Using a spatula or spoon, transfer 10.0 grams of each sample (not spiked samples) to an aluminum foil weighing vessel or petri dish. Dry the samples in a drying oven at 100°C for 2 hours, allow to cool, and re-weigh each to determine the moisture contents. The "% moisture" is the weight loss (in grams) multiplied by ten. Record the "% moisture" for later reporting or for calculation of residues on a "dry weight" basis.

2. Extraction

a. Crops and Most Processed Products

Weigh 25 grams of representative sample (12.5 grams of dried fodder, hay, or other bulky/low density samples) into a 250-mL polyethylene, wide-mouth, centrifuge bottle and add 100 mL of 95/5 (see Reagents and Standards (k) above). Insert the Tekmar homogenizer probe to about one-half the depth of the extract and gradually increase speed until large pieces are macerated. Continue at steady, medium speed until mixture appears homogeneous, but not longer than two minutes. Shut off motor and raise unit until bottom of shaft assembly is about one cm below top of bottle. Immediately rinse lower shaft and blade assembly with a few mL of 95/5 using a disposable pipet or TEFLON® wash-bottle, allowing the rinses to drain into the bottle. It is not necessary to attempt to recover small particles remaining on the blade after rinsing. Proceed to Centrifugaton (i.) or Filtration (ii.), depending on sample type.

i. Centrifugation

Starchy or Oily Commodities (grains, corn meal, etc.)

Balance samples in preparation for centrifugation using 95/5 and centrifuge samples for five minutes at 2000 rpm. Rinse a piece of fluted filter paper with 95/5 using a TEFLON® wash-bottle or disposable pipette and discard rinsate. While the filter paper is still wet, pour the extract through the filter paper into a 250-mL graduated mixing cylinder, leaving the solids in the centrifuge bottle. Add 100 mL of fresh 95/5 to the centrifuge bottle, cap and shake vigorously until the solids are dispersed and thoroughly rinsed. Balance, centrifuge and decant through the same filter paper into original graduated cylinder. Repeat solids rinse with a final 40-mL aliquot of 95/5 and combine with original extract. Add 95/5 to the graduated cylinder to bring final volume to 250 mL, cap and mix by tumbling.

ii. Filtration

Fibrous Crops (most watery crops, animal fodders, etc.)

Rinse a piece of filter paper with 95/5 using a TEFLON® wash bottle or disposable pipette and discard rinsate. While the paper is still wet, transfer the funnel and filter paper to a 250-mL graduated mixing cylinder. Pour the extract and solids into the filter paper. Use 25-50 mL of 95/5 to rinse the centrifuge bottle and carefully decant this over the solids in the filter paper. Pour fresh 95/5 slowly over the solids in the filter paper until the 250-mL mark is reached, being careful to distribute the solvent over the entire surface area of the solids for efficient rinsing. Cap cylinder and mix by tumbling. Proceed to Fortifications (sec. 3).

b. Soil (use for corn flour and starch also)

Transfer 50.0 grams of representative soil (25 grams of flour or starch) to a polyethylene centrifuge bottle using a spatula or small spoon. Add 100 mL of 95/5 (see Reagents and Standards (k) above) and place in a sonic bath. Add water to the sonic bath until the water level is approximately the same as the solvent level in the sample containers. Sonicate the samples for 20 - 30 minutes. During the sonication, shake each sample vigorously (for about 5 seconds) at 4-5 minute intervals, to dislodge trapped air and refresh solvent around the particles. After sonication, balance the bottles with 95/5 and centrifuge for 5 minutes at 2000 rpm. Decant the extracts into 250-mL graduated mixing cylinders (use pre-rinsed filter paper if necessary).

Add 100 mL of fresh 95/5 to the samples, cap and shake vigorously for about 30 seconds. Balance samples with fresh 95/5 and centrifuge at 2000 rpm for 5 minutes. Combine rinse with original extract. Repeat rinse with a final 40-mL aliquot of fresh 95/5 and combine with previous extractions. Bring final volume to 250 mL with 95/5, cap and mix by tumbling. Proceed to Fortifications (sec. 3).

c. Vegetable Oils (crude and refined)

Weigh 25.0 grams of oil into a 125-mL glass beaker. Decant the oil into a 250-mL separatory funnel. Rinse the beaker with two 50-mL aliquots of hexane and combine these with the oil. Swirl the separatory funnel vigorously until the mixture is homogeneous. Add 100 mL of 95/5 to the separatory funnel, stopper and shake vigorously for one minute. Allow the phases to separate and drain the lower phase into a 250-mL graduated mixing cylinder. Add a 100-mL aliquot of fresh 95/5 to the separatory funnel and repeat the partition. Combine the extracts in the graduated cylinder. Add fresh 95/5 to the cylinder until the 250-mL mark is reached. Cap and mix by tumbling. Proceed to next step.

Raw extracts for most commodities can be stored for up to 48 hours, at room temperature, without detectable loss of recoveries. Storage for longer periods should be in flammable-solvent approved refrigerators or freezers.

3. Fortifications

At least one spiked sample (fortification) must be analyzed concurrently with each set of samples to monitor the integrity of the method. The spiking levels should be in the range of the expected residue in samples, including the control.

a. All Samples (except oils)

During the course of weighing out samples in preparation for extraction, weigh out two subsamples of the check (untreated) sample. Using a transfer pipet, distribute 1.0 or 2.0 mL of TCA spiking standard over the surface of the sample contained in one of the centrifuge bottles. Leave the other check unspiked for analysis in order to determine background interference. Extract these samples along with treated samples. Proceed to Water Removal. (sec. 4).

b. Vegetable Oils

During the course of weighing out samples in preparation for extraction, weigh out two subsamples of the check (untreated) sample. Transfer samples to separatory funnels (as per method). Using a transfer pipette, transfer 1.0 mL of TCA spiking standard (0.5 µg/mL) to one of the samples contained in the separatory funnels. This results in a spiking level of 0.02 ppm. Since there is no other suitable solvent for TCA, aqueous spikes are required for all samples, including oils. Leave the other check unspiked for analysis in order to determine background interference. Extract these samples along with treated samples. Proceed to next step.

