Residues of 2,4-dichlorophenoxyacetic acid and 2,4-dichlorophenol in soybean forage and grain

SAMPLE INFORMATION

Field application. The field application was directed by Dr. Richard Fawcett, Plant Pathology Dept., Bessey Hall, Iowa State University, Ames, Iowa 50011, (515) 294-1160.

The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was applied preemergent on 2 Jul 1982 at 0, 1 and 2 lbs/acre. Harvest was 8 Nov 1982 for a treatment-harvest interval of 128 days. The control plots were treated with Lasso (2.5 lbs/acre), Sencor (5.0 lbs/acre), and Roundup (1.0 lb/acre).

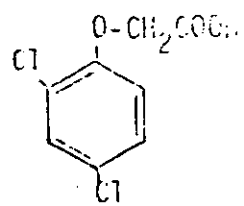
The 2,4-D was applied as the butoxyethanol ester using a custom sprayer employing a 30 GPA spray volume at a speed of 2 miles per hour.

The samples were received at the N.D. Satellite Laboratory on 17 and 20 of Jan 1983. They were shipped in plastic bags and were preserved with dry ice. The samples consisted of soybeans (grain) and plant foliage, which consisted of stems, stalks, leaves and pod shells hereafter referred to as forage. Upon receipt, the grain samples were placed in glass jars, sealed with aluminum foil-lined lids and placed in a freezer at -20°C. The forage was air-dried, ground in a Wiley mill, then stored in the same manner as the grain.

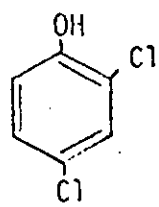
Storage-stability tests. Subsamples (25 g) of soybean grain were prepared by grinding whole soybeans in a coffee mill. Additions of 2,4-D as the butoxyethanol ester, and 2,4-dichlorophenol (DCP), both in acetone, were carried out and the meal thoroughly mixed.

Forage samples were air-dried, then ground through a Wiley Mill (#20 screen size). Subsamples of 15 g were fortified with 2,4-D (butoxyethanol ester) and DCP. Both sets of storage-stability samples were placed in glass sample bottles, sealed with aluminum foil-lined screw cap lids and placed in the freezer with the other field samples. The storage-stability samples were prepared on 3 Feb 1983.

RESIDUE ANALYSIS



2,4-Dichlorophenoxyacetic Acid
(2,4-D)



2,4-Dichlorophenol
(DCP)

Method of analysis. The analysis method is a total residue procedure adapted from Cook et al., J. Agric. Food Chem. 31, 268 (1983).

Special equipment.
(a) Coffee mill, Krups model 203
(b) Wiley mill equipped with #20 mesh screen
(c) Chromatographic column, 10X300 mm, Kontes 420100
(d) Chromatography column with reservoir, Kimax 17810-113
(e) Culture tubes with Teflon-lined screw caps, 25X150 mm, 25X200 mm
(f) Graduated cylinders with Teflon-lined screw caps, 100-mL

REAGENTS AND MATERIALS

(1) Sulfuric acid, ACS reagent grade, concentrated and 5 N solution
(2) Sodium hydroxide, analytical reagent, 5 N, 1 N and 0.5 N solutions
(3) Sodium hydrogen carbonate, ACS reagent, 0.25 N solution
(4) Sodium chloride, analytical reagent
(5) Alumina, Woelm, W-200, acid (Activity II)
(6) Ottawa sand, standard 20-30 mesh
(7) Pesticide grade solvents of hexane, diethyl ether, benzene, methanol and acetone
(8) Diazald reagent (N-methyl-n-Nitroso-p-toluene sulfonamide) Aldrich, 99%
(9) 2-(2-Ethoxyethoxy)ethanol
(10) Florisil, deactivated with 2% water (w/w)
(11) Sodium sulfate, anhydrous
(12) Celite 545, filter aid (leach with 10% aqueous NaOH before use)
(13) Standards: 2,4-dichlorophenol, 99%; from U.S. EPA Reference Standards Repository, Research Triangle Park, NC 27711. Lot 1281. 2,4-dichlorophenoxyacetic acid, butoxyethanol ester, 99%; from U.S. EPA Reference Standards Repository. 2,4-dichlorophenoxyacetic acid, methyl ester, analytical grade; Poly-Science Corporation, Evanston, Illinois.

Analysis procedure. Soybeans were ground in a coffee mill so as to pass through a #20 U.S. standard sieve.

Forage material was air dried to constant weight, then ground in a Wiley mill using a #20 U.S. standard sieve.

(1) Place a 25 g subsample in a 1-L r.b. flask containing an egg shaped magnetic stirrer bar. Add fortification at this point if so desired. Add 85 mL of 5 N H_2SO_4 and sufficient distilled water so that the bar magnet is able to rotate.

(2) Place the flask in a heating mantle resting on a magnetic stir plate, and attach a water-cooled condenser. Stir and heat the mixture at reflux for 2 hr. Begin timing when drops fall from the water condenser.

(3) Cool the hydrolyzed mixture in an ice bath and adjust the pH to 10 or more by adding 85 mL of 5 N NaOH through the condenser. Check the pH with indicator paper and add additional alkali if necessary. Filter

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the mixture with the aid of a slight vacuum through about 0.5 cm of filter aid on a glass-fiber filter disk in a 12.5-cm Buchner funnel. Rinse the flask and filter cake with two 20-30 mL portions of water. Transfer the filtrate to a 500-mL separatory funnel.

(4) Add 50 g of NaCl to the filtrate and adjust the pH of the solution to near 1 by addition of about 20 mL of 5 N H_2SO_4 (check with indicator paper). Without delay, extract the acidified solution with four 50-mL portions of hexanes-diethyl ether (1:1). Avoid vigorous shaking in order to diminish emulsion formation. If an emulsion forms, break it by adding 3 mL of methanol, or apply a slight vacuum to the separatory funne Collect the organic extracts in a centrifuge bottle. If the combined organic extract volume is less than 150 mL, centrifuge the emulsion to recover the remaining solvent. Transfer the organic extract back to a 500-mL separatory funnel, rinse the centrifuge bottle with 10 mL of diethyl ether and add the rinsings to the funnel.

(5) Extract the combined hexane-ether extracts and rinsings successively with 40, 20, and 20-mL portions of 0.5 N NaOH. Collect the alkaline extracts in a 100-mL graduated cylinder with a screw-cap top.

(6) Prepare a 4-g column of alumina, Grade II (4% water deactivated) in diethyl ether, using two cm of Ottawa sand as a top layer.

(7) Add 15 g of NaCl to the graduate cylinder from step 5 and acidify the alkaline extracts to a pH of 3 or lower by adding about 12 mL of 5 N H_2SO_4. Extract with four 12-mL portions of diethyl ether, transferring each ether extract to the alumina column by means of a Pasteur pipe Allow each 12-mL extract to just reach the top of the column bed before adding the next. Avoid getting any of the aqueous layer on the column and do not allow the column to run dry of ether. Collect the ether eluates in a 25X200 mm culture tube. These eluates contain the DCP fraction. Blow the column dry with a gentle stream of air or nitrogen after the last extract has drained.

(8) To the ether eluates in the culture tube add 5 mL of 1 N NaOH and shake for 3 minutes. Allow the layers to separate. Remove and discard the ether layer with a Pasteur pipet. Blow off the last traces of ether with a stream of air (or nitrogen). Add 1 g NaCl, and acidify the alkaline extracts to a pH less than 3 with 12 drops of concentrated H_2SO_4. Check the pH with indicator paper. Add 2.0 mL of benzene, shake for 3 minutes, allow the layers to separate, and transfer the benzene extract to a small screw-cap culture tube using a Pasteur pipet. Analyze by gas chromatograph

(9) Elute the alumina column (from the end of step 7) with 25 mL of 0.2% N aqueous $NaHCO_3$. Collect the eluate in a 25X150 mm culture tube. Add 7 g of NaCl to the eluate, then cautiously add 12 drops of concentrated H_2SO_4. Check with indicator paper for pH of 3 or lower. Extract three times with 5-mL portions of diethyl ether. Transfer the ether extracts to a 100-mL r.b. flask with a Pasteur pipet. This fraction contains the 2,4-D.

(10) Place 1 mL of 30% KOH, 1 mL of 2-(2-ethoxyethoxy)ethanol and 1 mL of diethyl ether in a diazomethane generator. Add about 0.2 g of Diazald. Pass N_2 through this solution and into the fraction containing 2,4-D for 60 sec. Dilute the 2,4-D fraction with 25 mL of benzene and 50 mL of hexane. PERFORM DERIVATIZATION IN HOOD!

(11) Prepare a column by plugging with glass wool, then dry pack with 4 g 2% deactivated Florisil. Cover the Florisil with a 1-cm layer of anhydrous Na_2SO_4. Prewash the column with 50 mL of diethyl ether.

Place a 250-mL r.b. flask under the column and pass the fraction containing methyl 2,4-D through the column. After the solution has passed through the column, rinse the derivatizing flask with 25 mL of diethyl ether-hexane (3:2) and pass the rinse through the column. Reduce the volume of the eluate to 3-5 mL on the rotary evaporator, then transfer to a 10-mL volumetric flask, rinsing with benzene.

(12) Determine the 2,4-D by gas chromatography.

Gas chromatography. Gas chromatography was performed with a Tracor model 550 chromatograph equipped with a Tracor 700 Hall conductivity detector in the reductive halogen mode. The column was glass, 1.8 mX2 mm (i.d.), and packed with 10% OV-1 on 80/100 mesh Gas-Chrom Q. The column was conditioned at 250°C for 63 hr. The detector was operated according to the manufacturers specifications, except that methanol was used as electrolyte solution.

Operating parameters:	Column	120°C (DCP) 170°C (methyl 2,4-D)
	Inlet	200°C
	Outlet	210°C
	Transfer line	270°C
	Detector furnace	900°C
	Carrier gas	He at 30 mL/min (DCP) He at 45 mL/min (methyl 2,4-D)
	Reaction gas	H_2 at 30 mL/min

Standard solutions. Stock solutions of 2,4-D and DCP were prepared in acetone at 100 µg/mL. These were diluted to 1.25 µg/mL for fortification purposes.

Methyl 2,4-D and DCP standards for gas chromatography were prepared in benzene, at 0.05-0.30 µg/mL.

Quantitation. The injection volume was 8.0 µL. The peak height was used to measure recorder response. Each sample and standard was injected twice and the mean of the responses used in the calculations. Samples were diluted to within the range of the standards when necessary. Standards giving a greater and lesser response than the sample were injected for quantitation.

The following illustrates a calculation for the recovery of 2,4-D added to a control sample of soybeans (grain).

Sample	Peak Height (chart units) Injection 1	Injection 2	Average
2,4-D 0.125 µg/mL*	21.5	19.5	20.5
Sample 1973R	18.5	17.5	18.0
2,4-D, 0.100 µg/mL*	18.5	15.0	16.8

*Weight of standards are expressed as weight of 2,4-D free acid, but the chemical form is the methyl ester.

