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THE DOW CHEMICAL COMPANY

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SIMULTANEOUS DETERMINATION OF O,O-DIMETHYL O-(3,5,6-TRICHLORO-2-PYRIDYL)PHOSPHOROTHIOATE (CHLORPYRIFOS-METHYL) AND 3,5,6-TRICHLORO-2-PYRIDINOL AS TOTAL PYRIDINOL IN GRAINS BY GAS CHROMATOGRAPHY

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1. Scope

A method is described for the quantitative determination of 3,5,6-trichloro-2-pyridinol, including that present as a moiety of chlorpyrifos-methyl, in wheat, corn, oats, barley, rice and milo. The procedure was validated for each grain using three combinations of the pyridinol and chlorpyrifos-methyl resulting in total pyridinol levels from 1.9 to 2.5 ppm.

2. Principle

A subsample of grain from a ground sample is comminuted in the presence of methanol. Sodium hydroxide is added and the sample is heated to release the pyridinol, then centrifuged. The pyridinol in an aliquot of the resulting solution is separated from the bulk of co-extractives, converted to the trimethylsilyl ether and quantified by gas chromatography with electron capture detection.

3. Special Equipment

a. Laboratory mill, Wiley standard Model 4, with 2 or 3-mm screen (Arthur H. Thomas Co., Philadelphia, PA).

b. Centrifuge tube, 50-ml, screw-cap (Corning Glass Works, Corning, NY, Cat. No. 8422). Use 24-mm foil-lined caps with liners cut from 0.015 inch Teflon sheet.

c. Homogenizer, Polytron Model PT 10-35, equipped with PT 10ST generator (Brinkman Instruments, Inc., Westbury, NY).

d. Steam bath.

e. Centrifuge, Sorvall Model GLC-2, with head and cups to accomodate item b (Dupont Instruments, Newtown, CT).

f. Capsule vials, screw-cap, 4-dram, 2-dram and 1-dram, Kimble No. 60910 (Owens-Illinois Co., Toledo, OH), with aluminum foil-lined caps.

g. Shaker, reciprocal, Eberbach 6010 (Eberbach Corp., Ann Arbor, MI).

h. Syringe, 10-µl, Hamilton 701 (Hamilton Co., Reno, NV).

i. Gas chromatograph, equipped with 3 mm i.d. x 6 ft borosilicate glass column and electron capture detector. The instrument used was a Barber-Colman Series 5000 with the detector and electrometer replaced by a Tracor linear EC detector.

4. Reagents and Materials

a. Methanol, distilled-in-glass (Burdick and Jackson Laboratories, Inc., Muskegon, MI).

b. Sodium hydroxide, 10% (w/v). Dissolve 10 g of A.C.S. grade pellets in 100 ml of distilled water.

c. Hydrochloric acid, 1:3. Dilute one part concentrated reagent with 3 parts distilled water.

d. Toluene, distilled-in-glass (Burdick and Jackson).

e. Sodium bicarbonate, 1% (w/v). Dissolve one g of A.C.S. grade reagent in 100 ml of distilled water.

f. N,O-bis-trimethylsilyl acetamide ("BSA") (Pierce Chemical Co., Rockford, IL; Cat. No. 38836).

g. Chlorpyrifos-methyl* spiking solution, 10 µg/ml in methanol.

h. 3,5,6-Trichloro-2-pyridinol* stock solution, 1 mg/ml in methanol.

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*Obtain from Sampling Coordinator, Agricultural Products Department, Dow Chemical U.S.A., P. O. Box 1706, Midland, Michigan 48640.

i. 3,5,6-Trichloro-2-pyridinol spiking solution, 5 µg/ml in methanol.

j. 3,5,6-Trichloro-2-pyridinol calibration standards. By appropriate dilution of the stock solution with distilled-in-glass toluene, prepare solutions covering the concentration range from 0.01 to 0.08 µg/ml.

k. Gas chromatographic column packing, 3% OV-7 on 100/120 mesh Gas Chrom Q (Applied Science Laboratories, Inc., State College, PA, Cat. No. 12710).

l. Silyl-8 GLC column conditioner(Pierce Chemical Co., Cat. No. 38014).

5. Gas Chromatography

a. Column

1. Fill a column with packing and condition at 225°C for 72 hours with a carrier gas flow of 70 ml/min.

2. Lower the temperature to 200°C and inject 50 µl of Silyl-8 onto the column. Allow to continue conditioning for at least 16 hours.

3. Lower the temperature to operating level, connect the column to the detector and allow to stabilize.

b. Typical Operating Parameters

1. Carrier gas: Nitrogen, 70 ml/min.

2. Purge gas: Nitrogen, 5 ml/min.

3. Temperatures (°C): Column - 150; injector - 200; detector - 350.

4. Linearizer settings: Standing current - 2×10^{-10} amp; relative pulse width - 4.0 microseconds; attenuator - 8.

c. Procedure

1. Fill the needle of a 10-µl syringe with toluene, using the conventional pumping action to eliminate entrapped air.

2. Carefully draw 2 µl of sample into the syringe.

3. Inject the sample into the column as rapidly as possible.

6. Preparation of Calibration Curve

a. Chromatograph 2-µl aliquots of the calibration standards (Note a).

b. Measure the heights of the peaks obtained, in terms of percent of full-scale recorder deflection.

c. Plot peak heights vs. corresponding concentrations of 3,5,6-trichloro-2-pyridinol.

A typical calibration curve is shown in Figure 1. Chromatograms from derivatized standards are presented in Figure 2.

7. Determination of Percent Recovery

a. Procedure

1. Put control grain through a Wiley mill.

2. Weigh a 5-g subsample into a 50-ml centrifuge tube.

3. Spike the sample at the desired concentrations of chlorpyrifos-methyl and 3,5,6-trichloro-2-pyridinol, using appropriate aliquots of the respective spiking solutions.

4. Add 15 ml (less total volume of spiking solutions used) of methanol, cool in an ice bath for 5 minutes and homogenize for half a minute. Rinse the generator with 5 ml of methanol, collecting the rinsings in the centrifuge tube.

5. Add 5 ml of 10% sodium hydroxide, cap the tube (Note b), mix by shaking and immerse in a steam bath to a depth of 1 1/2 inches for 30 minutes, shaking at 10-minute intervals. Cool to room temperature and centrifuge for 5 minutes at 3000 rpm.

6. Pipet 1 ml of the supernatant solution into a 4-dram vial. Add 4 ml of 1:3 hydrochloric acid and 10 ml of toluene. Cap the vial, shake for 2 minutes and centrifuge for 1 minute at 2000 rpm.

7. Pipet 4 ml of the toluene phase into a 2-dram vial. Add 2 ml of bicarbonate solution, cap the vial, shake for 2 minutes and centrifuge for 1 minute at 2000 rpm. Discard the toluene phase (Note c).

8. Pipet 1 ml of the aqueous phase into a 2-dram vial. Add 1 ml of 1:3 hydrochloric acid and 2 ml of toluene, cap the vial, shake for 2 minutes and centrifuge for 1 minute at 2000 rpm.

9. Transfer 1 ml of the toluene solution to a 1-dram vial, add 1 µl of "BSA", cap the vial and allow to stand at room temperature for 10 minutes.

10. Inject 2 µl of sample into the gas chromatograph (Note d).

b. Calculations

1. Measure the height of the peak obtained in terms of percent of full-scale deflection.

2. Determine the concentration of 3,5,6-trichloro-2-pyridinol in the solution injected by reference to the calibration curve. Multiply the concentration value by 50 to obtain gross ppm in the fortified sample.

3. Analyze an unfortified control sample, using the procedure described above, and calculate the blank value, if any, in terms of equivalent ppm 3,5,6-trichloro-2-pyridinol.

4. Calculate the percent recovery as follows:

$$\text{percent recovery} = \frac{(\text{ppm in spiked sample} - \text{ppm blank}) \times 100}{\text{ppm Added}}$$

Recoveries were determined for samples spiked with chlorpyrifos-methyl only and samples spiked with two combinations of chlorpyrifos-methyl and 3,5,6-trichloro-2-pyridinol. The average recovery for 19 determinations was 99 percent (Table I).