4. Water Removal and Hexane Partition (all samples)

Transfer 40.0 mL of sample extract to a silanized, 40-mL centrifuge tube and add 4 drops of 10% sodium acetate solution using a disposable pipette. Concentrate to 4-5 mL using the N-EVAP with the water bath at 60°C. Use a rapid blow-down rate while maintaining the end of the blow-down tube at 10-15 mL above the top of the solvent. As the individual samples reach the 4-5 mL volume, raise the air delivery tube, add 20-25 mL of hexane (while sample is still in hot water bath), stopper and shake vigorously for about 15 seconds. Place tube in a tube rack. Repeat until all samples have been partitioned with warmed hexane. Discard hexane (upper) phases using disposable pipettes. Leave 0.5-1.0 mL of hexane to

avoid removing any of the aqueous phase. The remaining hexane will be largely removed in the next step.

Add about 10 mL of fresh hexane to each tube, using it to rinse the sides of the tubes. Do not shake or swirl this mixture, or difficult emulsions will occur with some samples. Remove and discard the hexane phases using disposable pipettes.

Transfer the remaining solution (5-6 mL), including any emulsion, to silanized 15-mL centrifuge tubes. Use 5 mL of methanol (NOT 95/5) to rinse the 40-mL tube and add this to the 15-mL tube. Mix by gentle vortexing. Concentrate to 2-3 mL using the N-EVAP at 60°C and a rapid blow-down rate.

Remove tube from N-EVAP, add 5 mL of methanol and mix by gentle vortexing. Concentrate solution to 1 mL using N-EVAP and a rapid blow-down rate.

Remove tube from N-EVAP, add 5 mL of methanol and mix by gentle vortexing. Concentrate solution to 1 mL using N-EVAP and a rapid blow-down rate. Repeat this concentration to 1 mL three additional times to insure complete removal of water. Remove from N-EVAP and proceed to next step.

5. Methylation

a. Samples

Add methanol to the 2.0-mL mark and mix by gentle vortexing. Very carefully add 4 drops of concentrated sulfuric acid, cap and mix by gentle vortexing. Stopper loosely and place in water bath at 60°C. Check stoppers to make certain they are sitting loosely. Allow samples to sit in water bath for 2 hours. Remove samples from water bath and allow them to cool to room temperature.

At this point, the samples may be allowed to sit at room temperature for up to 72 hours without detectable loss of recovery. Alternately, the water bath can be shut off after at least one hour at the reaction temperature and the samples allowed to remain in it for up to 72 hours.

Add 4.0 mL of hexane to each sample using a transfer pipette. Cap with a TEFLON® stopper and mix by gentle vortexing.

** In order to prevent analyte decomposition, the following step must be performed on each sample individually, rather than as a set.

Add 6 mL of 10% sodium chloride solution to the sample, stopper with TEFLON® stopper and immediately shake (vigorously) for 1 minute.

Allow phases to separate while proceeding to next sample. If emulsions persist centrifuge gently (a slow speed is adequate). At least 3 mL of the hexane phase should be clear before proceeding to the next step.

Transfer 3.0 mL of the hexane (upper) phase to a 15-mL, graduated centrifuge tube using a disposable pipette. These tubes should be calibrated for 3.0 mL using a 3-mL transfer pipette.

The TCA methyl ester is very stable in hexane. Solutions can be stored at any convenient point throughout the remainder of this method, assuming containers are tightly stoppered to prevent evaporation.

b. GLC Standard

Transfer an aliquot of the most concentrated TCA standard (1000-2000 µg/mL) equivalent to 10,000 µg of TCA to a 100-mL volumetric flask and dilute to the mark with methanol. Cap and mix by shaking. Transfer 1.0 mL of this standard to a 100-mL volumetric flask and dilute to the mark with methanol. This final standard is 1.0 µg/mL TCA in methanol (necessary for conversion to methyl ester).

Transfer 25.0 mL of the 1.0 µg/mL standard into a 40-mL centrifuge tube, add 20 drops of concentrated sulfuric acid, cap and mix by gentle vortexing. Place in water bath at 60°C and make sure stopper is fitted loosely. Allow to react for 2 hours.

Remove from water bath and allow to cool to room temperature. Carefully transfer the 25 mL of acidic methanol to a 250-mL separatory funnel using a disposable pipette. Add 100.0 mL of hexane to the separatory funnel using a transfer pipette. Add 100 mL of water to the separatory funnel, using some of it to rinse the reaction tube (centrifuge tube). Immediately stopper and shake the mixture vigorously for one minute.

Drain and discard aqueous (lower) phase. Drain hexane phase into a suitable air-tight container such as a volumetric flask fitted with a TEFLON® stopper.

This standard is 0.25 µg/mL TCA as the methyl ester, and will be used for preparation of all GLC standards. Dilutions of 1:100, 2:100, 4:100, 8:100 and 16:100 will result in GLC standards of 0.0025, 0.005, 0.01, 0.02 and 0.04 µg/mL, respectively. Since the GLC standard concentrations are based on equivalent TCA (prior to methylation) it is not necessary to use a conversion factor for calculation of residue values. If, however, methyltrichloroacetate is used directly to prepare GLC standards, a conversion factor must be applied. The 0.25 µg/mL standard should be prepared fresh every 2-3 months. The dilutions should be made more frequently, depending on amount of usage.

6. Silica Column Clean-Up

a. Column Conditioning

Clean-up columns must be conditioned just prior to introduction of the sample matrix. Attach a 20-mL syringe to the top of the Baker MISER multiport valve and put one of the valve, solvent lines into a 250-mL Erlenmeyer flask containing hexane. Affix one of the pre-packed columns to the bottom of the valve and fill the syringe with hexane.

Force 7-8 mL of hexane through the column with enough pressure to expel all the air (voids) from the column. A well-conditioned column should have a gelled look throughout the packing with no powdery appearance. Remove the column while there is still hexane remaining above the top of the frit. Go to the next step (sample introduction) before the column begins to go dry.

b. Sample Introduction

In the following clean-up step, the first 5 mL of hexane will be discarded and the following 12 mL of hexane saved for analysis. This will necessitate a 15-mL centrifuge tube for measuring waste (5 mL) and a 15-mL tube for each sample (12 mL).

Place the prepared column in a 15-mL centrifuge tube so that the column flange rests on the lip of the tube. Using a disposable pipet, transfer the hexane sample matrix (3 mL) to the top of the prepared column in two or three small aliquots, forcing each aliquot onto the column packing using a rubber syringe bulb for air pressure. This technique may also be used in place of the Baker MISER when few samples require analysis. Discard the first 5 mL of hexane*. Do not allow any part of the packing to go dry.