$20.5 - 16.8 = 3.7$

$18.0 - 16.8 = 1.2$

$0.125\ \mu g/mL - 0.100\ \mu g/mL = 0.025\ \mu g/mL$

$$\frac{3.7}{1.2} = \frac{0.025}{(x-0.10)} \qquad x = 0.108\ \mu g/mL$$

correction for control: $0.108 - 0.019 = 0.089\ \mu g/mL$

2,4-D (free acid) in final extract $= 0.089\ \mu g/mL \times 10\ mL$

$= 0.89\ \mu g$

2,4-D recovered $= \frac{0.89\ \mu g \text{ recovered}}{1.25\ \mu g \text{ added}} (100)$

$= 71\%$

Detection limit. The detection limit was determined by the response of the Hall detector in the presence of sample matrix to be 0.05 ppm. At the time when linearity of 2,4-D and DCP were being established, 0.40 ng of either compound produced a response of about 8% FSD at an attenuation of 4 and range setting of 1.

Moisture content of forage. Moisture loss of the forage was determined by weighing 25-30 g of the tissue to the nearest 0.01 g and allowing the material to air dry to a constant weight. The same samples were further dried at 105°C for 2 hr. These samples were not analyzed.

Moisture content of forage (%)	
air drying	45.7 ± 2.9% n = 4
oven drying	50.9 ± 2.6% n = 4

RESULTS AND DISCUSSION

The forage was air dried in order to pass through the Wiley mill. This was necessary for preparation of storage stability samples and efficient extraction of DCP and 2,4-D residues. Forage data are expressed as the air-dried weight of tissue.

All samples contained less than 0.05 ppm of 2,4-D and DCP.

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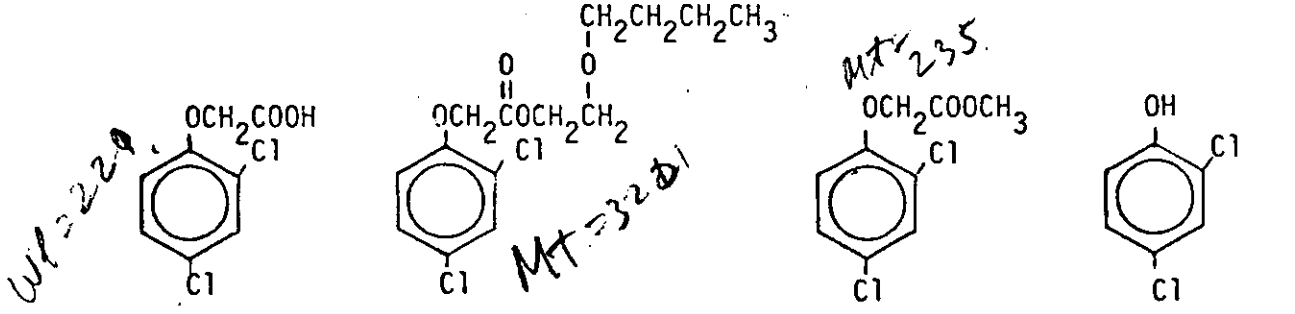
ANALYSIS

1. Method description. The samples were analyzed by two methods.

Method one, described by Cook et al. (1983), allows for the determination of 2,4-D and DCP. The level of quantitation is 0.05 ppm for each analyte. A 2-hour hydrolysis in this procedure converts the LV ester of 2,4-D to the free acid. The hydrolysis also converts metabolites of 2,4-D such as the amino acid conjugates and 2,4-D-glucose ester to the parent herbicide (Chkanikov et al., 1976).

Method 2, described by Yip (1972), is more sensitive than method 1 (0.02 ppm), but does not measure DCP. There is no treatment in the method to convert the LV ester of 2,4-D to the free acid, thus fortifications for recovery purposes must be done with 2,4-D free acid.

The same gas chromatographic method and quantitation method were used for both analysis procedures.



2,4-D free acid form | 2,4-D LV ester (butoxyethyl ester) | 2,4-D methyl ester | 2,4-dichlorophe (DCP)

2. Analytical standards. The butoxyethanol ester of 2,4-D and the DCP standard were obtained from the Pesticides & Industrial Chemicals Repository, Research Triangle Park, NC 27711. The ester was 94.8% pure and was from lot # C837 (code 2960). The DCP was 99.0% pure and was from lot # 1281 (code P306). The stock standard solution of the 2,4-D ester was prepared in acetone at 100 µg/mL. Dilutions of this standard were made in methanol for the purpose of spiking the storage-stability samples. A stock standard solution of DCP was prepared in benzene at 500 µg/mL. This solution was diluted with methanol to 12.5 µg/mL for the purpose of spiking storage-stability samples. Chromatography working standards of DCP were prepared by diluting the stock standard with hexane to give solutions in the range of 0.05 to 0.25 µg/mL.

A standard of 2,4-D methyl ester, "quant" grade, was obtained from Polyscience Corp., Evanston, IL. The stock-standard solution was prepared in acetone at 100 µg/mL and diluted with hexane to 0.02 to 0.15 µg/mL to prepare chromatographic standards. When analytical standards of 2,4-D were derivitized with diazamethane, the peak heights obtained on chromatography were within 2% of those obtained with molar equivalents of the 2,4-D methyl ester standard.

3. Method 1 (Cook et al.)

A. Materials and equipment

Coffee-bean mill
Wiley mill with No. 20 mesh screen
Chromatography column, 10 x 300 mm
Culture tubes with Teflon-lined screw caps, 25 x 150 mm, 25 x 200 mm
Graduated cylinders with Teflon-lined screw caps, 100 mL
Centrifuge bottles, 250 mL
Centrifuge
Glass wool
Celite 545 (filter aid)
Ottawa sand, 20-30 mesh
Volumetric flask, 10 mL
Flasks, round bottom with 24/40 ⅀ joints
Water condensers with 24/40 ⅀ joints
Magnetic stirring bars, oval, 1.5 inch
Magnetic stirring plates
Diazomethane generator (Schlenk & Gellerman, 1960)
Suction flask, 1 L
Buchner funnel, 12.5 cm
Glass-fiber filter disks, 12.5 cm
Separatory funnels, 500 mL

B. Reagents

NaOH, KOH, conc. H_2SO_4, $NaHCO_3$, NaCl, anhydrous Na_2SO_4; analytical reagents
Hexane, diethyl ether, benzene, methanol and acetone; Pesticide grade
Diazald reagent (N-methyl-N-nitroso-p-toluene sulfonamide) Aldrich Chemical Co., 99%
Alumina, Woelm, W-200, acid (Activity II)
Florisil, 2% deactivated with water
2-(2-ethoxyethoxy)ethanol

C. Solutions

0.5, 1.0 and 5 N NaOH
0.25 N $NaHCO_3$
5 N H_2SO_4
30% (w/v) KOH

D. Extraction and Cleanup procedure (Cook, et al.)

Soybeans were ground in a coffee mill so as to pass through a #20 U.S. standard sieve.

Straw was ground in the Wiley mill to pass through a #20 U.S. standard sieve.

(a) Place a 25-g sample in a 1-L r.b. flask. Spike sample at this point if desired. Add an egg-shapead magnetic stirring bar. Add fortification at this point if so desired. Add 85 mL of 5 N sulfuric acid and 100 mL water.

(b) Place the flask in a heating mantle resting on a magnetic stir plate. Attach a water-cooled condenser. Stir and heat the mixture at reflux for 2 hr. Begin timing when liquid begins to fall from the water condenser.

(c) Cool the hydrolyzed mixture in an ice-water bath and adjust the pH to 10 or more by adding 85 mL of 5 N NaOH. Check with pH-indicating paper and add additional alkali if necessary. Filter the mixture with suction through a 0.5-cm pad of filter aid layered over a glass-fiber filter disk. Use a 12.5-cm Buchner funnel. Rinse the flask twice with 25-mL portions of water and filter the rinses. Transfer the combined filtrates to a 500-mL separatory funnel.

(d) Add 50 g of NaCl to the filtrate solution. Adjust the pH of the solution to near 1 by adding 20 mL of 5 N sulfuric acid. Check with pH-indicating paper. Without delay extract the acidified solution with four 50-mL portions of hexane-diethyl ether (1:1). If an emulsion forms, transfer to a 250-mL centrifuge bottle and centrifuge for 5-10 min at 1000 x g. Transfer the organic extracts to another 500-mL separatory funnel.

(e) Extract the combined hexane-ether extracts successively with 40, 20 and 20-mL portions of 0.5 N NaOH. Collect the alkaline extracts in a 100-mL graduated cylinder with a screw-cap top.

(f) Plug the bottom of a 10 x 200 mm chromatography column with a small amount of glass wool. Over the plug place 4 g of alumina, grade II (4% water deactivated) in diethyl ether. Place a 2-cm layer of Ottawa sand over the alumina bed.

(g) Add 15 g of NaCl to the alkaline extract from step (e). Bring the pH of this solution to 3 or less with 12 mL of 5 N sulfuric acid. Check with pH-indicating paper.

(h) Extract the acidified aqueous solution with four 12-mL portions of diethyl ether, transferring each ether extract to the alumina column by means of a transfer pipet. Allow each 12-mL extract to just reach the top of the column bed before adding the next. Avoid getting any of the aqueous layer on the column and do not allow the column to run dry of ether. Collect the ether eluates in a 25 x 200 culture tube. These eluates contain the DCP. Blow the column dry with a gentle stream of nitrogen gas after the last extract has drained.

(i) To the ether eluates add 5 mL of 1 N HaOH and shake for 3 minutes. Allow the layers to separate. Remove and discard the ether layer with a transfer pipet. Blow off the last trace of ether with a stream of nitrogen gas. Add 1 g of NaCl. Acidify the aqueous extract

to a pH of less than 3 with 12 drops of concentrated sulfuric acid. Check with pH-indicating paper. Add 2.0 mL of benzene (accurate volume), and shake three minutes. Allow the layers to separate, and transfer the benzene layer to a small screw-cap culture tube with a transfer pipet. Analyze by gas chromatography for DCP.

(j) Elute the column from step (h) with 25 mL of 0.25 M $NaHCO_3$. Collect the eluate in a 25 x 150 mm culture tube. Saturate the eluate with 7 g of NaCl, then cautiously add 12 drops of concentrated sulfuric acid. Check with pH-indicating paper to assure pH of 3 or less. Extract three times with 5-mL portions of diethyl ether. Transfer the ether extracts to a 100-mL r.b. flask with a transfer pipet. This is the 2,4-D fraction.

(k) PERFORM THIS STEP IN THE HOOD. Prepare a diazomethane generator (Schlenk and Gellerman, 1960). In tube 1 place 10 mL of diethyl ether. In tube 2 place 1 mL of diethyl ether, 1 mL of 30% KOH and 1 mL of 2-(2-ethoxyethoxy)ethanol. To start the reaction place 200 mg of Diazald in tube 2 and place the ether solution from (j) in tube 3. Flush the system gently with N_2 until the ether solution in tube 3 remains yellow (about 10 minutes).

(l) Plug a 10 x 300 mm chromatography column with glass wool. Dry pack with 4.0 of 2% deactivated Florisil. Cover the Florisil with a 1-cm layer of Na_2SO_4. Prewash the column with 50 mL of diethyl ether.

(m) Pass the derivatized fraction from step (k) through the column. Collect in a 250-mL r.b. flask. Rinse the derivatizing flask with 25 mL of diethyl ether-hexane (3:2) and pass the rinse through the column. Reduce the volume of the eluates to 3-5 mL on the rotary evaporator. Do not allow bath to exceed 40°C. Transfer the solution to a 10-mL volumetric flask, rinsing with benzene. Dilute to the mark and mix.

(n) Analyze by gas chromatography.