Typical chromatograms from recovery studies involving the six grains are shown in Figures 3 through 8.

8. Analysis of Samples of Grain Treated With Chlorpyrifos-methyl

a. Procedure

1. Analyze samples using the procedure described for recovery determinations (Section 7a), omitting addition of spiking solution(s).

b. Calculations

1. Measure peak heights, determine concentrations of 3,5,6-trichloro-2-pyridinol in the solutions injected and calculate gross ppm values as described for recovery determinations (Section 7b, steps 1 and 2).

2. Subtract any significant blanks (in terms of equivalent ppm of 3,5,6-trichloro-2-pyridinol), obtained from analyses of control samples, to obtain net ppm of the pyridinol.

3. Calculate the corrected concentration of total 3,5,6-trichloro-2-pyridinol as follows:

$$\text{ppm total 3,5,6-trichloro-2-pyridinol} = \frac{\text{net ppm} \times 100}{\text{percent recovery}}$$

9. Notes

a. Prior to chromatographing a series of calibration standards, condition the column by injecting the most concentrated standard until the response is consistent. Best precision is obtained if a constant interval of 12 minutes between injections is maintained.

b. Examine centrifuge tubes carefully and reject those with imperfections which result in the loss of methanol when the capped tubes are heated. Marking the liquid level in the tube before heating allows detection and rejection of samples that lose methanol.

c. The toluene can best be removed using a disposable pipet connected to a vacuum line. Removal of a small amount of the aqueous phase, in order to ensure complete removal of the toluene, is permissible.

d. When chromatographing samples, observe the precautions regarding column conditioning and injection interval given in Note(a). Check calibration frequently.

* *

This method has been found to be satisfactory for its intended purpose when used by a qualified analyst. However, users are cautioned to confirm its suitability for themselves. Written permission from Dow Chemical U.S.A. is required before it may be published as a whole or in part.

TABLE I. RECOVERY OF 3,5,6-TRICHLORO-2-PYRIDINOL ADDED TO GRAIN IN FREE FORM AND/OR AS A MOIETY OF CHLORPYRIFOS-METHYL.

Grain	ppm Added			ppm Pyridinol Found	Percent Recovery
	Chlorpyrifos-Methyl	Pyridinol	Total (as Pyridinol)		
Wheat	4.0	0	2.46[1/]	2.52	102
Oats				2.40	98
Barley				2.51	102
Corn				2.46	100
Milo				2.48	101
Rice				2.56	104
Wheat	3.0	0.5	2.35	2.35	100
Wheat				2.52	107
Oats				2.14	91
Barley				2.31	98
Corn				2.21	94
Milo				2.29	97
Rice				2.32	99
Wheat	1.5	1.0	1.92[2/]	1.89	98
Oats				1.82	95
Barley				1.90	99
Corn				1.89	98
Milo				2.00	104
Rice				1.97	103
				Mean =	99±2[3/]

[1/] Spiked samples stored in refrigerator for 16 hours before analysis.

[2/] Spiked samples stored in refrigerator for 64 hours before analysis.

[3/] 95% confidence limit of the mean.

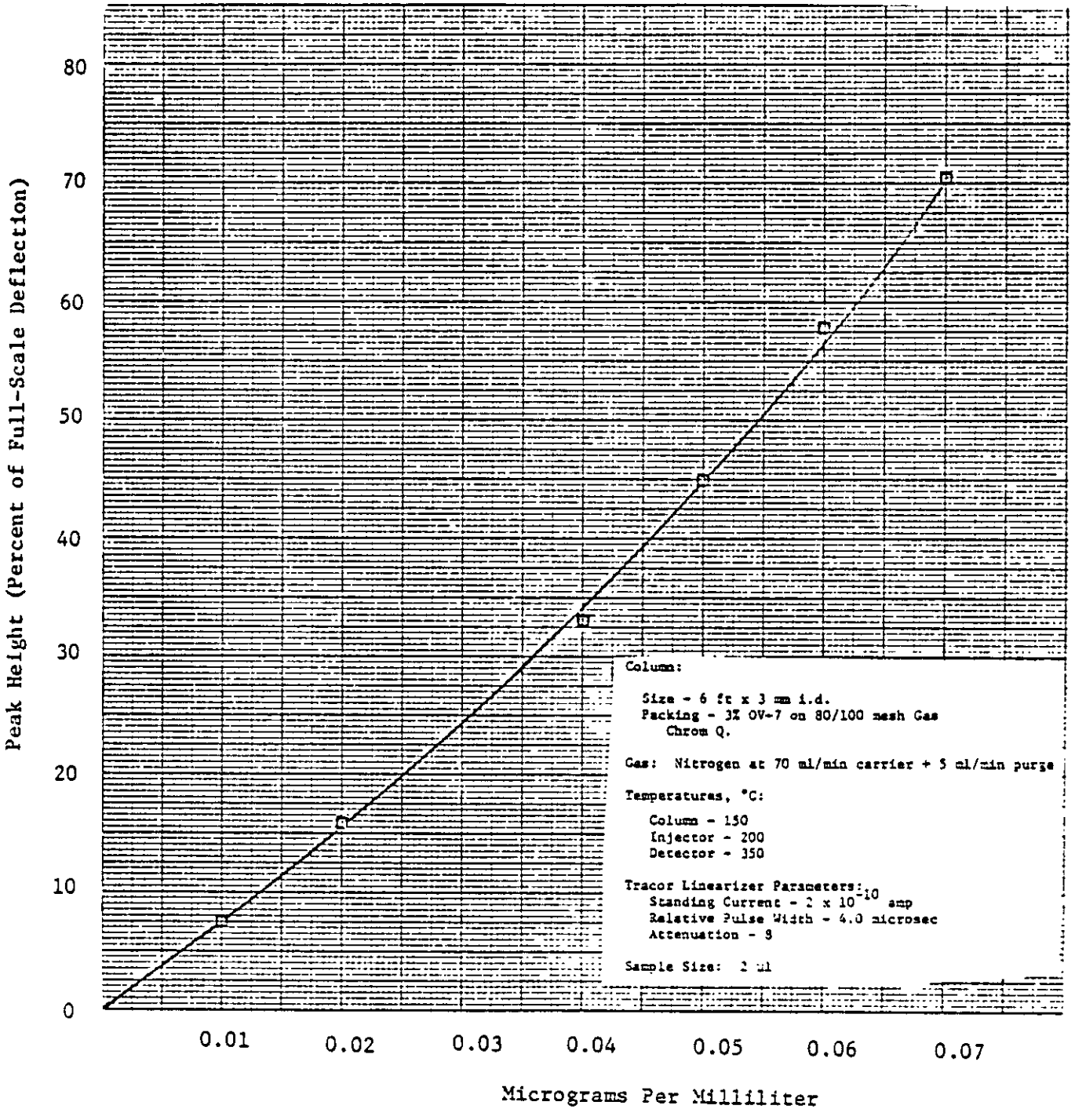


FIGURE 1. CALIBRATION CURVE FROM GAS CHROMATOGRAPHY OF 3,5,6-TRICHLORO-2-PYRIDINOL:TRIMETHYLSILYL ETHER STANDARDS.