Use 1 mL of hexane to rinse the sample container and add this to the top of the column. Fasten the column to the bottom of the Baker MISER and a clean, labeled, 15-mL centrifuge tube to the bottom of the column for sample collection. Force the hexane through until 4-5 mm remains above the top of the packing. Fill the BAKER-MISER syringe with hexane and continue forcing hexane through the column until a total volume of 12.0 mL is collected*. Stopper tube and mix by shaking. Proceed to next step.

*NOTE: Sample matrix and silica packing may vary somewhat, altering profile of compound from clean-up column. It is helpful to retain the 5-mL hexane discard aliquot of spiked samples and a 2-mL post-cut (following the 12-mL heart cut) for analysis along with samples. If any analyte is found in one of these cuts, the profile should be adjusted accordingly by taking more or less than the 5-mL discard. Keep the heart-cut at 12.0 mL, however.

INSTRUMENTATION AND QUANTITATION

1. GLC Conditions

Instrument	:	HEWLETT-PACKARD, model 5890.
Detector	:	Electron capture, 300°C
Column	:	Capillary, 30 M x 0.56 mm, DB-1 (J&W MEGABORE), 90°C.
Carrier gas	:	He, 9 psi at inlet, 12 mL/min through column, 13 mL/min at split vent.
Injector	:	Standard HP split liner packed with 0.5-1.0 cm of glass wool, 220°C.
Make-up gas	:	Argon/methane, 30 mL/min.
Septum purge	:	1.0 mL/min.
Typical response	:	0.04 ng of TCA (as methyl ester) should give a response of about 40% FSD, with a noise level of <1% and a retention time of about 2.5 minutes.

2. Sample Analysis

a. Calibration

Use standards of methylated TCA in the range of 0.0025 to 0.04 µg/mL and hexane as solvent. Make a 2-µl injection of the highest concentration standard (0.04 µg/mL). Adjust the attenuation until the TCA response is approximately 80% of full scale. Inject the remainder of the standards for construction of a calibration curve. Using the above parameters, a non-linear curve usually results, requiring a second-order best-fit. Do not assume linearity until all the standards have been injected and respective responses measured. If a data system is used for construction of the calibration curve, five standard concentration levels should be injected in the range of 0.0025 to 0.04 µg/mL TCA. The origin should be included as a data point whether the curve is linear or non-linear.

b. GLC Analysis

Inject cleaned-up samples with intermittent injections of TCA (methylated) standards. Construct a calibration curve by plotting µg/mL of standard injected versus peak height (or area). Determine µg/mL of TCA in samples from calibration curve and calculate ppm of TCA according to the following equation:

$$\frac{\text{µg/mL TCA}}{\text{grams/mL sample}} = \text{ppm TCA}$$

INTERFERENCES

1. Labware

Interfering peaks which may be encountered during GLC analysis are generally the result of exposure of solvents to soft plastic or rubber, such as pipette bulbs, plastic tubing, rubber stoppers. Care should be taken to limit solvent exposure only to inert materials such as glass, metal or inert plastics (linear polyethylene, fluorocarbons, etc.). Since this can be a random and nonreproducible occurrence, reagent blanks may fail to detect such interferences.

2. Sample Matrices

No interfering GLC peaks have been observed due to sample matrix. However, some sample matrices (such as corn stover) may result in two or three GLC peaks with a retention time much greater than the analyte which may interfere with subsequent injections. These peaks are much broader than the analyte peak and should not result in mistaken peak identity. Time between injections should be adjusted so that these peaks never occur in the area of the analyte.

CONFIRMATORY TECHNIQUE - GLC/MSD

An apparent TCA residue may be confirmed using a mass selective detector in the SIM mode. The GLC column suggested for GLC-EC analyses would be inappropriate for the MSD detector because of the high flow rate; however, any non-polar column suitable for GLC-MSD analyses should be adequate for confirmation of this compound.

Single or multiple ion chromatograms of m/e 59 (base peak) and the isotope pair 117/119 were used successfully for confirmation of TCA as the methyl ester. If the amount of TCA to be confirmed is less than 0.05 ppm, the cleaned-up extract will have to be concentrated. Concentration of the 12 mL after final clean-up, to 1 mL, should be adequate for confirmation at 0.01 ppm. This

concentration will result in losses of 10-20% of the methyl ester, however, because of its volatility. Concentration below 1 mL should not be attempted.

APPROXIMATE TIME REQUIRED FOR ANALYSIS

A "set" of samples is a group of samples which are carried through extraction, clean-up and quantitation together. A typical set of samples would include one untreated sample, one or two spikes and two to five treated samples. A set of samples will require 1.5-2 days for TCA residue determination.

RESULTS AND DISCUSSION

ACCURACY

Accuracy of the method appears to be generally consistent for all sample types with an expected mean value of about 82% recovery from spiked samples. Recoveries generally range (all samples) from about 70% to 90%. Spiking levels ranged from 0.01 to 1.0 ppm, usually in the 0.02-0.05 ppm range. Sample types included various soils, lettuce (Romaine), radishes, corn grain, green forage, stover, corn oils and other fractions derived from dry milling of corn, soybeans and foliage, wheat grain and straw, and table beets.

PRECISION

The standard deviation for this method was calculated to be 9.87. The total population (126) of recovery values was used to determine the standard deviation. The population parameter 'n-1' (125) was used for the calculation. This standard deviation results in a variance of 97.4.

LIMITS OF DETECTION AND QUANTITATION

The limits of detection and quantitation for this method were based on a detector (EC) response for the analyte which was at least 5X the background noise level. Generally, the signal-to-noise (s/n) level was about 10/1. Noise here does not include low-level interferences from sample matrices, but electrical noise which can vary with EC source age/usage and line voltage fluctuations. With time and use, s/n can vary somewhat as compound response factor varies with the condition of the inlet, column and gas purity. The following definitions for detectable levels will therefore assume an acceptable s/n which may range from about 5/1

to >10/1, to allow for variations from instrument to instrument and within the same instrument with time and use.

1. Limit of Detection (LOD) - herein defined as the amount (in nanograms) of analyte which results in a detector response that is at least five times the background noise level. For TCA, the LOD should be about 0.005 ng giving >5% full scale response with a 1% noise level (i.e., s/n = 5/1).