4. Method 2 (PAM I)

A. Materials and equipment

Homogenizer, Tekmar, SD45
Glass beaker, heavy walled
Buchner funnel, 12.5 cm
Filtering flask, 1 L
Filter disks, glass fiber, 12.5 cm
Glass wool
Flask, round bottom, with 24/40 ȝ joint
Rotary evaporater
Chromatography column, 10 x 300 mm
Volumetric flask, 10 mL

B. Reagents

NaCl, NaOH, KOH, anhydrous Na_2SO_4; Analytical reagent
Solvents: 95% ethanol, $CHCl_3$, isooctane, diethyl ether, hexane; Reagent grade or Pesticide grade when available
Florisil
Diazald (N-methyl-N-nitroso-p-toluene sulfonamide) Aldrich Chemical Co.
2-(2-ethoxyethoxy)ethanol

C. Solutions

10% (w/v) H_2SO_4
Saturated aqueous solution of NaCl
3% (w/v) NaOH
30% (w/v) KOH

D. Extraction and Cleanup Procedure (PAM I method)

Soybeans were ground in a coffee mill so as to pass through a #20 U.S. standard sieve.

Straw was ground in the Wiley mill to pass through a #20 U.S. standard sieve.

(a) Place 50 g of ground sample in heavy-walled beaker. Spike at this point if desired. Add 50 mL 95% ethanol, 20 mL 10% H_2SO_4 and 400 mL $CHCl_3$. Blend briefly with homogenizer. Add ca 20 g NaCl and blend at high speed 2 minutes. (The amount of solvent recommended in the PAM I procedure was insufficient to suspend the sample, thus less sample and additional solvent are used here.)

(b) Filter by suction. Do not wash residue with $CHCl_3$. Remove any aqueous layer in the filtrate by aspiration. Transfer the $CHCl_3$ extract to a graduated cylinder and record the volume. The centrifugation step described in the PAM I procedure was found to be unnecessary with the soybean samples.

(c) Transfer the $CHCl_3$ extract to a 500-mL separatory funnel. Add 25 mL 3% NaOH and 50 mL water. Shake vigorously. Add 10 mL of saturated NaCl and 20 mL 95% ethanol. Shake again. Allow the phases to separate. Draw off the $CHCl_3$ layer and discard. Add 20 mL $CHCl_3$ to the aqueous extract, shake and again discard the $CHCl_3$ layer.

(d) Add to the aqueous solution, 25 mL of 10% H_2SO_4 and check to ascertain acidity is less than pH 3. Extract the acidified solution successively with 50, 25 and 25-mL portions of $CHCl_3$. Drain each $CHCl_3$ extract through a dense glass-wool plug in the apex of a glass funnel. Collect the $CHCl_3$ extracts in a 250-mL r.b. flask. Rinse the plug with $CHCl_3$. A flocculent precipitate is removed by the plug.

(e) Add 3 mL of isooctane to the combined $CHCl_3$ extracts and take to just dryness on the rotary evaporator with the bath temperature at 40°C. Take up the residue with 10 mL diethyl ether.

(f) CARRY OUT THIS STEP IN THE FUME HOOD. Prepare a diazomethane generator (Schlenk and Gellerman, 1960). In tube 1 place 10 mL of diethyl ether. In tube 2 place 1 mL diethyl ether, 1 mL 30% KOH and 1 mL of 2-(2-ethoxyethoxy)ethanol. To start the reaction place 200 mg of Diazald in tube 2 and place the ether solution from step (e) in tube 3. Flush the system gently with N_2 gas until the ether solution in tube 3 remains yellow (about 10 minutes). Standards of 2,4-D free acid may be derivitized at this step in the same volume of ether.

(g) Plug the bottom of a 10 x 300 mm chromatography column with a small amount of glass wool. Add 6 g of Florisil (directly from manufacturers bottle, without further activation) to the column. Tap down and add a 1-cm layer of anhydrous Na_2SO_4. Wash the column with diethyl ether.

(h) The methylated sample from step (f) is diluted with 20 mL of diethyl ether and 50 mL hexane. Pass this solution through the Florisil column. Collect the eluate in a 250-mL r.b. flask. Rinse the solution onto the column with diethyl ether-hexane (3:2). CH_2Cl_2 was not used here as it gave occasional interference during gas chromatographic analysis.

(i) Reduce the volume of the column eluates to 3-5 mL on the rotary evaporator using a water bath at 40°C. Transfer the concentrate to a 10-mL volumetric flask, rinsing with hexane. Dilute to the mark and mix.

(j) Carry out G.C. analysis.

5. Gas chromatography. Gas chromatography was performed with a Tracor model 550 chromatograph equipped with a Tracor 700 Hall conductivity detector in the reductive halogen mode. The column was glass, 1.8 mX2 mm (i.d.), and packed with 10% OV-1 on 80/100 mesh Gas-Chrom Q. The column was conditioned at 250°C for 63 hr. The detector was operated according to the manufacturers specifications, except that methanol was used as electrolyte solution.

Operating parameters:	Column	130°C (DCP) 180°C (methyl 2,4-D)
	Inlet	210°C
	Outlet	270°C
	Transfer line	315°C
	Detector furnace	900°C
	Carrier gas	He at 30 mL/min (DCP) He at 35 mL/min (methyl 2,4-D)
	Reaction gas	H_2 at 30 mL/min

6. Quantitation. The injection volume was 7.0 µL. The peak height was used to measure recorder response. Each sample and standard was injected twice and the mean of the responses used in the calculations. Samples were diluted to within the range of the standards when necessary. Standards giving a greater and lesser response than the sample were injected for quantitation.

a. The following illustrates a calculation for the recovery of 2,4-D butoxyethyl ester added to a control sample of soybean straw analyzed by method 1 (Cook et al.).

Sample	Peak Height (chart units) Injection 1	Injection 2	Average
2,4-D (0.10 µg/mL)[b]	52.0[a]	48.0	50.0
Sample 2195C	52.0	48.0	50.0
2,4-D (0.05 µg/mL)[b]	25.0	24.0	24.5

[a] Chromatogram shown in Figure 7.
[b] Weight of standards are expressed as weight of 2,4-D free acid, but the chemical form is the methyl ester. Sample spiked with butoxyethyl ester of 2,4-D (1.82 µg), equivalent to 1.25 µg 2,4-D free acid.

Subtract background of control: 50 - 1.5 = 48.5 chart units

$$50.0 - 24.5 = 25.5$$
$$48.5 - 24.5 = 24.0$$
$$0.10 - 0.05 = 0.05 \text{ µg/mL}$$

$$\frac{25.5}{24.0} = \frac{0.05}{(X - 0.05)}$$

$$X = 0.097 \text{ µg/mL 2,4-D free acid in final solution}$$
$$\text{2,4-D recovered} = 0.097 \text{ µg/mL (10 mL)}$$
$$\text{" "} = 0.97 \text{ µg}$$
$$\text{Percent recovery} = \frac{0.97 \text{ µg recovered}}{1.25 \text{ µg added}} (100) = 78\% \quad \text{(2195C in Table 4)}$$

b. The following illustrates a calculation for the recovery of 2,4-D free acid added to a control sample of soybean seed analyzed by method 2 (PAM I).

Sample	Peak Height (chart units) Injection 1	Injection 2	Average
2,4-D (0.10 µg/mL)[b]	83[a]	81	82
Sample 2185-Y-14 [b]	75	74	74.5
2,4-D (0.05 µg/mL)	33	30	31.5

[a] Chromatogram shown in Figure 13.
[b] Standards are 2,4-D methyl ester but weight expressed as 2,4-D free acid.

Subtract background of control: 74.5 - 11.8 = 62.7

$$82 - 31.5 = 50.5$$
$$62.7 - 31.5 = 31.2$$
$$0.10 - 0.05 = 0.05$$

$$\frac{50.5}{31.5} = \frac{0.05}{(X-0.05)}$$

X = 0.0809 µg/mL 2,4-D in final 10 mL of extract

2,4-D recovered = 0.0809 µg/mL (10 mL)
" " = 0.809 µg

$$\frac{\text{Volume of } CHCl_3 \text{ recovered (step b)}}{\text{Volume of } CHCl_3 \text{ used (step a)}} = \frac{400 \text{ mL}}{400 \text{ mL}} = 1.0 \text{ (correction factor for loss of } CHCl_3 \text{ extract)}$$

2,4-D added in spike = 1.0 µg
Correction for loss of $CHCl_3$ extract = 1.0 µg (1.0) = 1.0 µg

$$\text{Percent recovery} = \frac{0.809 \text{ µg recovered (100)}}{1.0 \text{ µg added (corrected value)}}$$

Percent recovery = 81% (sample 2185-Y-14, Table 4)

7. Limit of quantitation. Methyl 2,4-D at an equivalent weight of 0.07 ng of 2,4-D free acid gave a peak height of 17-20 chart units. This constituted the limit of detection for the instrument (Figure 1). The limit of quantitation was 0.02 ppm for method 2 (PAM I), which gave a peak height of 170-200 chart units when 7 µL of a 1.0 µg/10 mL 2,4-D standard was injected (0.7 ng total). The limit of quantitation for method 1 was 0.05 ppm. In both methods, the limit of quantitation was the lowest level of fortification which gave reproducible recovery of the spiked material.

DISCUSSION

1. Straw. The foliage material received at the analysis laboratory was dry and did not contain the beans. In this report we have referred to this material as straw but we are uncertain as to what the proper designation should be. The moisture content was determined by an AOAC method using 25-g samples.

Replicate	Moisture (%)
1	4.2
2	4.1
3	4.6
4	4.9
$\bar{x}$ =	4.5

2. Analysis method 1 includes a hydrolytic step. This allows for the release of 2,4-D from certain plant metabolites such as the glucose ester and amino acyl conjugates (Chkanikov et al., 1976). The butoxyethyl ester is also hydrolyzed by this step. Earlier investigators (Bristol et al., 1982; Chow et al., 1971; Cook et al., 1983; Crosby, 1964; Lokke, 1975) have confirmed that a hydrolytic step in the cleanup or extraction step releases bound or conjugated herbicide residues. The hydrolysis also increases the interfering material in the sample matrix and thus a more extensive cleanup protocol is necessary. The increased complexity of the cleanup explains the higher limit of quantitation obtained compared to method 2.

3. Method 2, originally described by Yip (1971), does not include a hydrolytic step and thus does not convert the butoxyethyl ester of 2,4-D to the free acid form. Thus samples fortified with the ester gave low recovery as much of the ester is lost in step c. The ester does not transfer from the $CHCl_3$ phase to the 3% NaOH aqueous phase (Table 4). However, spiking with the free acid form of the herbicide gave adequate recovery (Table 4). The simpler extraction protocol and cleanup gives a lower limit of quantitation than does method 1.

4. The limit of detection for method 1 was 0.05 ppm, which was the lowest level of reproducible recovery above 70%. Fortification at 0.02 ppm gave recoveries of 51 ± 7% with this method for 2,4-D and 50 ± 14% for DCP.

5. Analysis of the samples was begun shortly after arrival in the laboratory and storage stability samples were not analyzed. They will be analyzed after 8 months of storage to supply data for another group of samples from a different growing region.