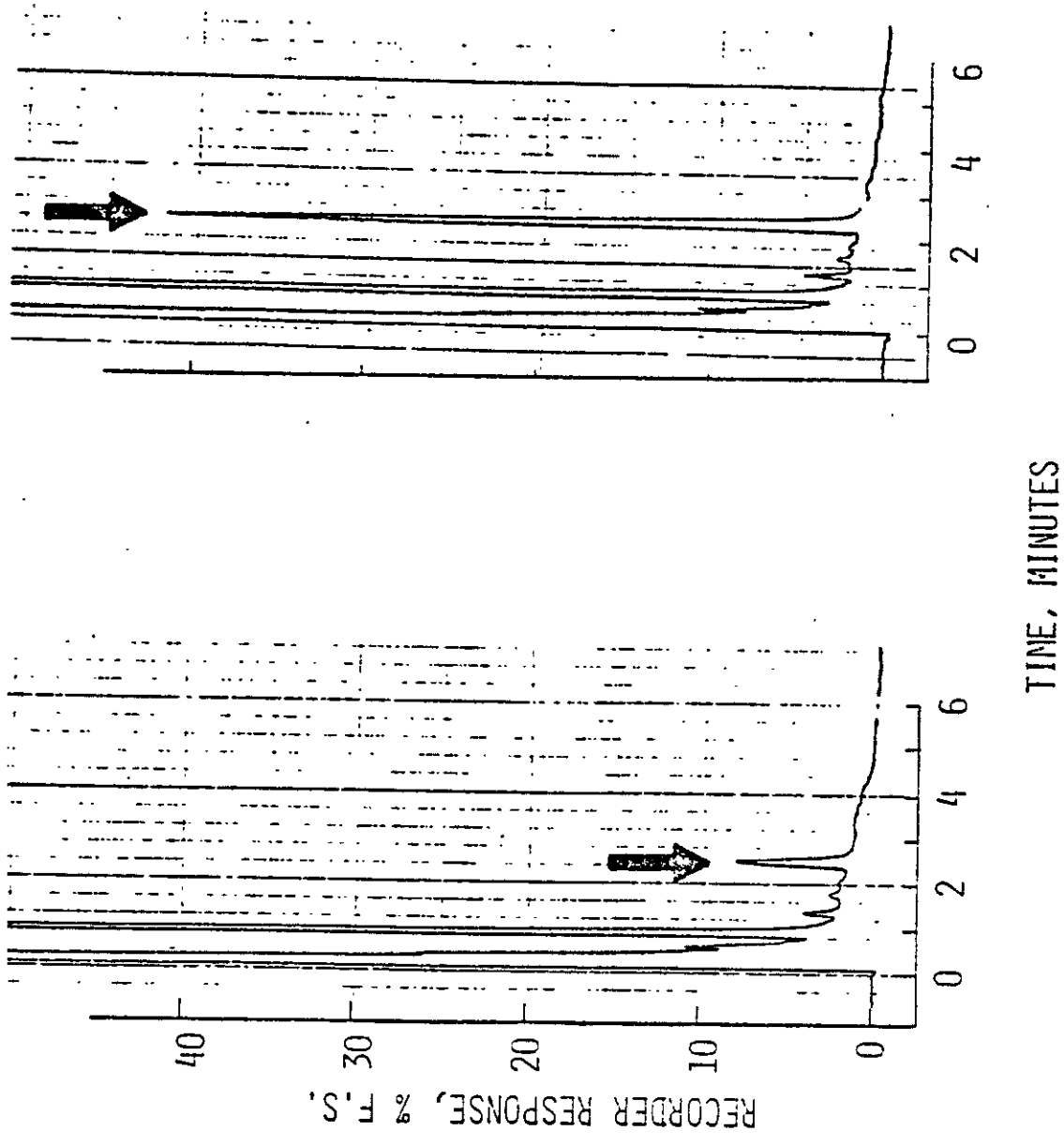
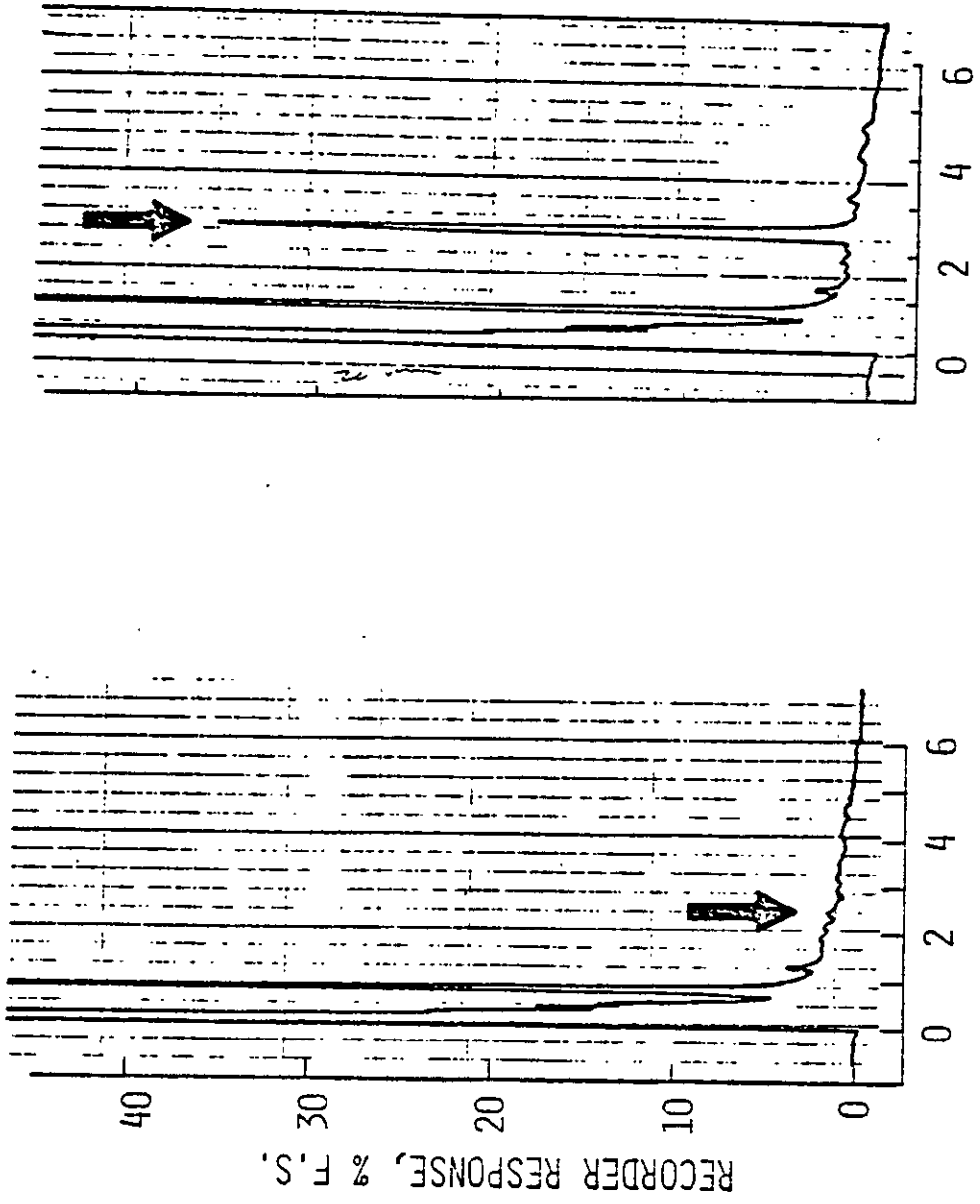