2. Minimum Quantifiable Concentration (MQC) - herein defined as the practical, lower-limit of concentration (ppm) achievable for this method, resulting from an analyte response greater than, or equal to, the LOD (where "practical" implies sufficient tolerance/ruggedness built into the method to allow for experimental variations). Since the method as written will generally result in injections equivalent to 0.5 mg (or more) of sample, the resultant MQC for TCA is 0.01 ppm.

LIMITATIONS

This method was designed with relatively broad tolerances to allow for column variations, sample matrix effects, volumetric variations, etc. While it is assumed that some material changes can be made to take advantage of existing equipment, certain steps must be strictly followed for successful analysis.

1. Silanization - Loss of compound may occur if the silane treatment is inadequate or silane groups have been removed (through washing, use, etc.). No more than three sample sets should be run on glassware prior to re-silanizing. Only the glassware used for concentration of TCA extract needs to be silanized. Glassware used after methylation of TCA need not be silanized. If low recoveries occur, inadequate silanization should be suspected.

2. Pressure vs. Vacuum - Solvent delivery to Si clean-up column must be via pressure as stated in the method. A vacuum manifold, while faster, causes voiding in much of the column resulting in irreproducibility.

3. Compound Degradation - TCA will degrade rapidly in some common solvents such as acetone and acetonitrile, and slower in other solvents such as methylene chloride and methanol (unless water is present). The solvents given in the method should not be substituted unless stability is carefully determined for TCA in the substitute. Analytical steps should proceed as rapidly as practical (especially during concentration of TCA) unless a BREAK-POINT is indicated.

CONCLUSION

The residue method as described will work for a wide variety of sample types. Extraction techniques vary depending upon the nature of the sample, but subsequent clean-up and quantitation are identical for all sample types, simplifying the overall method. Complete conversion of the TCA to the methyl ester requires removal of water (to <1%) via a series of concentration steps. While this is somewhat tedious, the modifications described for the concentration apparatus make it the most effective technique. Adequately silanized glassware is also important for success during the concentration step.

Previous methods for TCA generally require the use of ethyl ether, or rigorous efforts to provide a chloroform-free environment, both of which have been avoided in this method. Solvent volumes, partition coefficients, column profiles, etc., were selected which both minimize solvent consumption (and disposal) and maximize tolerances for method ruggedness.

CERTIFICATION

ANALYTICAL METHOD FOR THE DETERMINATION OF
RESIDUES OF TRICHLOROACETIC ACID IN CROPS AND SOIL

We, the undersigned, declare that the work described in this report was performed under our supervision and that this report, to the best of our knowledge, provides an accurate record of the procedures and results.

Report by: G. F. Barber — Date: 8/4/89
G. F. Barber
Study Director

Approved by: R. D. Collins — Date: 8/17/89
R. D. Collins
Environmental Studies
Supervisor

Date Study Initiated: December 21, 1987

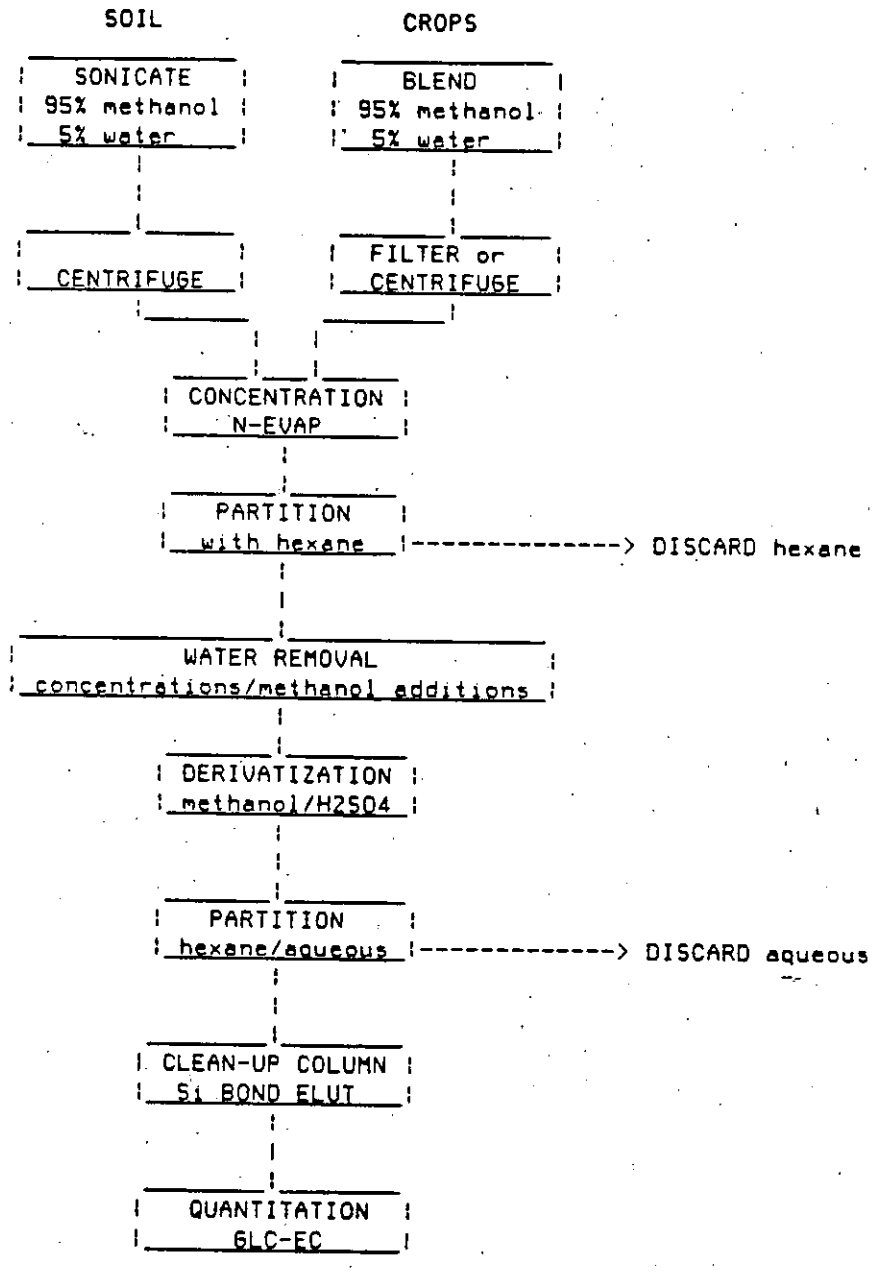
Date Study Completed: August 4, 1989 (Final Report Issued)