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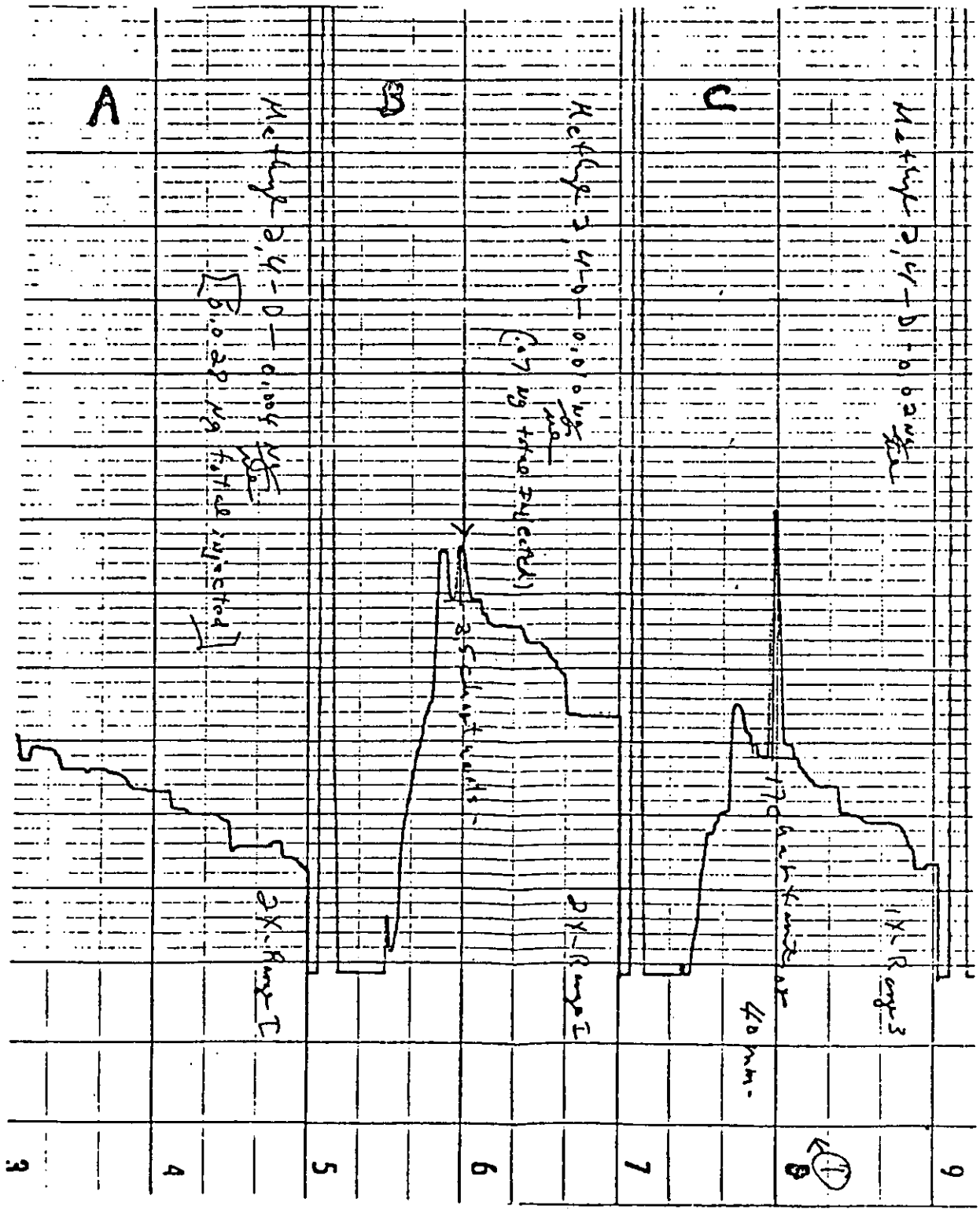

Figure 1. Gas chromatography of methyl 2,4-D standards at or near the limit of detection. 7 µL injection volume.
A. 0.004 µg/mL methyl 2,4-D; attenuation 2, conductivity range 1.
B. 0.01 µg/mL methyl 2,4-D; attenuation 2, conductivity range 1.
C. 0.02 µg/mL methyl 2,4-D; attenuation 1, conductivity range 3.

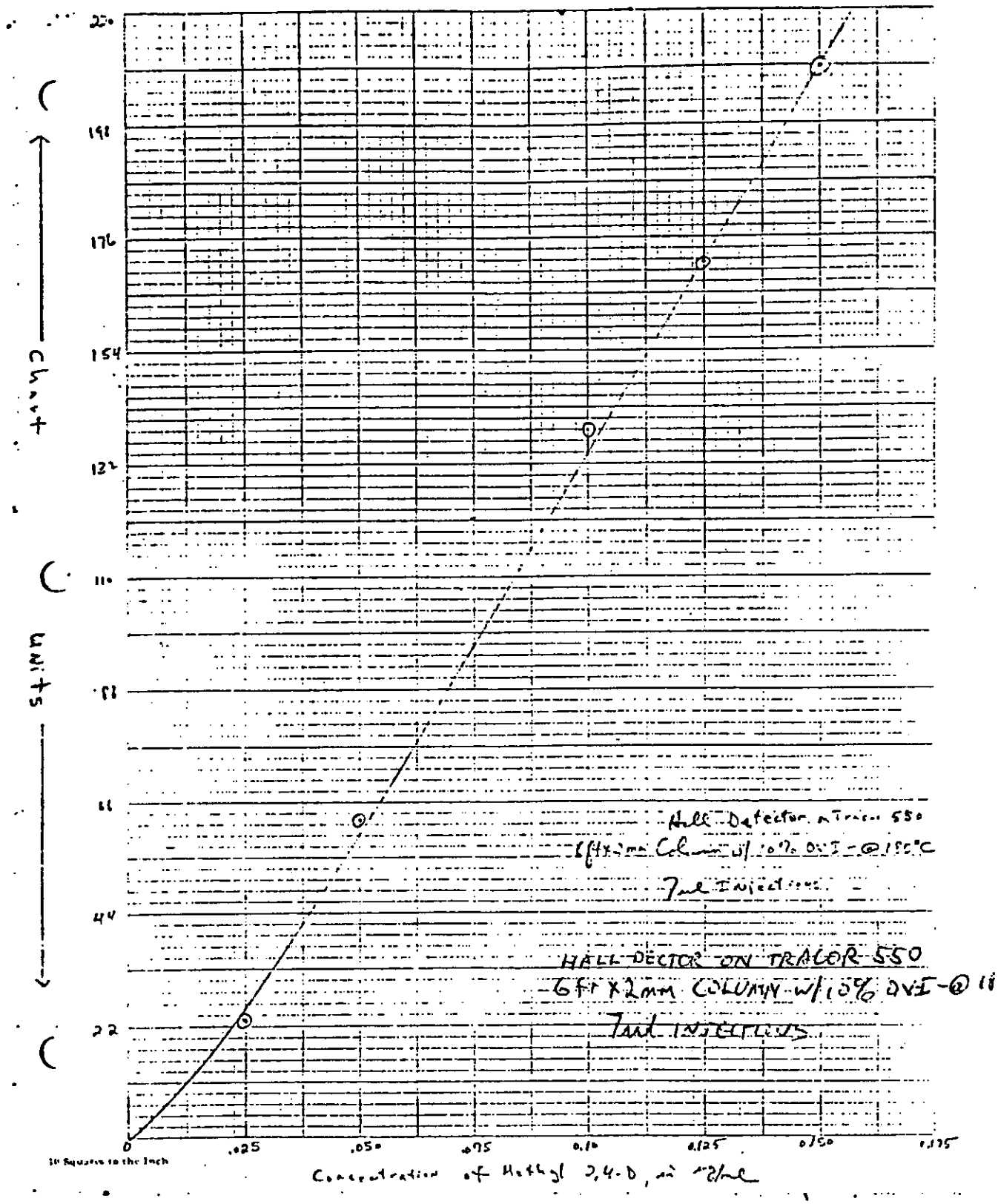

Figure 2. Response curve using methyl 2,4-D.

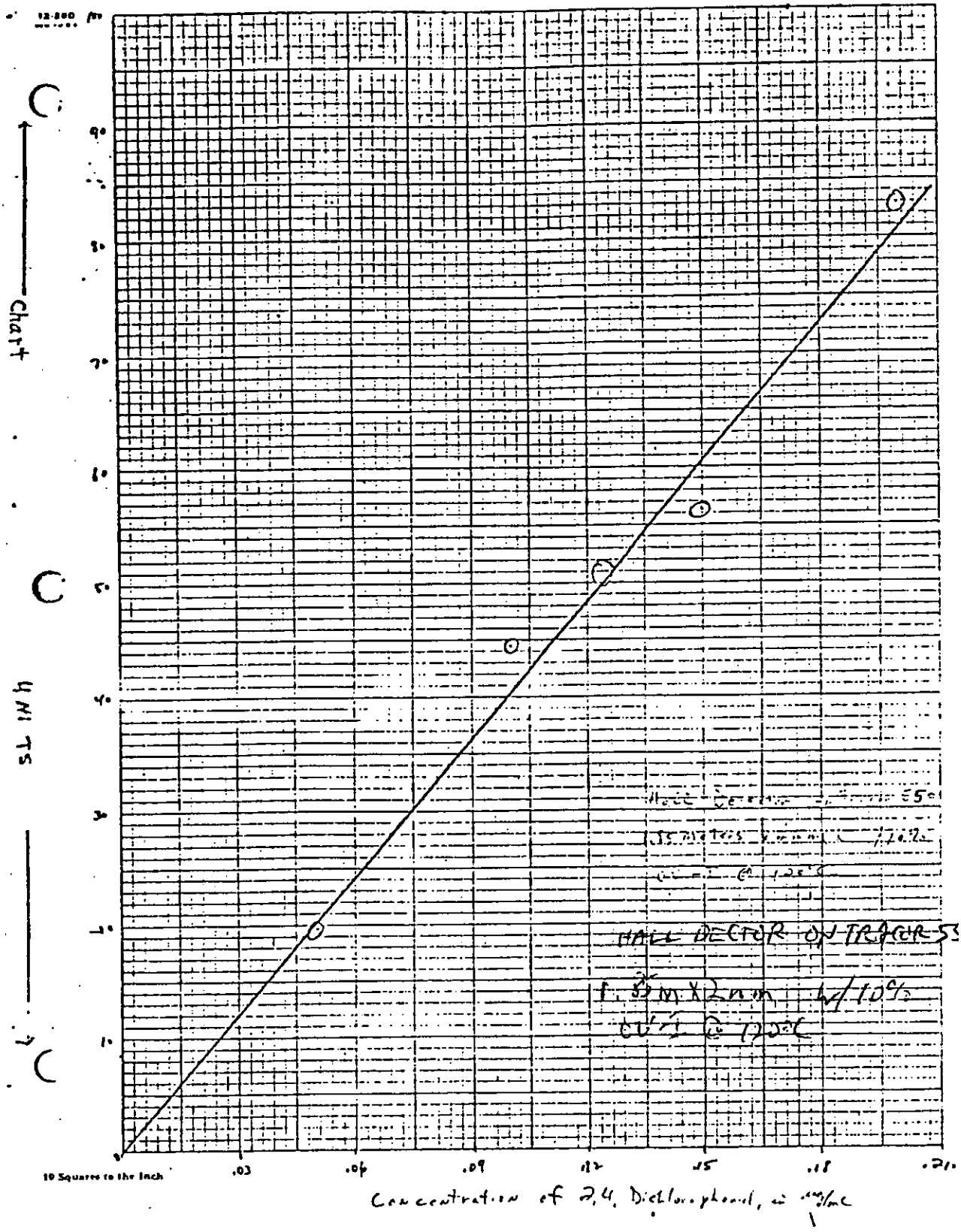

Figure 3. Response curve using 2,4-dichlorophenol (DCP).