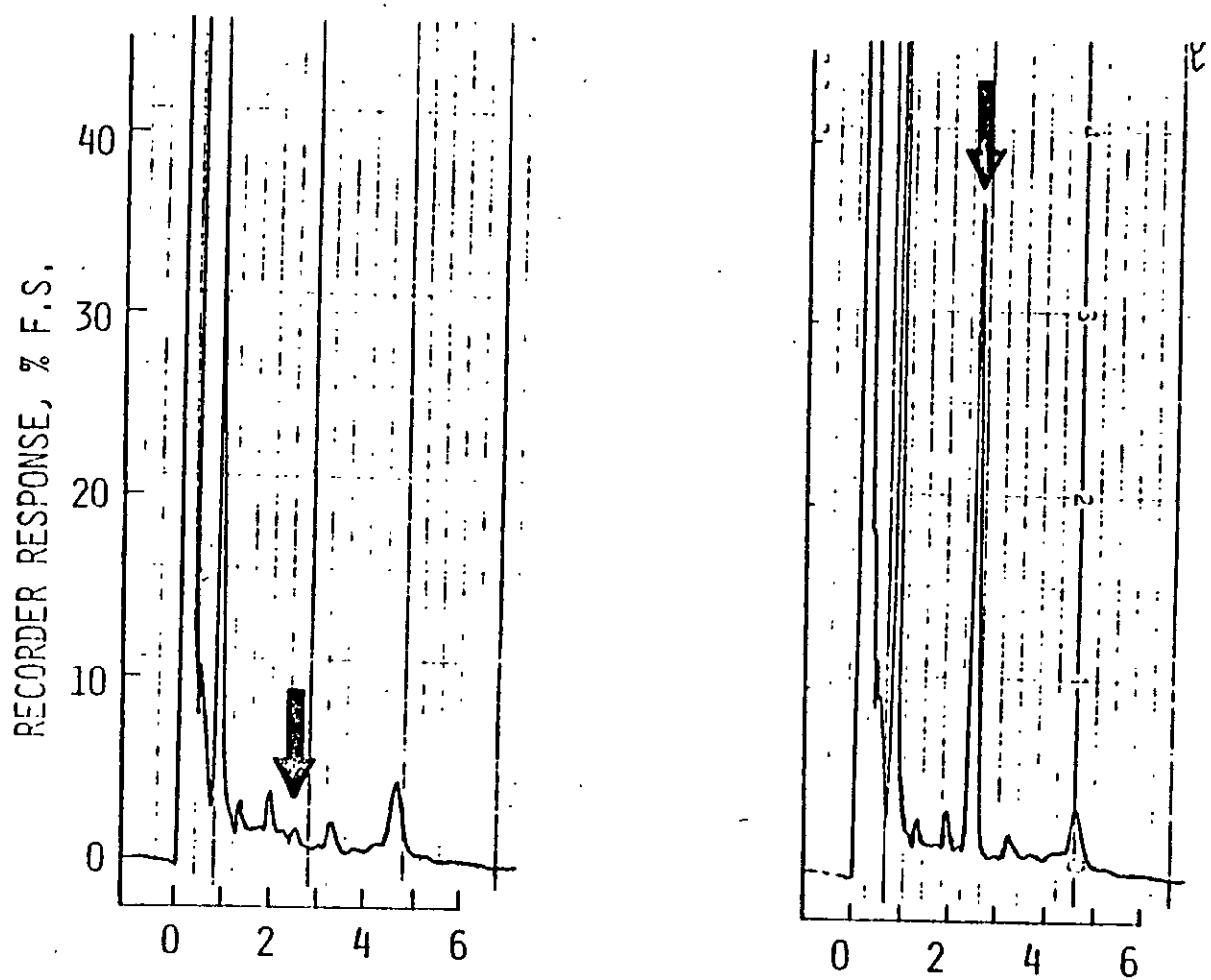
FIGURE 2. TYPICAL CHROMATOGRAMS FROM 3,5,6-TRICHLORO-2-PYRIDINOL: TRIMETHYL SILYL ETHER STANDARDS.



Control
Blank Equivalent to
0.02 ppm Pyridinol

Spiked: 3 ppm Chlorpyrifos-methyl
+ 0.5 ppm Pyridinol
Recovery = 100%

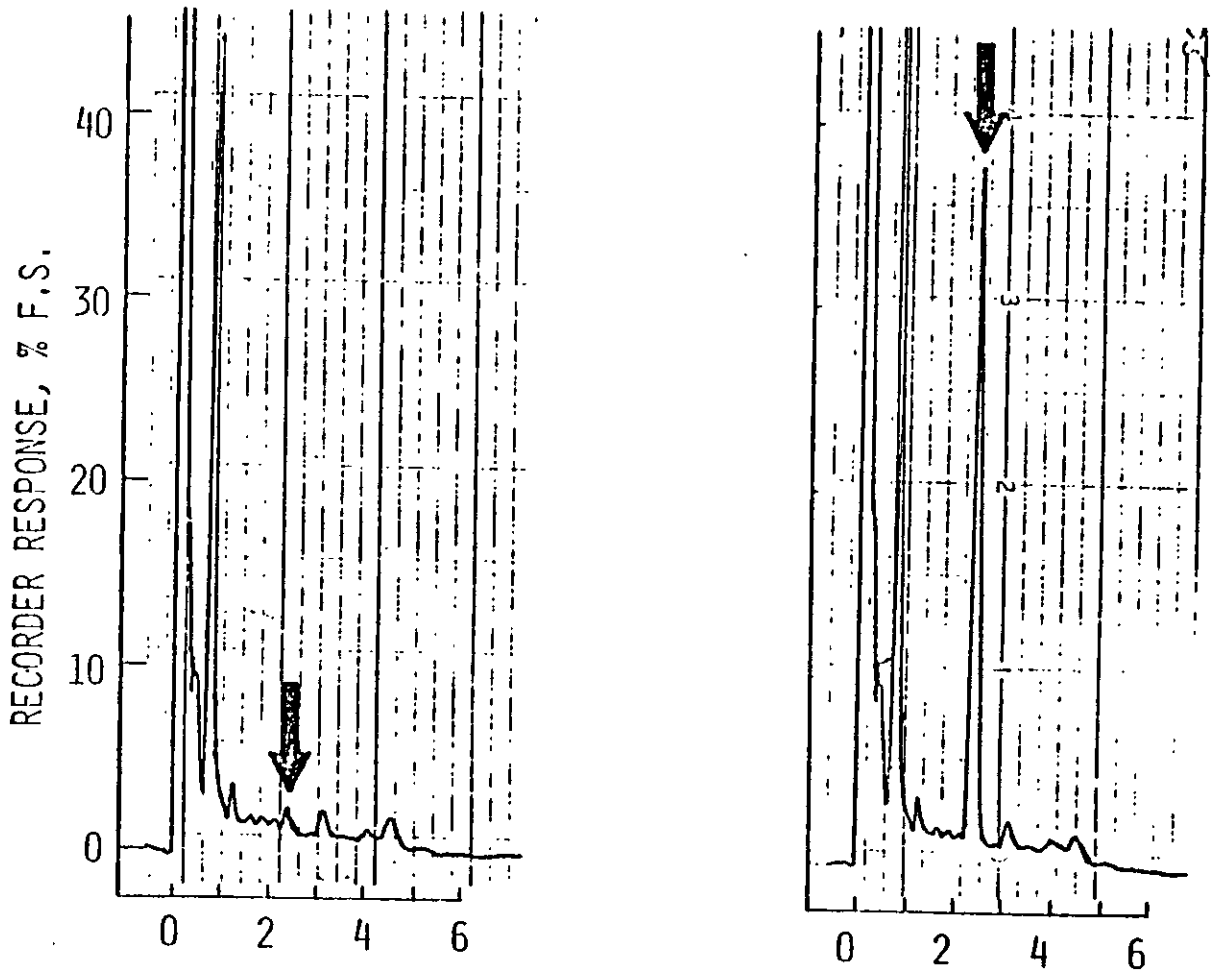
FIGURE 3. TYPICAL CHROMATOGRAMS: RECOVERY OF 3,5,6-TRICHLORO-2-PYRIDINOL FROM WHEAT SPIKED WITH THE PYRIDINOL AND CHLORPYRIFOS-METHYL.