Notebook References: E52289

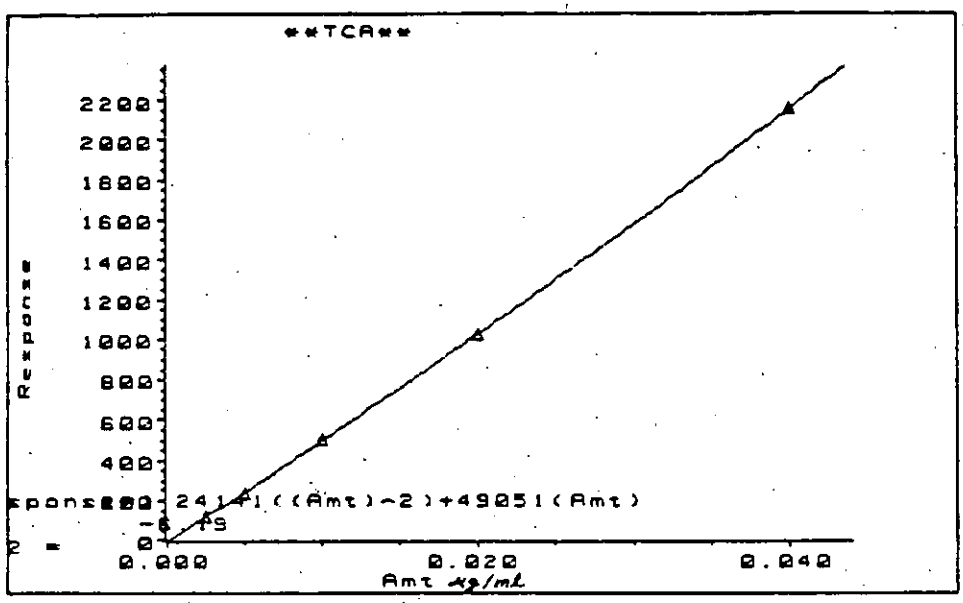
Sponsor: E. I. du Pont de Nemours & Company, Inc.
Agricultural Products Department
Research and Development Division
Experimental Station
P. O. Box 80402
Wilmington, Delaware 19880-0402

APPENDIX

METHOD FLOW CHART



TYPICAL CALIBRATION CURVE

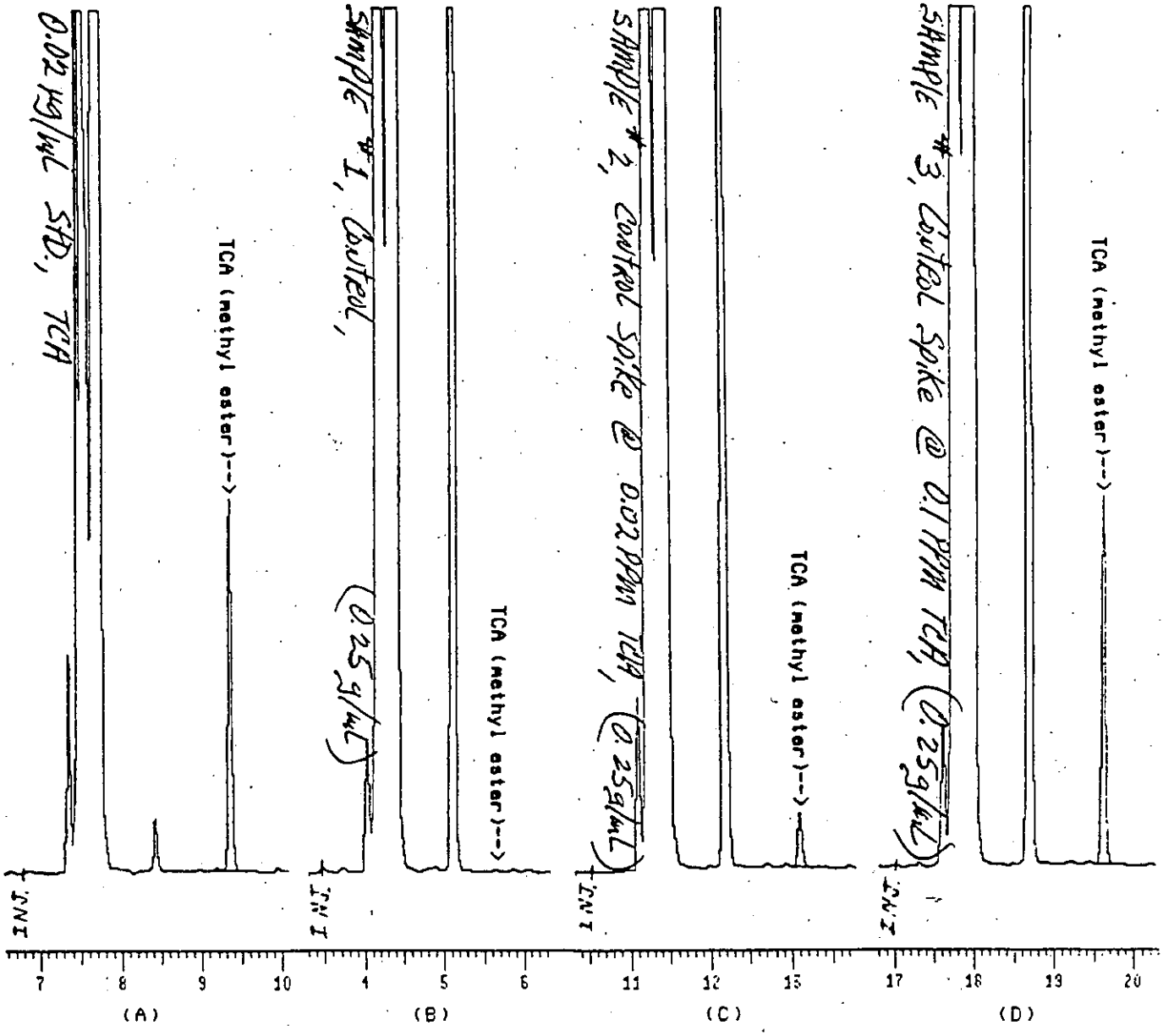


*** Calibration Table ***

Last Update: 3 Nov 88 3:24 pm
Reference Peak Window: 5.00 % of Retention Time
Non-Reference Peak Window: 5.00 % of Retention Time
Sample Amount: 0.000 Uncalibrated Peak RF: 0.000 Multiplier: 4.000