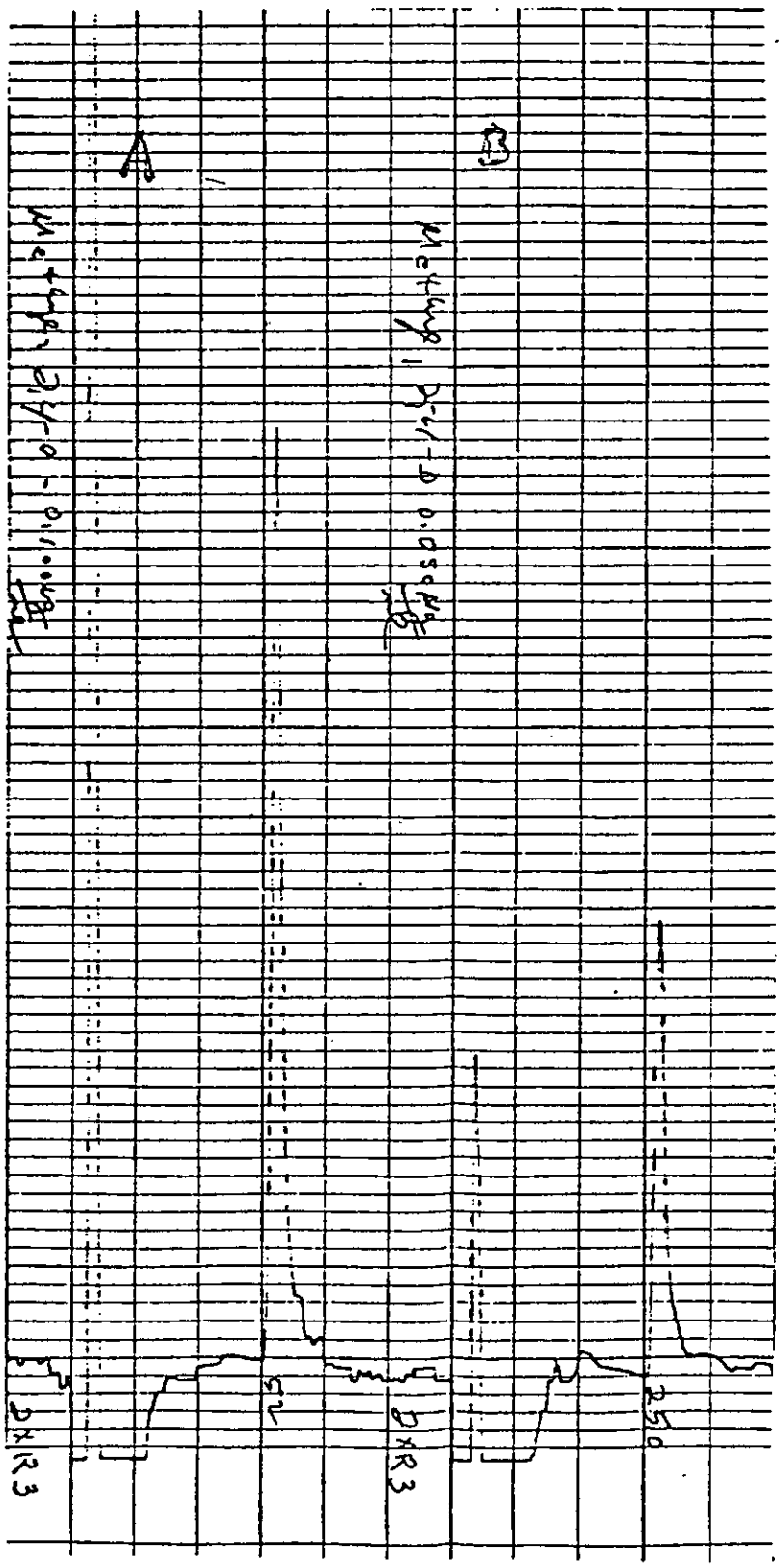

Figure 4. Gas chromatograms of methyl 2,4-D standards. 7 µL injected.
A. 0.10 µg/mL methyl 2,4-D; Attenuation 2, conductivity range 3.
B. 0.05 µg/mL methyl 2,4-D; Attenuation 2, conductivity range 3.

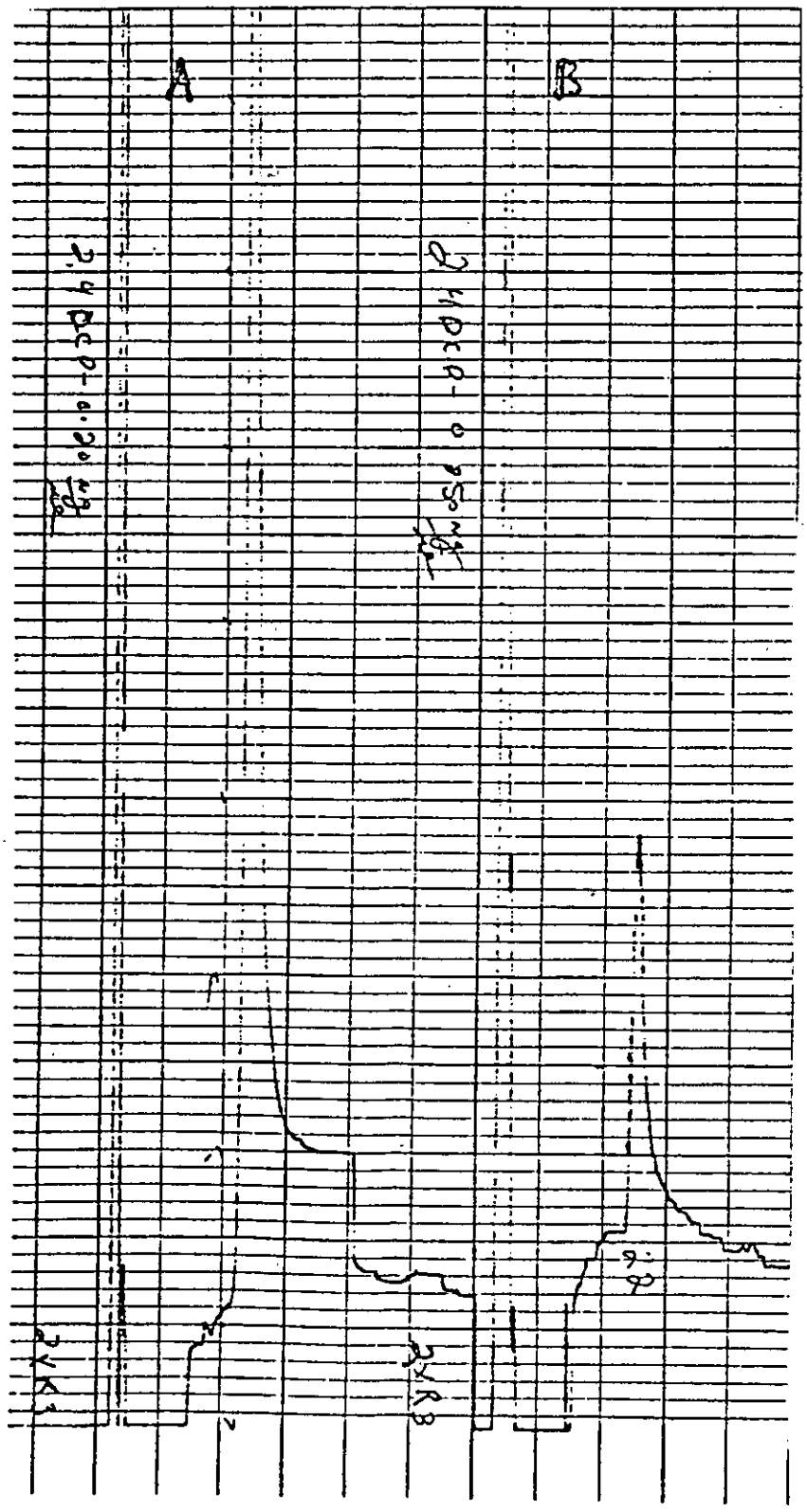

Figure 5. Gas chromatograms of DCP standards. 7 µL injected.
A. 0.20 µg/mL DCP; attenuation 2, conductivity range 3.
B. 0.05 µg/mL DCP; attenuation 2, conductivity range 3.

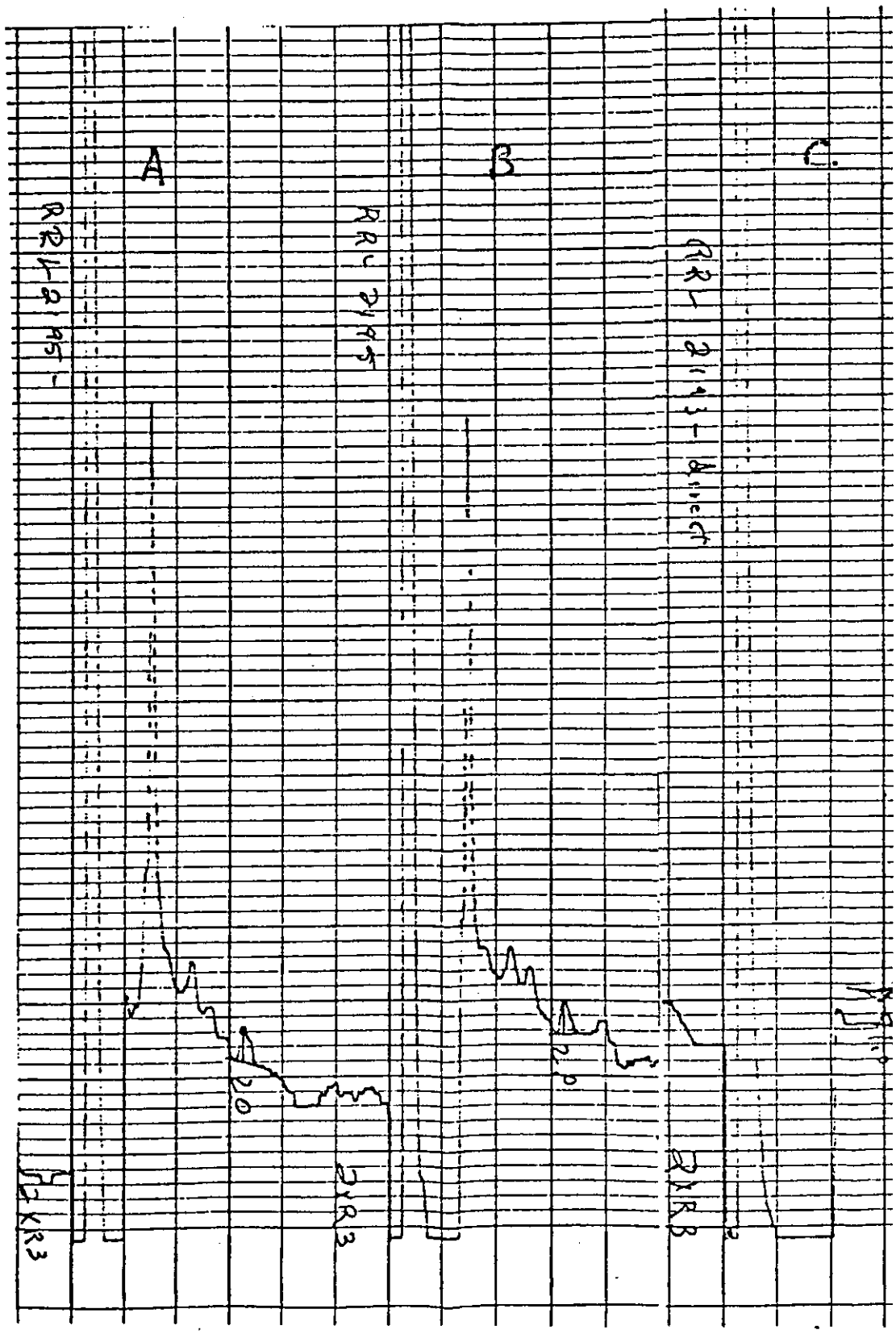

Figure 6. Gas chromatograms of control straw samples 2195 and 2193, analyzing 2,4-D by method 1 (Cook et al.).
Attenuation 2, conductivity range 3.
A. Sample 2195; B. Repeat injection of A. C. Sample 2193.