Control
Blank Equivalent to
0.05 ppm Pyridinol

Spiked: 3 ppm Chlorpyrifos-methyl
+ 0.5 ppm Pyridinol
Recovery = 91%

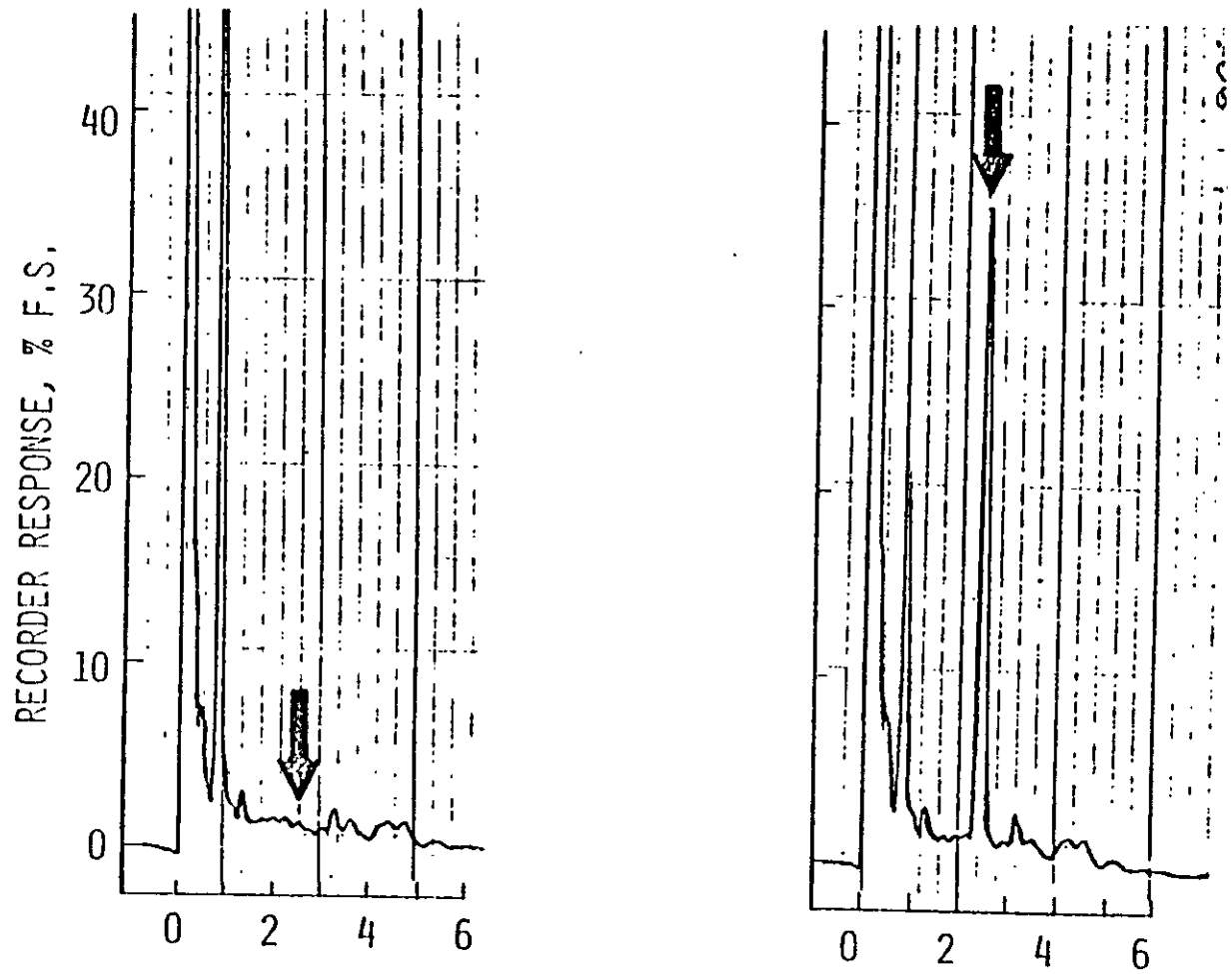
FIGURE 4. TYPICAL CHROMATOGRAMS: RECOVERY OF 3,5,6-TRICHLORO-2-PYRIDINOL FROM OATS SPIKED WITH THE PYRIDINOL AND CHLORPYRIFOS-METHYL.



Control
Blank Equivalent to
0.08 ppm Pyridinol

Spiked: 3 ppm Chlorpyrifos-methyl
+ 0.5 ppm Pyridinol
Recovery = 98%

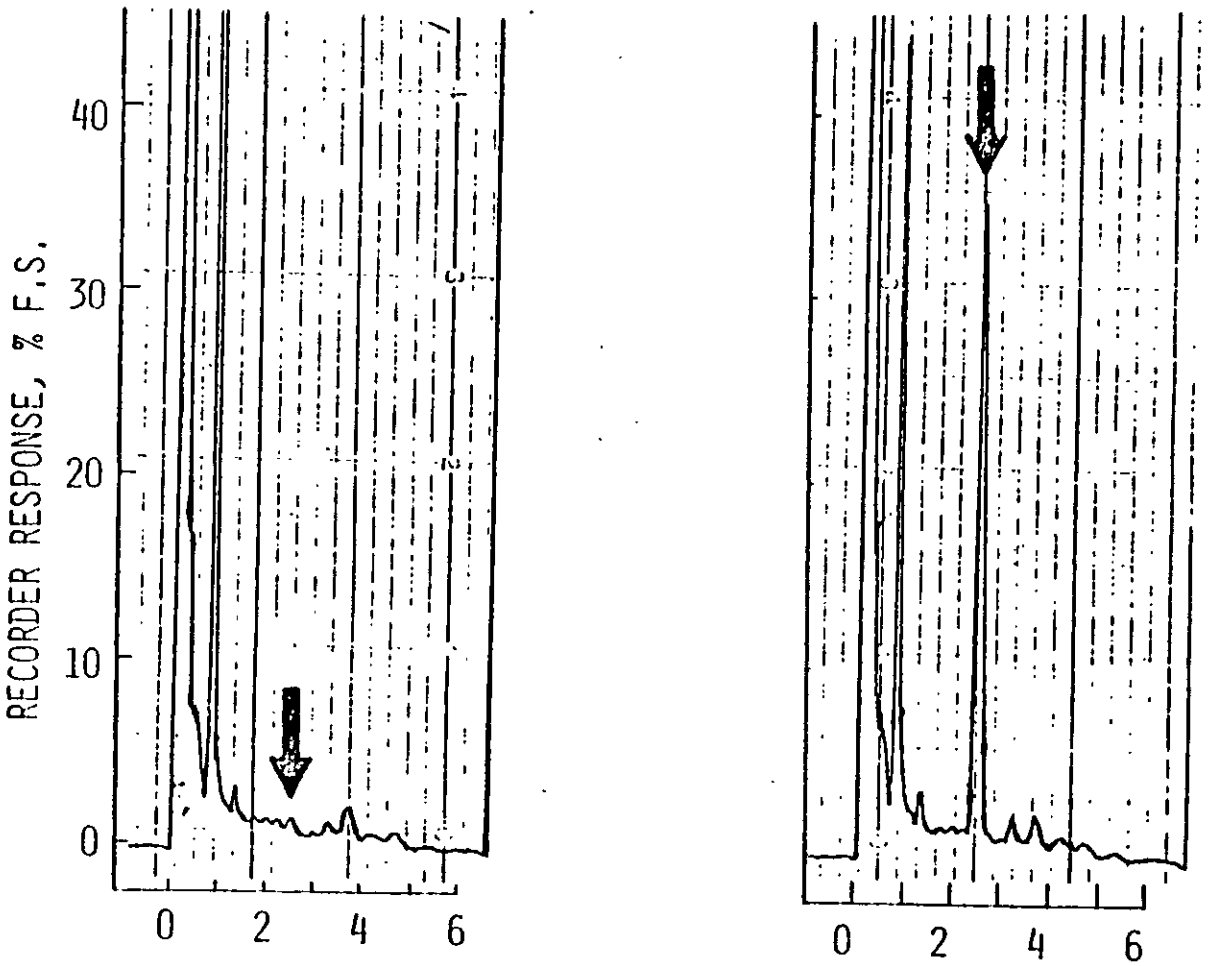
FIGURE 5. TYPICAL CHROMATOGRAMS: RECOVERY OF 3,5,6-TRICHLORO-2-PYRIDINOL FROM BARLEY SPIKED WITH THE PYRIDINOL AND CHLORPYRIFOS-METHYL.