Ret Time	Pk#	Signal Descr	Amt PPM	Lvl	[Area]	Pk-Type	Partial Name
12.282	1	6C Signal 2	0.010000	5	500.54	1	**ICA**
			0.020000	4	1026.0		
			2.500e-3	3	114.95		
			5.000e-3	2	233.39		
			0.040000	1	2154.0		

TYPICAL GLC CHROMATOGRAMS OF CORN GRAIN FOR TCA ANALYSIS



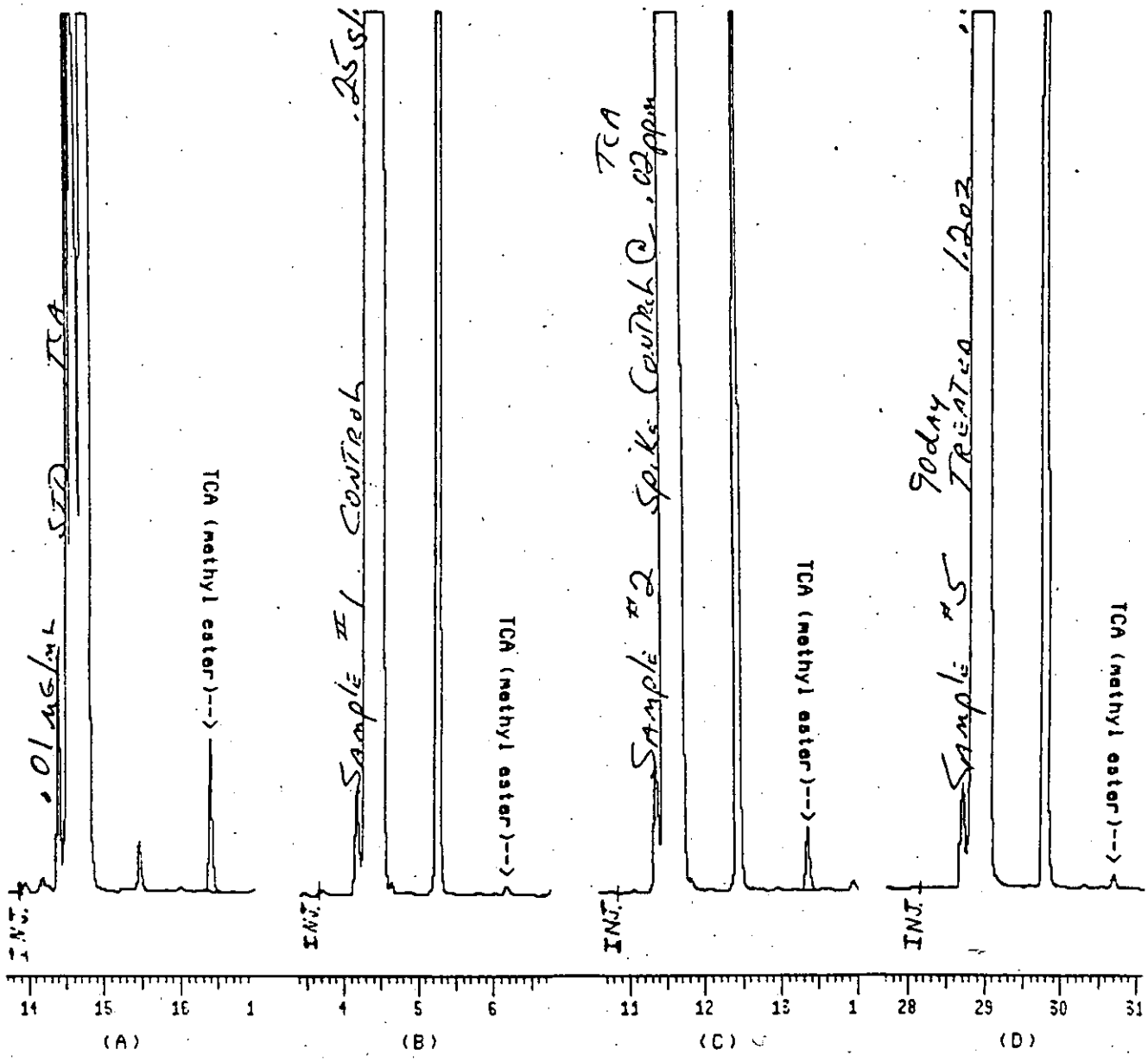
(A) TCA (as methyl ester) standard, 0.02 ug/ml.

(B) Control sample, 0.25 g/ml.

(C) Control sample fortified at 0.02 ppm TCA, 0.25 g/ml.

(D) Control sample fortified at 0.1 ppm TCA, 0.25 g/ml.