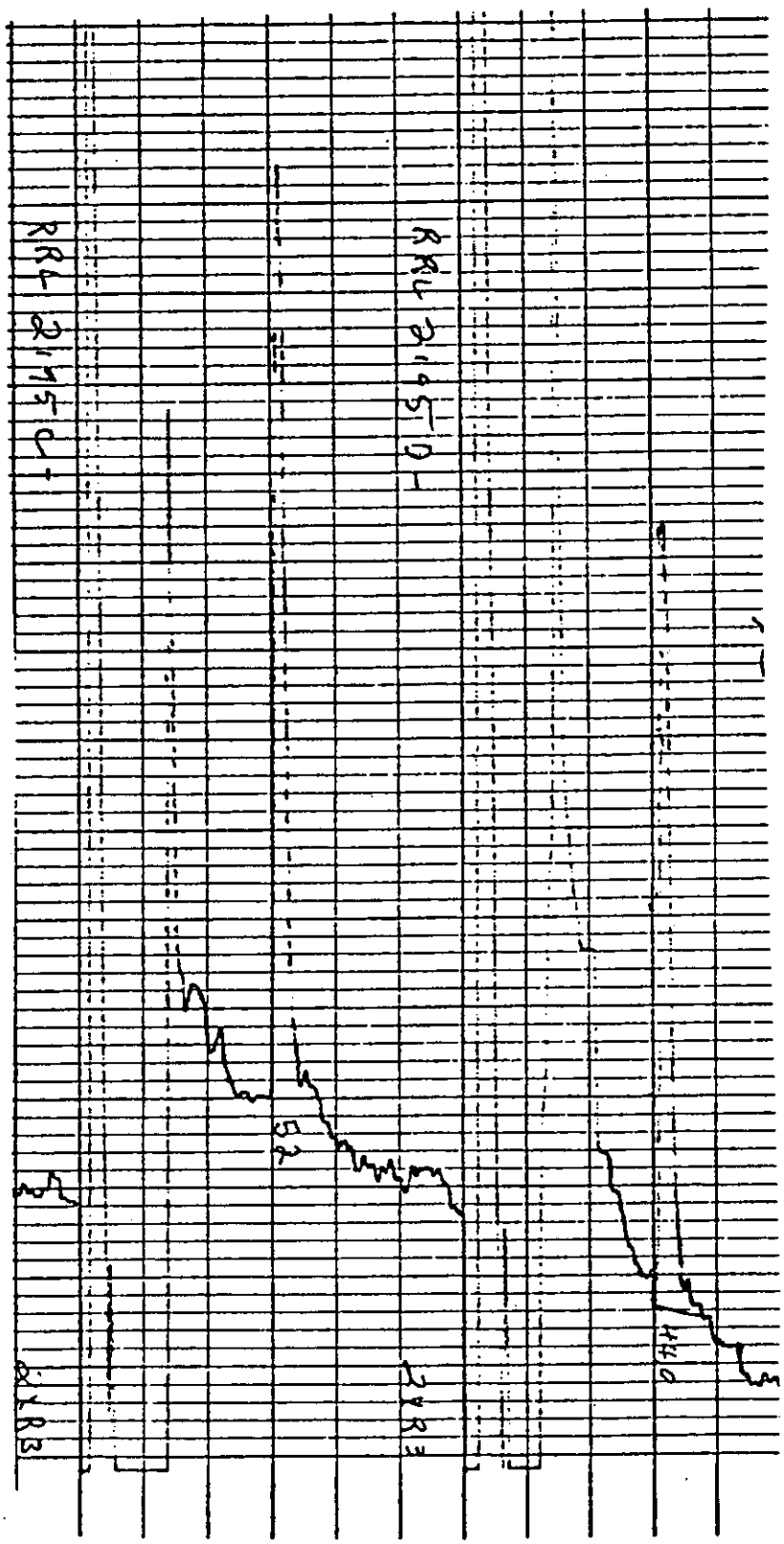

Figure 7. Gas chromatograms of two spiked samples of straw control tissue; samples 2195C and 2195D.
Spiked with 2,4-D LV ester and analyzed by method 1 (Cook et al.).

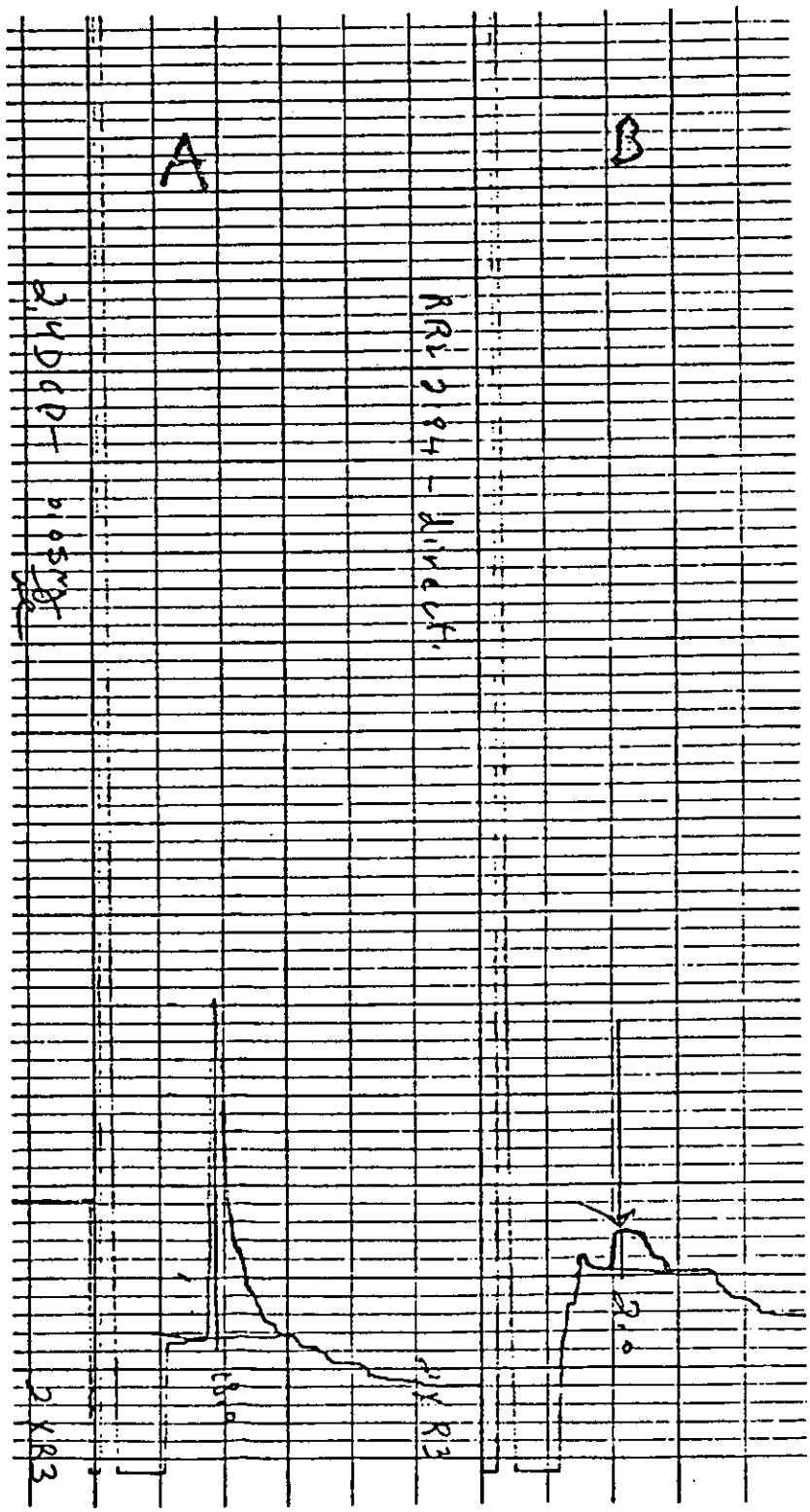

Figure 9. A. Chromatogram of 0.05 µg/mL DCP standard, 7 µL injection volume. B. Control seed sample analyzed for DCP; 7 µL injected.
Attenuation 2; conductivity range 3.