Control
Blank Equivalent to
0.02 ppm Pyridinol

Spiked: 3 ppm Chlorpyrifos-methyl
+ 0.5 ppm Pyridinol
Recovery = 94%

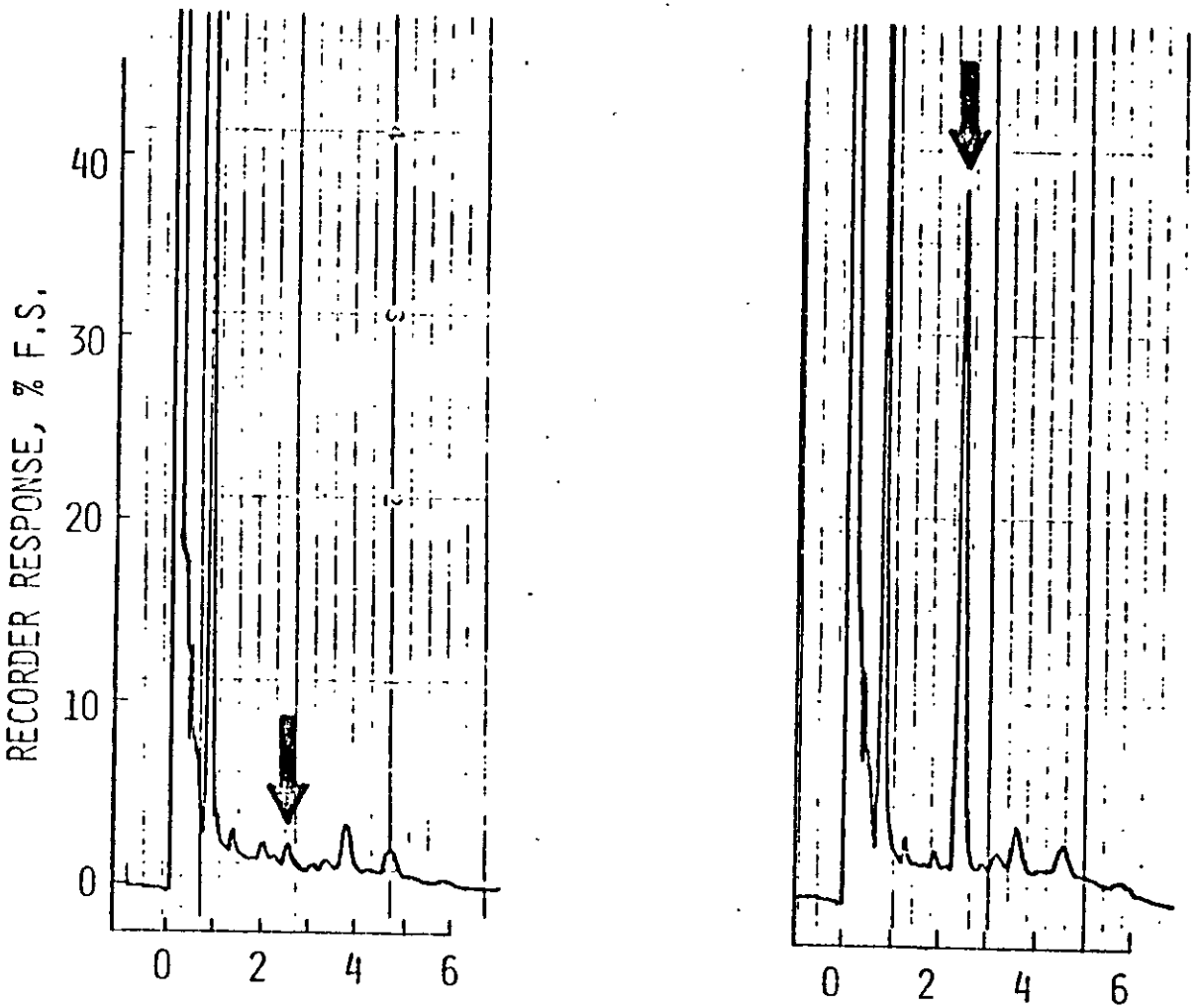
FIGURE 6. TYPICAL CHROMATOGRAMS: RECOVERY OF 3,5,6-TRICHLORO-2-PYRIDINOL FROM CORN SPIKED WITH THE PYRIDINOL AND CHLORPYRIFOS-METHYL.



Control
Blank Equivalent to
0.05 ppm Pyridinol

Spiked: 3 ppm Chlorpyrifos-methyl
+ 0.5 ppm Pyridinol
Recovery = 97%

FIGURE 7. TYPICAL CHROMATOGRAMS: RECOVERY OF 3,5,6-TRICHLORO-2-PYRIDINOL FROM MILO SPIKED WITH THE PYRIDINOL AND CHLORPYRIFOS-METHYL.



Control
Blank Equivalent to
0.1 ppm Pyridinol

Spiked: 3 ppm Chlorpyrifos-methyl
+ 0.5 ppm Pyridinol
Recovery = 99%

FIGURE 8. TYPICAL CHROMATOGRAMS: RECOVERY OF 3,5,6-TRICHLORO-2-PYRIDINOL FROM RICE SPIKED WITH THE PYRIDINOL AND CHLORPYRIFOS-METHYL.

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DETERMINATION OF RESIDUES OF CHLORPYRIFOS-METHYL IN BOVINE TISSUES

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Agricultural Products Department

1. Scope

This method has been designed for the determination of chlorpyrifos-methyl [O,O-dimethyl O-(3,5,6-trichloro-2-pyridyl)phosphorothioate] in muscle, liver, kidney and fat tissues of cattle. The method has been validated for residues as low as 0.01 ppm. For residues greater than 0.10 ppm, the final solution is diluted to a concentration appropriate for gas chromatography.

2. Principle

Residues of chlorpyrifos-methyl are extracted from muscle, liver and kidney tissues with acetone and from fat with hexane. The acetone is evaporated from the muscle, liver and kidney filtrate and the compound is then extracted with hexane. The hexane extracts from all tissues are partitioned with acetonitrile, the acetonitrile is evaporated and residues taken up in hexane and cleaned-up on a deactivated silica gel column. The eluate is evaporated and the residue is taken up in a known volume of acetone. Residues are determined quantitatively by gas chromatography using flame photometric detection.

3. Special Equipment

a. Jar, 4-oz, square powder, Brockway Glass Co., Inc., Brockway, Pennsylvania 15824.

b. Vial, capsule, 12-dram, Owens-Illinois, Toledo, Ohio 43612.

c. Caps, Poly-Seal liner, 24-mm and 38-mm, Poly-Seal Corp., Baltimore, Maryland 21233.

d. Funnel, Buchner, 60-ml.

e. Funnel, separatory, 125-ml and 250-ml.

f. Flask, Erlenmeyer, 125-ml with 24/40 standard taper ground glass joint, Corning Glass Works, Inc., Corning, New York 14830.

g. Column, Snyder evaporation, 15 cm, 3 section with 24/40 standard taper ground glass joint, catalog No. K503000, Kontes Glass Company, Vineland, New Jersey 08360.

h. Transfer pipet, disposable, 5 3/4 inch, catalog No. 269-183, Curtin Matheson Scientific Co., Houston, Texas 77002.

i. Liquid chromatographic column, 1 cm i.d. x 15 cm long, having a coarse fritted disc and a stopcock with a plug assembly made of Teflon and fitted with a metering valve, Corning Glass Works, Inc., Corning, New York 14830.

j. Homogenizer, Willems Polytron, Model PT10-35, equipped with PT20ST generator, Brinkman Instruments, Inc., Westbury, New York 11590.

k. Filtrator assembly, catalog number 9-788, Fisher Scientific Co., Pittsburgh, Pennsylvania 15219.

l. Evaporating assembly, see Figure 1 and Note 1.

m. Shaker, table model, set at 190 excursions/minute and adjusted to accomodate item (a).

n. Shaker, wrist action, set at 300 excursions/minute and adjusted to accommodate item (b).

o. Hot plate.

p. Water bath, 45°C, consisting of a 20 x 30 cm baking dish placed on a hot plate.

q. Centrifuge, with head and cups to accommodate item (b).

r. Gas chromatographic column, U-shaped, borosilicate glass, 4 mm i.d. x 1.8 m long.

s. Syringe, 10-µl, No. 701, Hamilton Co., Reno, Nevada 89501.

t. Recorder, 1-mv, Hewlett-Packard, Avondale, Pennsylvania 19311.

u. Gas chromatograph, equipped with flame photometric detector, Model 222, Tracor Inc., Austin, Texas 78710.

v. Filter drier, No. 23557-0001, Tracor, Inc., Austin, Texas 78710.