TYPICAL GLC CHROMATOGRAMS OF CORN GREEN FORAGE FOR TCA ANALYSIS



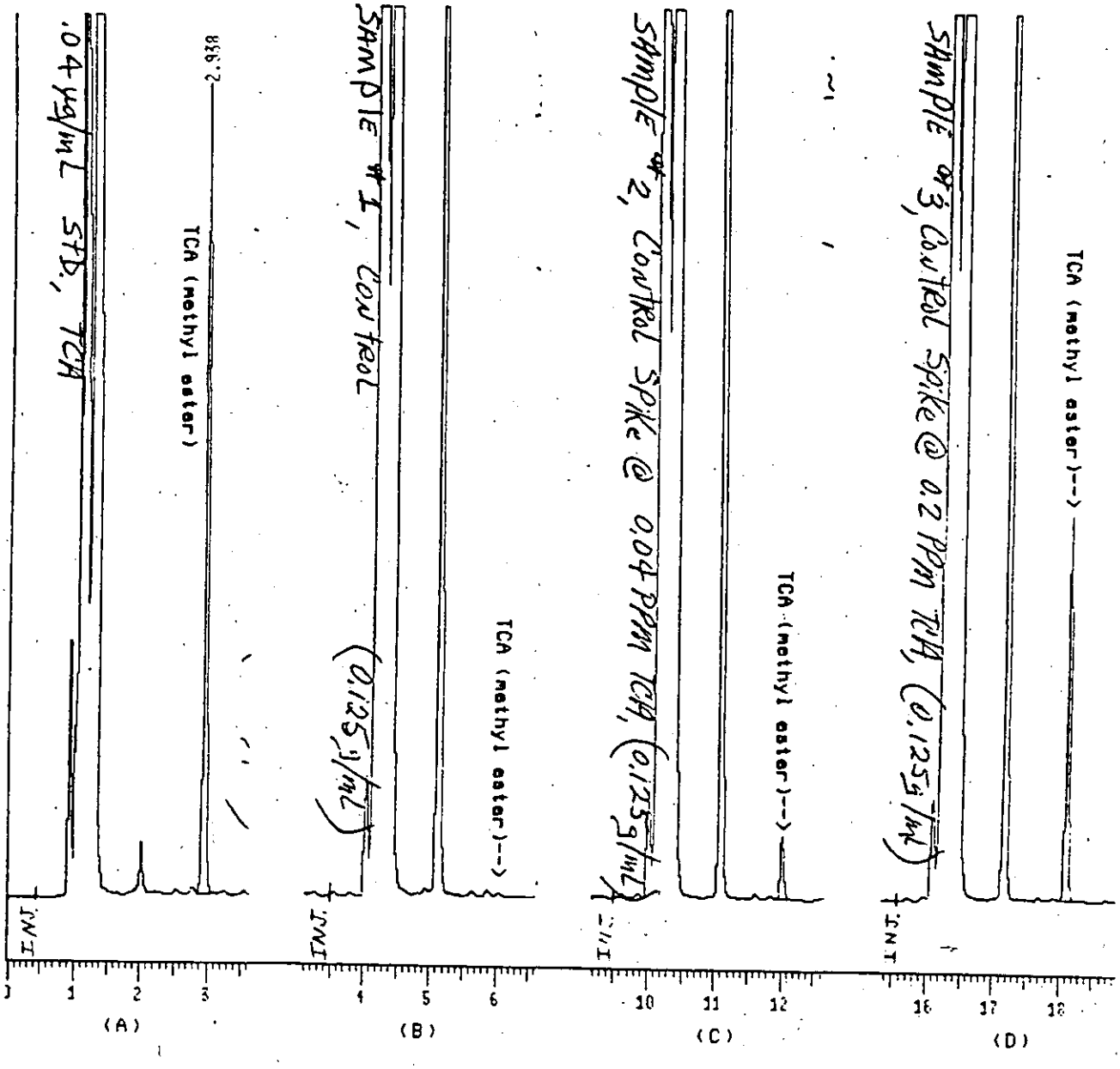
(A) TCA (as methyl ester) standard, 0.01 ug/ml.

(B) Control sample, 0.25 g/ml.

(C) Control sample fortified at 0.02 ppm TCA, 0.25 g/ml.

(D) Field treated sample, 1.2 oz. FORTRESS to soil, 0.25 g/ml.

TYPICAL GLC CHROMATOGRAMS OF CORN STOVER FOR TCA ANALYSIS



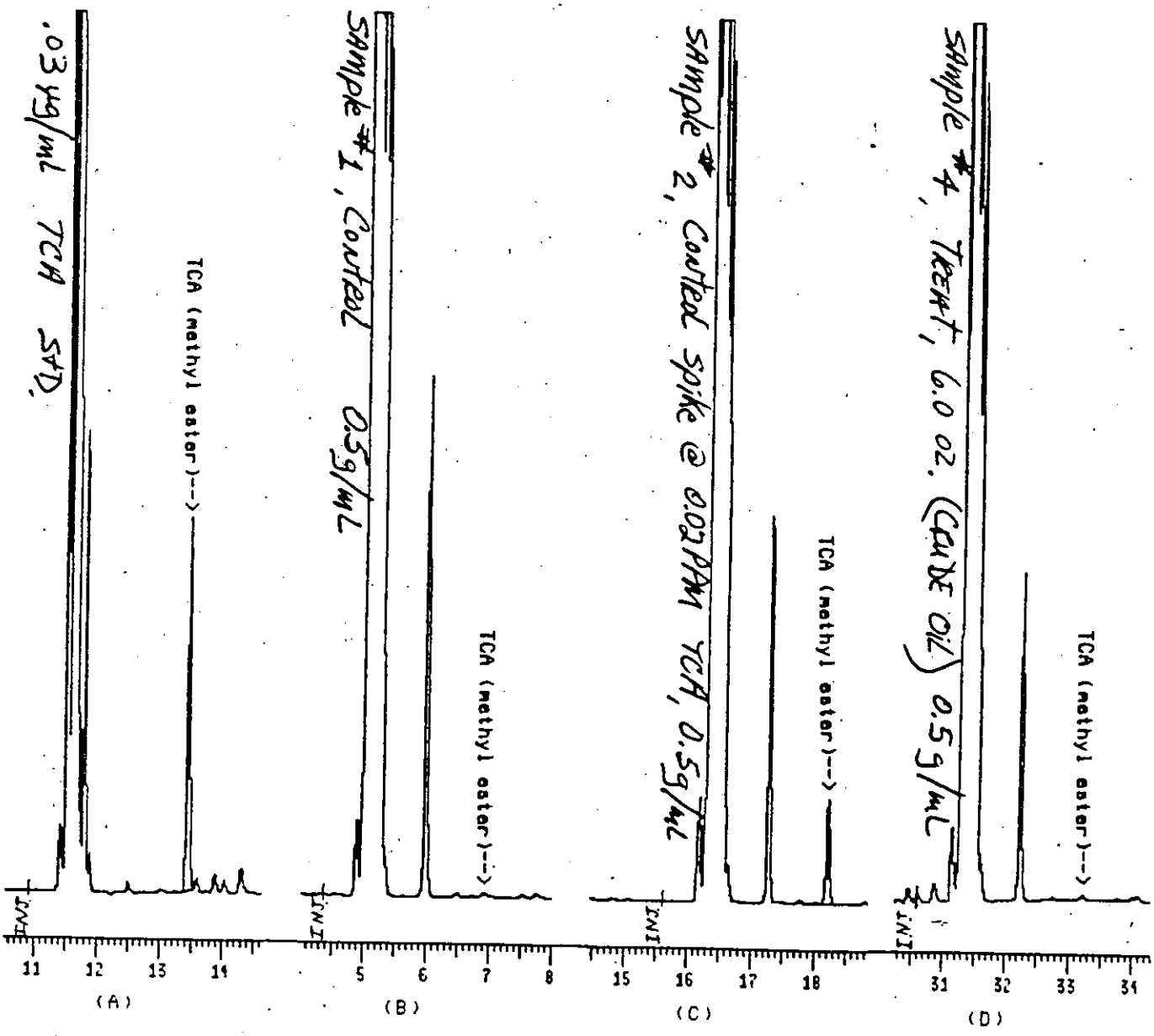
(A) TCA (as methyl ester) standard, 0.04 ug/ml.