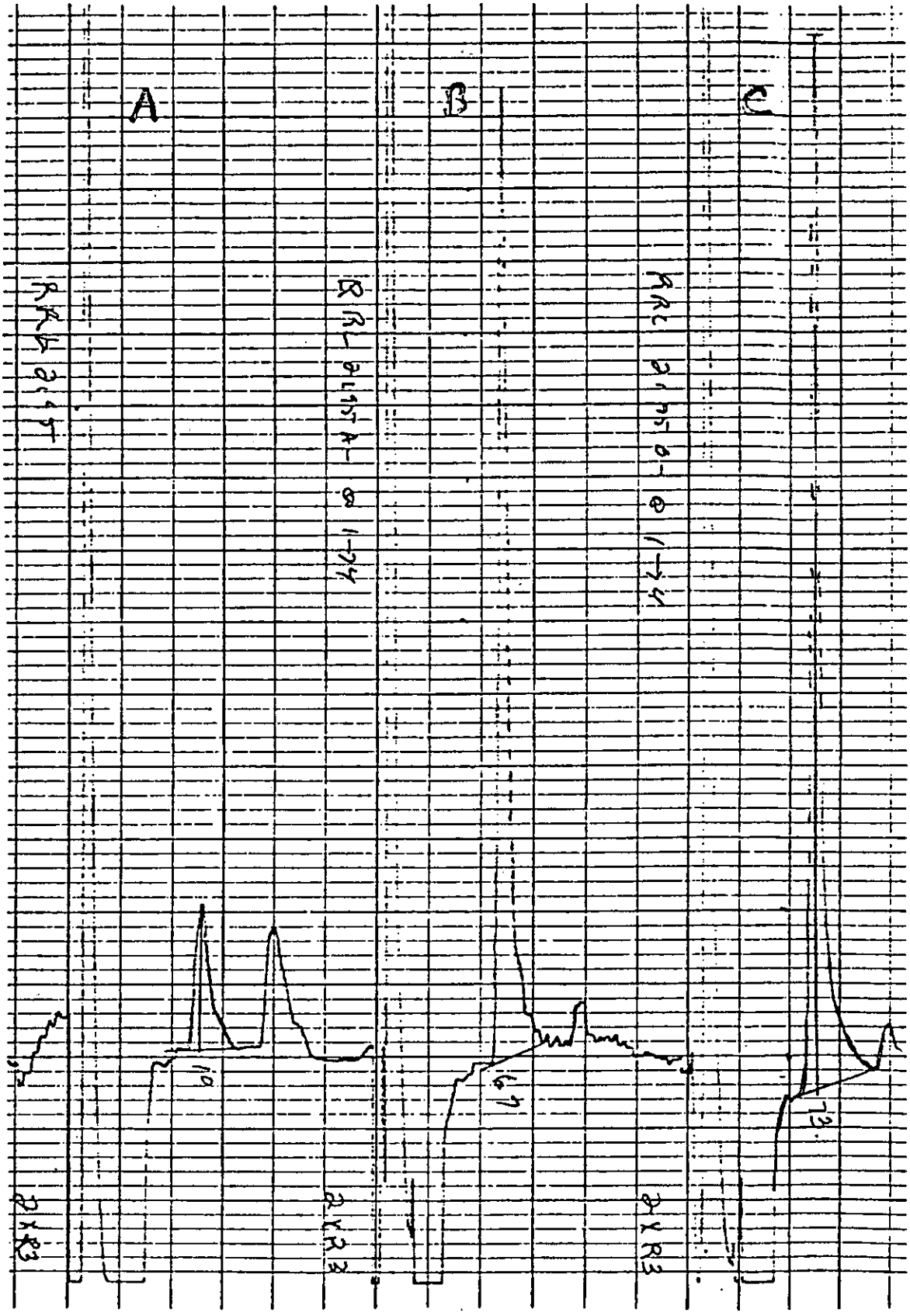

Figure 8. Gas chromatograms of straw samples analyzed for DCP.
A. Straw sample 2195 (control).
B. Straw sample 2195A spiked with 0.05 ppm DCP. Diluted 1 mL to 4 mL prior to analysis.
C. Straw sample 2195B spiked with 0.05 ppm DCP. Diluted 1 mL to 4 mL prior to analysis.
Attenuation 2, conductivity range 3.

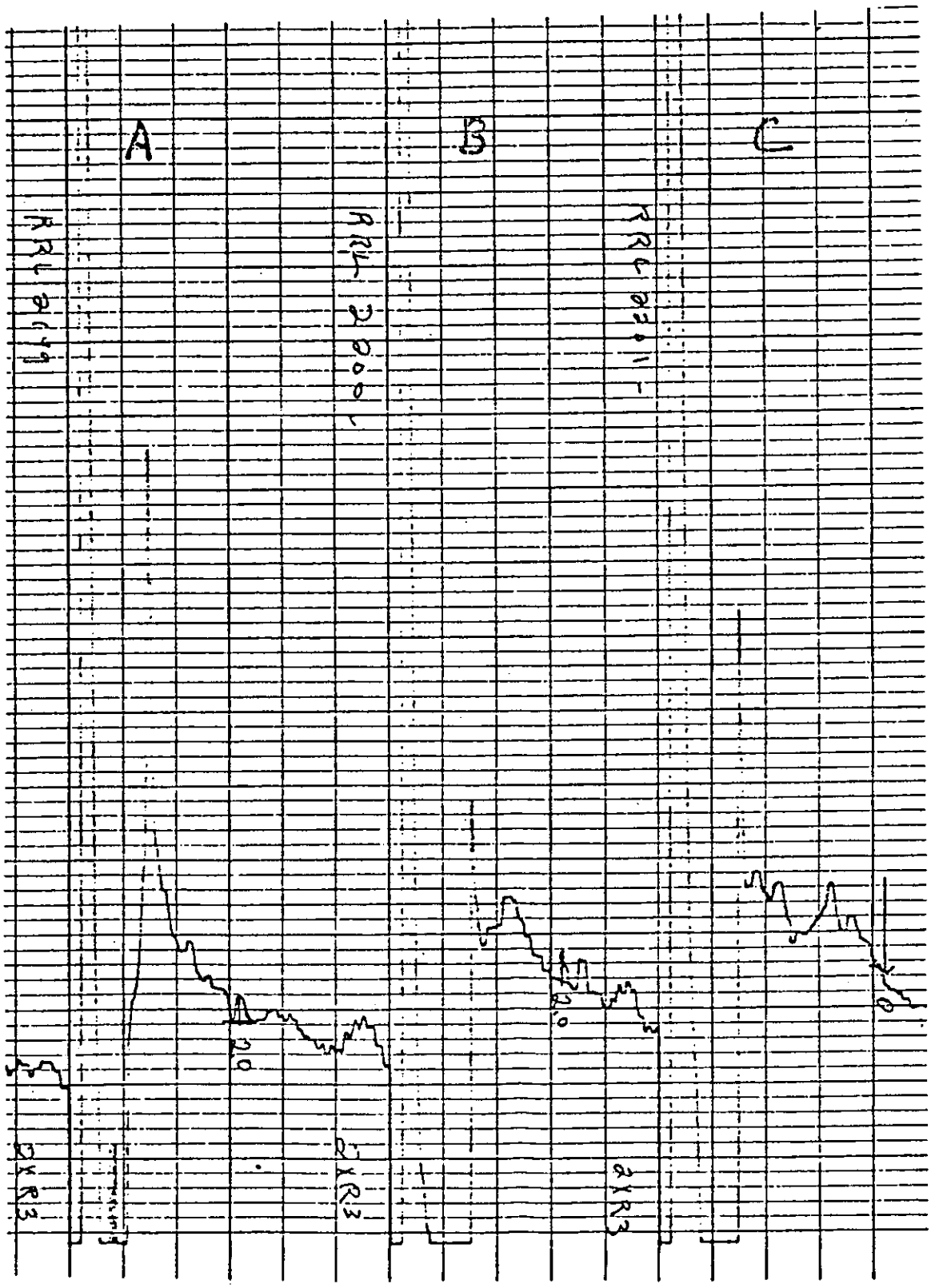

Figure 10. Gas chromatograms of straw samples treated at 2 lb/acre 2,4-D and analyzed for 2,4-D by method 1 (Cook et al.). A. Sample 2199; B. Sample 2200; C. SAmple 2201. Attenuation 2; conductivity range 3.

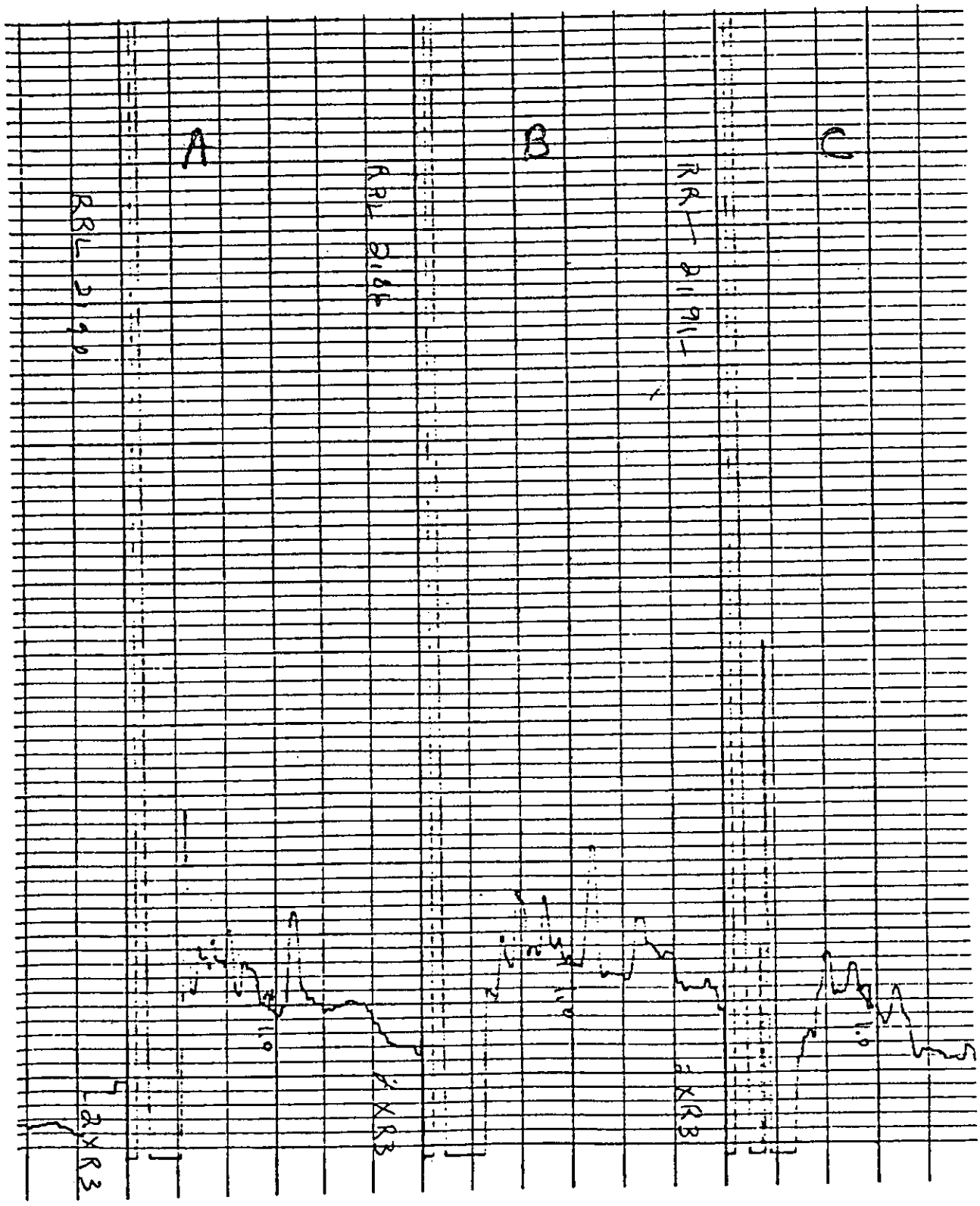

Figure 11. Gas chromatograms of sample 2186 (seed control) and samples 2190 and 2191 (seed from 2 lb/acre plots) and analyzed for 2,4-D by method 2 (PAM I).
Attenuation 2, conductivity range 3.
A. 2190; B. 2186; C. 2191.