4. Reagents and Materials

a. Hexane, distilled in glass, Burdick and Jackson Laboratories, Inc., Muskegon, Michigan 49440.

b. Acetone, distilled in glass, Burdick and Jackson Laboratories, Inc., Muskegon, Michigan 49440.

c. Acetonitrile, distilled in glass, Burdick and Jackson Laboratories, Inc., Muskegon, Michigan 49440.

d. Silica gel, Woelm, ICN Pharamaceuticals, Inc., Cleveland, Ohio 44101.

e. Silica gel, 20% deactivated, add 20 ml water to 80 g Woelm silica gel. Shake to mix well and allow to stand overnight.

f. Filter aid, Hyflo Super-Cel, Johns-Manville Products Corp., New York, New York 10001.

g. Prepurified nitrogen, passed through filter drier, Air Reduction Co., Albion, Michigan 49224.

h. Compressed air, passed through filter drier, Valley Oxygen Co., Bay City, Michigan 48706.

i. Hydrogen, passed through filter drier, Air Reduction Co., Albion, Michigan 49224.

j. Oxygen, passed through filter drier, Air Reduction Co., Albion, Michigan 49224.

k. Chromatographic column packing, QF-1 mixed phase on 80/100 mesh Gas Chrom Q, Applied Science Laboratories, Inc., State College, Pennsylvania 16801.

1. Chlorpyrifos-methyl [O,O-dimethyl O-(3,5,6-trichloro-2-pyridyl)phosphorothioate]stock solution, 1 mg/ml. Dissolve 50 mg of analytical grade chlorpyrifos-methyl* in 50 ml acetone.

m. Chlorpyrifos-methyl gas chromatographic standards. Make appropriate dilutions of the stock solution with acetone to cover the concentration range of 0.05 µg/ml to 1.0 µg/ml.

5. Gas Chromatography

a. Column Preparation

1. Pack a column with the packing material by tapping the column on a hard surface to settle the packing while filling. Plug the ends with silanized glass wool. The level of packing should be a few mm below the zones heated by the inlet block when the column is installed in the chromatograph.

2. Install the column in the chromatograph without attaching the column to the detector. Heat to 220°C for 24 hours.

3. Connect the column to the detector and lower the temperature to the operating level.

b. Typical Operating Conditions

Tracor 222 gas chromatograph equipped with flame photometric detector.

Carrier gas - nitrogen, 70 ml/min.

Burner gas - oxygen, 10 ml/min.
- hydrogen, 50 ml/min.
- air, 90 ml/min.

Column temperature, 200°C
Detector temperature, 195°C
Inlet temperature, 215°C
Attenuation - 256×10^2, using a 1-mv recorder.
Chart speed - 15 in/hr.

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*Available from Sampling Coordinator, Agricultural Products Department, Dow Chemical USA, Midland, Michigan 48640.

c. Injection Technique

1. Fill the syringe needle with acetone, avoiding entrapped air.

2. Draw 5 µl of sample into the syringe.

3. Inject into the chromatographic column as rapidly as possible.

6. Preparation of Calibration Curve

a. Chromatograph 5-µl aliquots of appropriate chlorpyrifos-methyl chromatographic standards.

b. Measure the heights of the peaks obtained in terms of percent of full-scale deflection.

c. Plot peak heights versus corresponding concentrations.

d. A typical calibration curve and typical chromatograms for standards are shown in Figures 2 and 3, respectively.

7. Recovery of Chlorpyrifos-Methyl From Fortified Bovine Muscle, Liver and Kidney

a. Weigh 10 g of sample into a 4-oz bottle.

b. Fortify the sample at the desired concentrations by adding an appropriate aliquot of the working standard.

c. Add 30 ml acetone and blend the sample with a Polytron ST-20 generator for one minute. Add 1 g of filter-aid and place on a shaker for 5 minutes.

d. Filter the sample through a 5-mm pad of filter-aid in a 60-ml Büchner funnel using a filtrator assembly. Collect the filtrate in a 125-ml Erlenmeyer flask. Rinse the bottle and filter pad with 60 ml acetone.

e. Using a hot plate and Synder column, evaporate the acetone until water begins to condense on the sides of the flask. Cool and transfer the remaining water and co-extractives to a 12-dram vial with a disposable pipet. Rinse the flask with 10 ml hexane, transferring to the vial.

f. Add 5 ml water to the vial. Cap and shake for 5 minutes. Centrifuge at 2000 rpm for 2 minutes to separate the layers. Transfer the hexane to a 125-ml separatory funnel. Add an additional 10 ml hexane to the vial. Shake, centrifuge and transfer the hexane to the funnel.

g. Add 10 ml acetonitrile to the separatory funnel, shake the funnel and allow the layers to separate for 5 minutes. Transfer the acetonitrile to a 125-ml Erlenmeyer flask. Repeat the partition two more times.

h. Evaporate the acetonitrile using a hot plate and a Snyder column until only 2 to 3 ml remain in the flask. Cool the flask and place on the evaporating assembly in a 45°C water bath. Evaporate the acetonitrile with a jet of dry air (see note 1). When all of the acetonitrile has evaporated, cool the flask.

i. Dissolve the contents of the flask in 5 ml hexane.

j. Place 2 g of deactived silica gel in a liquid chromatographic column and prewash with 5 ml hexane. Transfer the hexane from step i. to the column collecting the eluate in a 125-ml Erlenmeyer flask. Rinse with 10 ml hexane and add to the column. Continue eluting the column with an additional 50 ml hexane.

k. Evaporate the hexane using the procedure in step h.

l. Add exactly 2 ml acetone to the flask and swirl.

m. Inject 5 µl of the acetone solution into the gas chromatograph.

n. Measure the height of the peak obtained for chlorpyrifos-methyl in terms of percent full scale deflection and determine the concentration in the sample by reference to the calibration curve.

o. Calculate the apparent ppm of chlorpyrifos-methyl in the fortified sample and compare with the theoretical value to determine the percent recovery.

8. Recovery of Chlorpyrifos-Methyl From Fortified Bovine Fat

Analyze the sample following the procedure described in paragraph 7. with the following exceptions:

a. In steps c. and d. use hexane instead of acetone.

b. Omit steps e. and f. and transfer the hexane extract to a 250-ml separatory funnel.

c. In step g. partition with three 20-ml aliquots of acetonitrile.

9. Analysis of Bovine Tissues for Residues of Chlorpyrifos-Methyl

a. Procedure

Analyze the sample following the procedure described in paragraph 7. or 8. omitting the addition of standard solutions.

b. Calculations

1. From the peak obtained and the calibration curve, calculate the concentration of chlorpyrifos-methyl in the final solution. Multiply the concentration in µg/ml times 0.2 to get gross ppm residue.

2. Subtract the apparant ppm of chlorpyrifos-methyl blank, obtained from the analysis of control tissue, giving net ppm of chlorpyrifos-methyl residue.

3. Calculate the corrected residue as follows:

$$\text{ppm chlorpyrifos-methyl} = \frac{\text{net ppm chlorpyrifos-methyl}}{\text{\% Recovery}} \times 100$$

10. Validation of Method

The recovery data obtained using this method are given in Table I. Typical chromatograms from fortified samples and control samples are shown in Figures 4 through 7.

11. Notes

1. Evaporation of acetonitrile or hexane to dryness may result in loss of chlorpyrifos-methyl if not done carefully. The last 2 to 5 ml are evaporated by adjusting the evaporating assembly so that flasks can be tilted to a 45° angle in the 45°C water bath with the air jet extending into the flask. The air flow is adjusted to 200 ml/min. As soon as the solvent evaporates, remove the flask and cool.

* *

This method has been found to be satisfactory for its indended purpose when used by a qualified analyst. However, users are cautioned to confirm its suitablity for themselves. Written permission from The Dow Chemical Company is required before it may be published as a whole or in part.