(B) Control sample, 0.125 g/ml.

(C) Control sample fortified at 0.04 ppm TCA, 0.125 g/ml.

(D) Control sample fortified at 0.2 ppm TCA, 0.125 g/ml.

TYPICAL GLC CHROMATOGRAMS OF CORN OIL (CRUDE) FOR TCA ANALYSIS



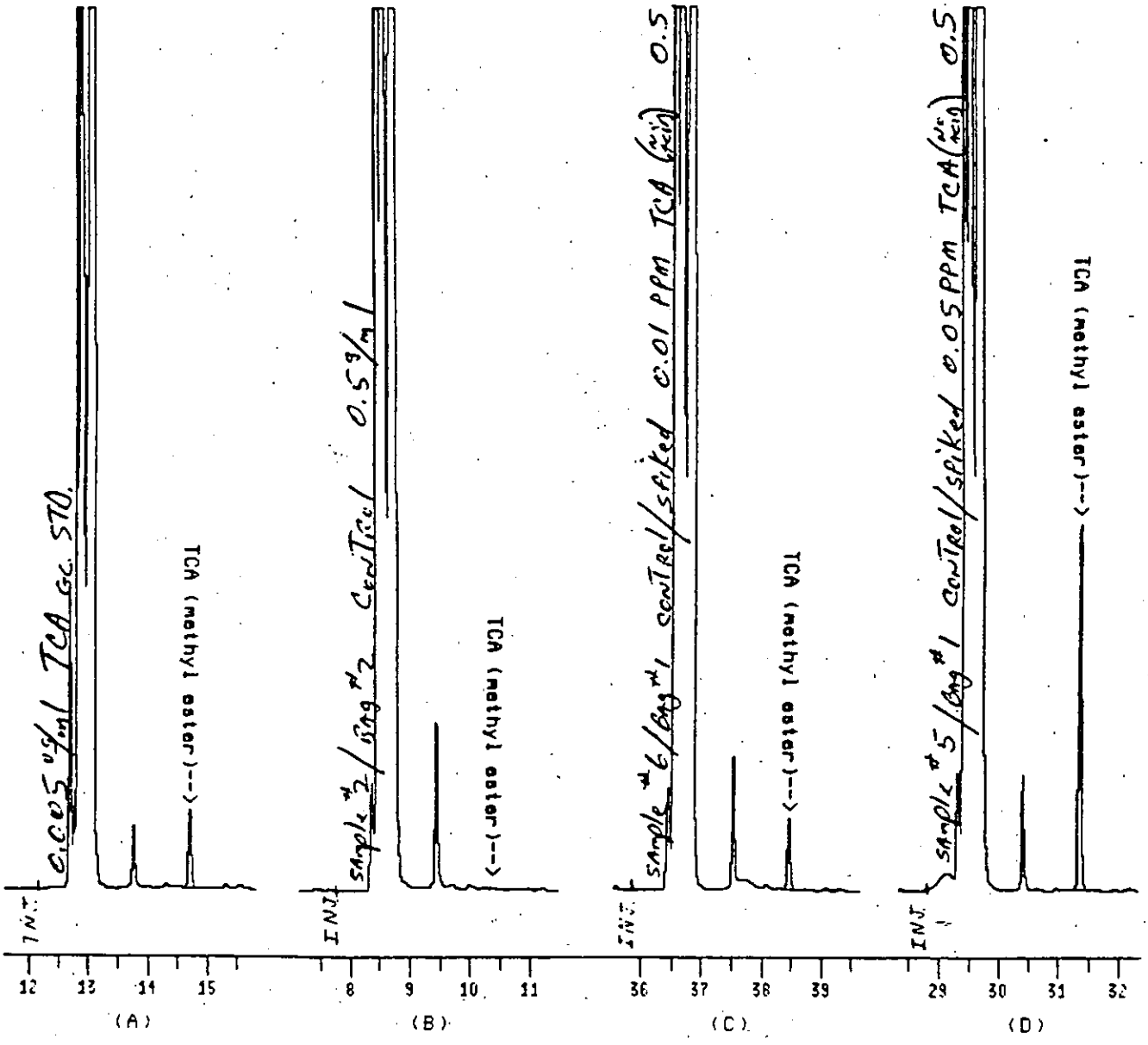
(A) TCA (as methyl ester) standard, 0.03 ug/ml.

(B) Control sample, 0.5 g/ml.

(C) Control sample fortified at 0.02 ppm TCA, 0.5 g/ml.

(D) Treated sample, 6 oz. FORTRESS/1000 feet to soil, 0.5 g/ml.

TYPICAL GLC CHROMATOGRAMS OF SOIL FOR TCA ANALYSIS



(A) TCA (as methyl ester) standard, 0.005 ug/ml.

(B) Control sample, 0.5 g/ml.

(C) Control sample fortified at 0.01 ppm TCA, 0.5 g/ml.

(D) Control sample fortified at 0.05 ppm TCA, 0.5 g/ml.

EXTRACTION EFFICIENCY STUDY

To demonstrate the extraction efficiency of the method, samples of corn forage and stover from the plant metabolism study of DPX-43898 were extracted by this method and by the more exhaustive procedure used for the plant metabolism study (Reference 1). The latter procedure involved two extractions with 90% methanol in water followed by acetone. Total radioactivity extracted was slightly higher for the exhaustive procedure. After concentration by rotary evaporation, all samples were partitioned with hexane to remove lipids. The samples were further concentrated on an N-EVAP and centrifuged to clarify the extracts. These solutions were analyzed directly by HPLC followed by fraction collection and liquid scintillation counting. The former (residue) method gave 99% and 89% of the latter (exhaustive) procedure for trichloroacetic acid in forage and stover, respectively, indicating that the extraction procedure is adequate.

REFERENCE

1. Woodward, M. D., Bolton, E. E., Jr., 1989. "Fate of Soil Applied DPX-43898 in Corn Plants". Du Pont Report No. AMR-1174-88. E. I. du Pont de Nemours and Company, Inc., Wilmington, Delaware.