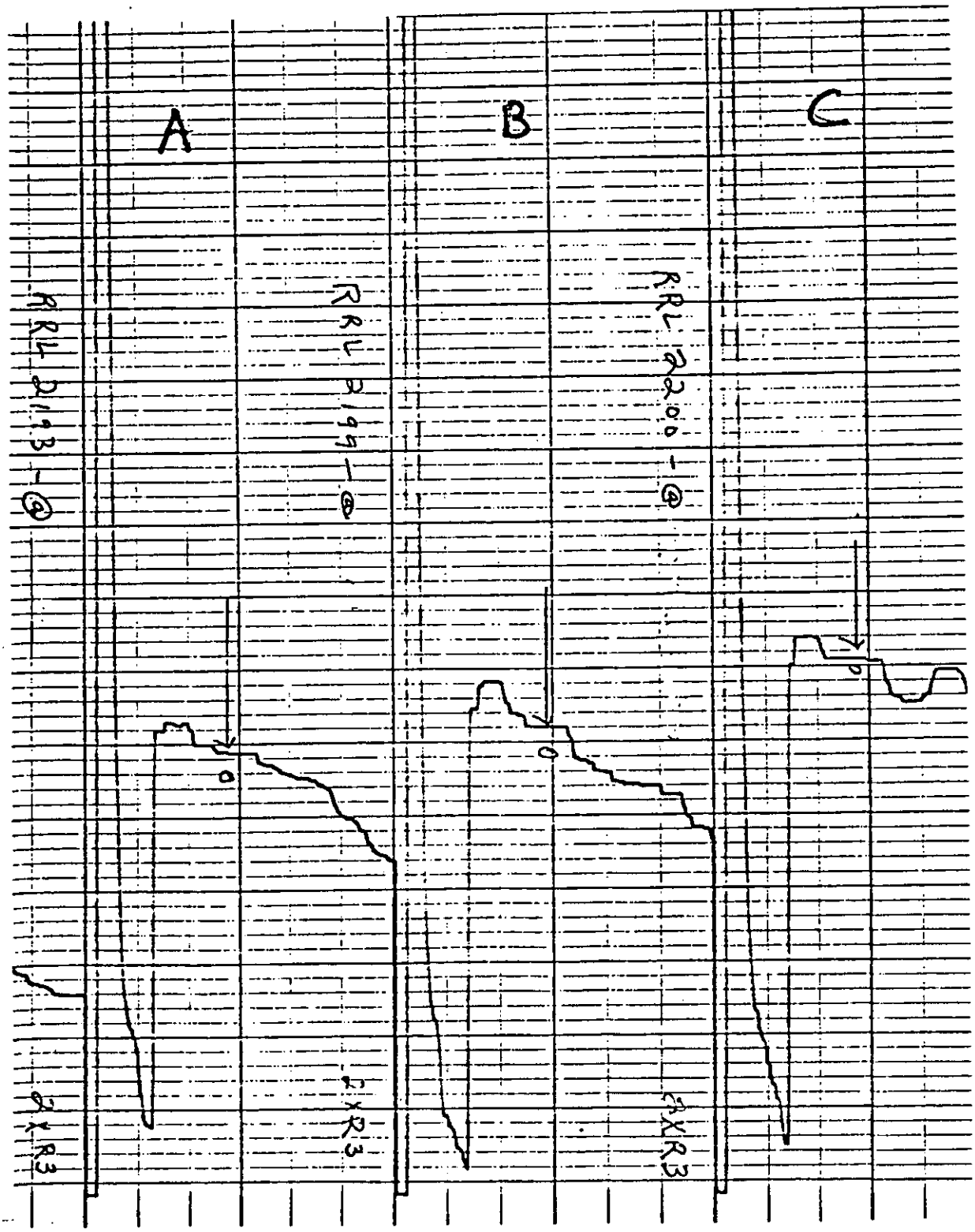

Figure 12. Gas chromatograms of sample 2193 (straw control) and samples 2199 and 2200 (straw from 2 lb/acre plots). Analyzed for 2,4-D by method 2 (PAM I). Attenuation 2, conductivity range 3. A. 2193. B. 2199; C. 2200.

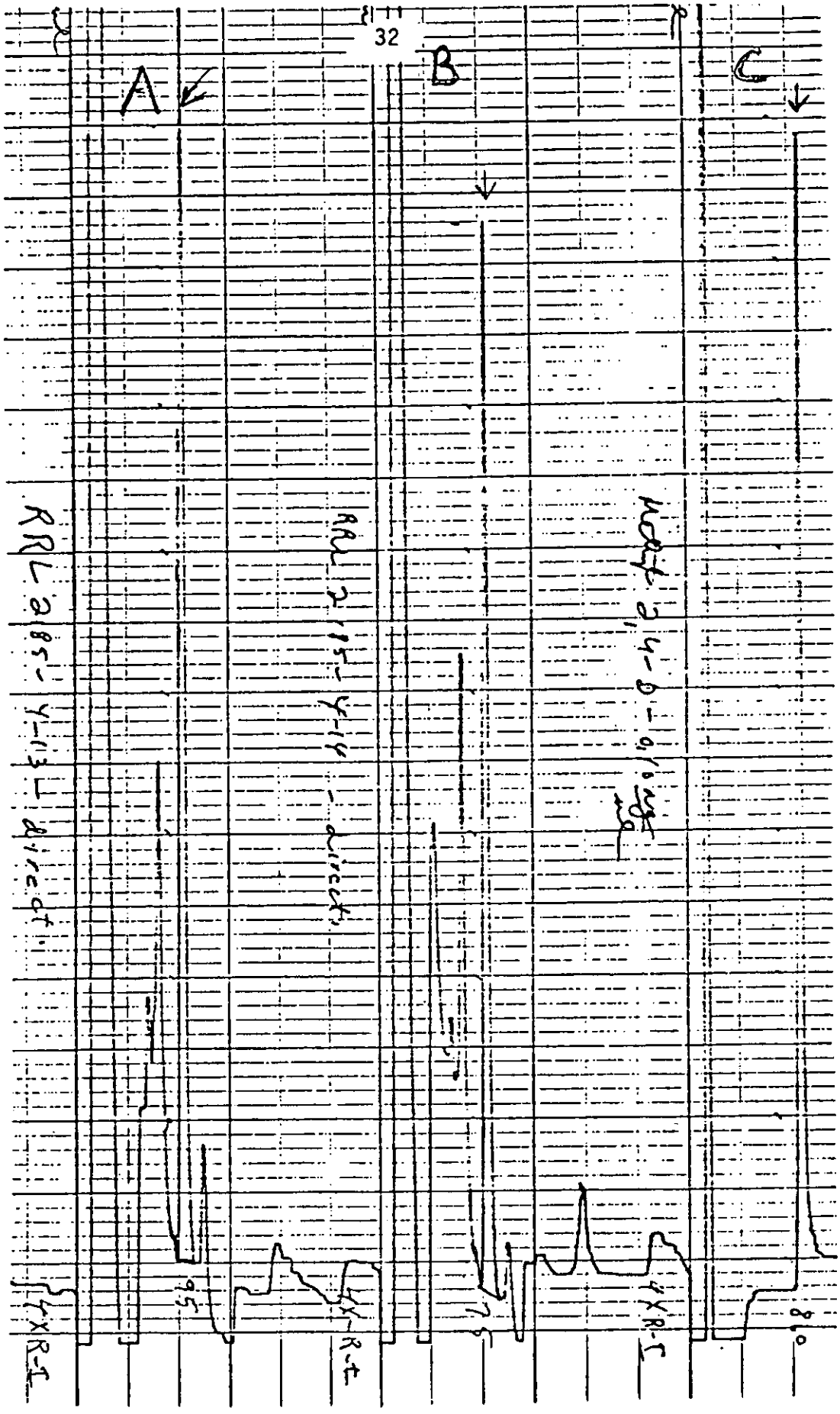

Figure 13. A and B. Chromatograms of two control samples (seed) fortified with 2,4-D (0.02 ppm) and analyzed for 2,4-D by method 2 (PAM I).
C. 0.10 µg/mL methyl 2,4-D standard.
Injection volume: 7 µL. Attenuation 4, conductivity range 1.

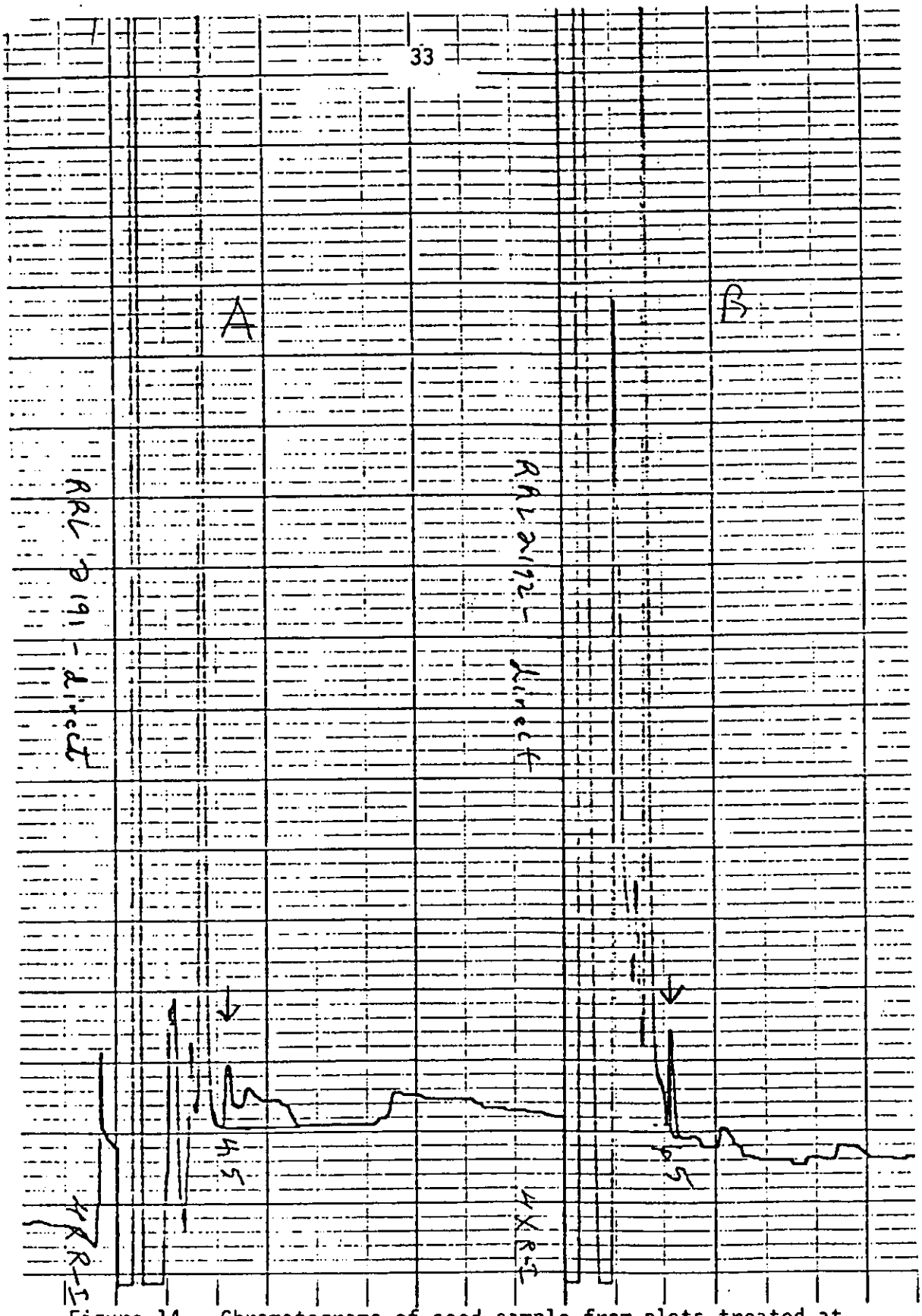

Figure 14. Chromatograms of seed sample from plots treated at 2 lb/acre 2,4-D. Analyzed for 2,4-D by method II (PAM I). A. Sample 2191; B. Sample 2192. Attenuation 4, conductivity range 1. Volume injected: 7 uL.