TABLE I. RECOVERY OF CHLORPYRIFOS-METHYL FROM BEEF TISSUES FORTIFIED WITH KNOWN AMOUNTS.

Substrate	ppm Added	ppm Found	% Recovery
Muscle	0.01	0.0084	84
	0.01	0.0084	84
	0.02	0.015	75
	0.02	0.016	80
	0.02	0.017	85
	0.02	0.017	85
	0.05	0.043	86
	0.05	0.046	92
	0.05	0.044	88
	0.05	0.047	94
	0.10	0.082	82
	0.10	0.088	88
			Av. = 85±3 1/
Liver	0.01	0.0080	80
	0.01	0.0087	87
	0.02	0.016	80
	0.02	0.017	85
	0.05	0.053	106
	0.05	0.043	86
	0.10	0.091	91
	0.10	0.091	91
	0.20	0.163	82
	0.20	0.178	89
	0.50	0.392	78
	0.50	0.413	83
			Av. = 87±5 1/

TABLE I (CONT'D). RECOVERY OF CHLORPYRIFOS-METHYL FROM BEEF TISSUES FORTIFIED WITH KNOW AMOUNTS.

Substrate	ppm Added	ppm Found	% Recovery
Kidney	0.01	0.0106	106
	0.01	0.0116	116
	0.02	0.020	100
	0.02	0.018	90
	0.05	0.047	94
	0.05	0.050	100
	0.10	0.091	91
	0.10	0.088	88
	0.20	0.178	89
	0.20	0.181	90
	0.50	0.457	91
	0.50	0.439	88
			Av. = 95±6[1/]
Fat	0.01	0.0089	89
	0.01	0.0082	82
	0.02	0.016	80
	0.02	0.014	70
	0.05	0.044	88
	0.05	0.039	78
	0.10	0.078	78
	0.10	0.083	83
	0.20	0.151	76
	0.20	0.151	76
	0.50	0.381	76
	0.50	0.409	82
			Av. = 80±3[1/]

[1/] 95% confidence limit for the mean.

EVAPORATING ASSEMBLY

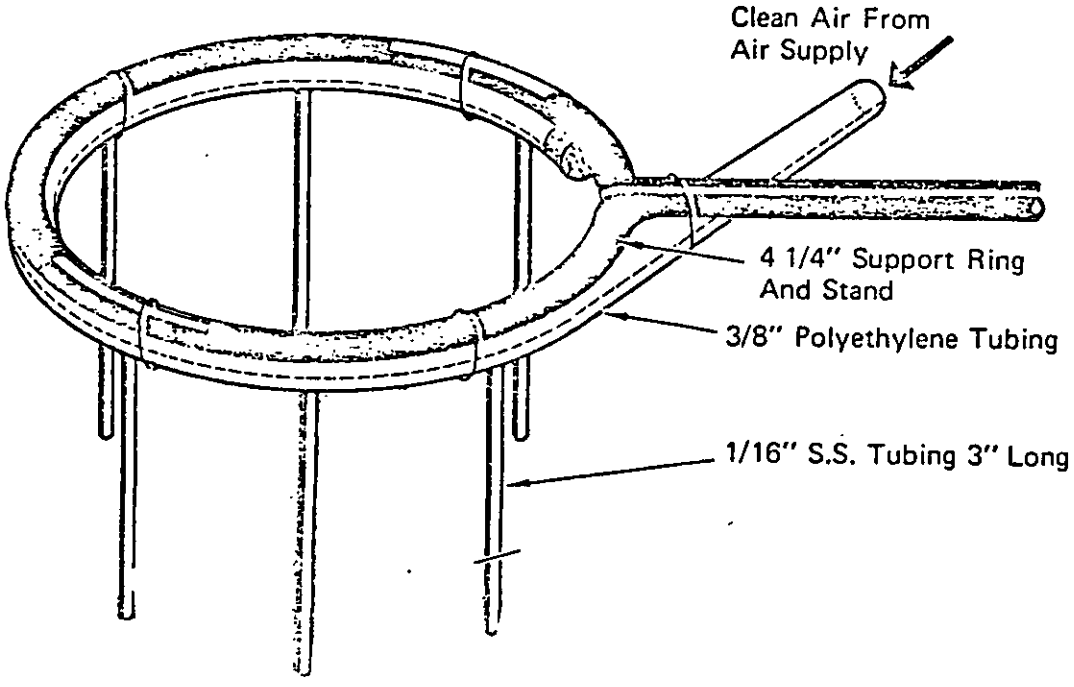


FIGURE 1.

Column: 6 ft x 3 mm, 11% OV-17 and QF-1 mixed phase on 80/100 mesh Gas Chrom Q.

Temperatures: Column - 200°C
Detector - 195°C
Inlet - 215°C

Carrier gas: Prepurified nitrogen, 70 ml/min.

Burner gases: Air - 90 ml/min
Oxygen - 10 ml/min
Hydrogen - 50 ml/min

Detector: Flame Photometric Detector

Attenuation - 256 x 10^2 using a 1-mv recorder

Sample size - 5 µl

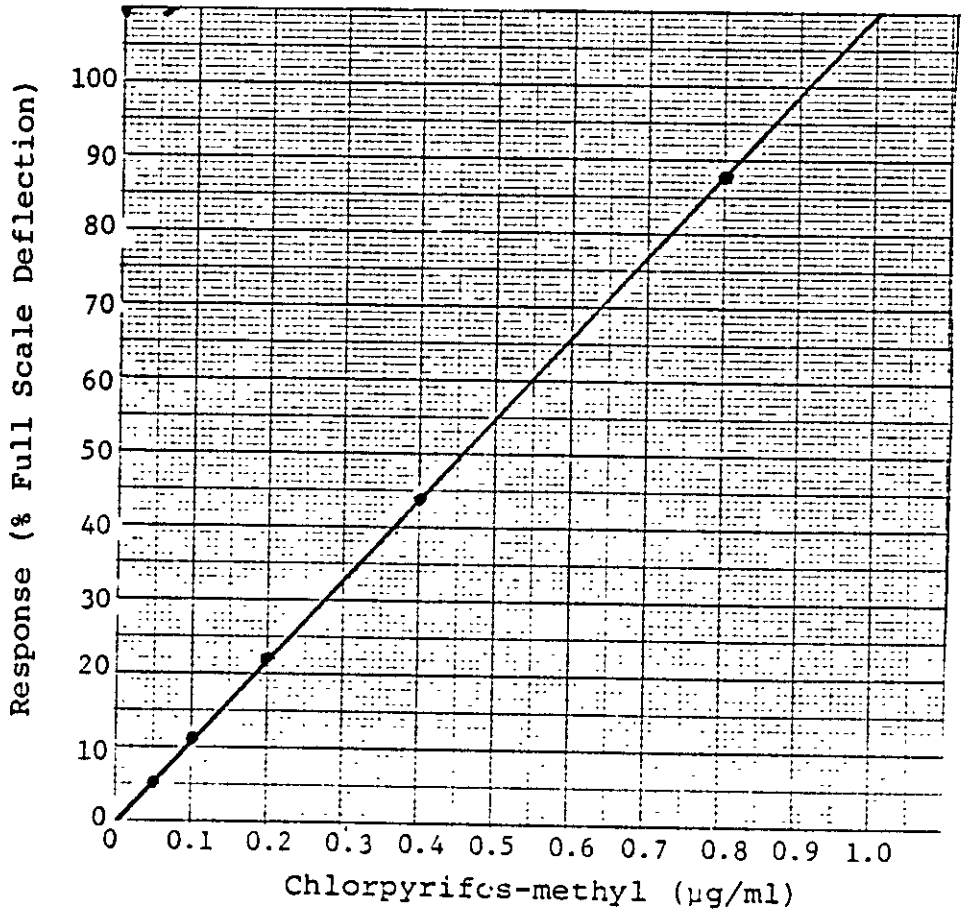


FIGURE 2. TYPICAL CALIBRATION CURVE FOR DETERMINATION OF RESIDUES OF CHLORPYRIFOS-METHYL.

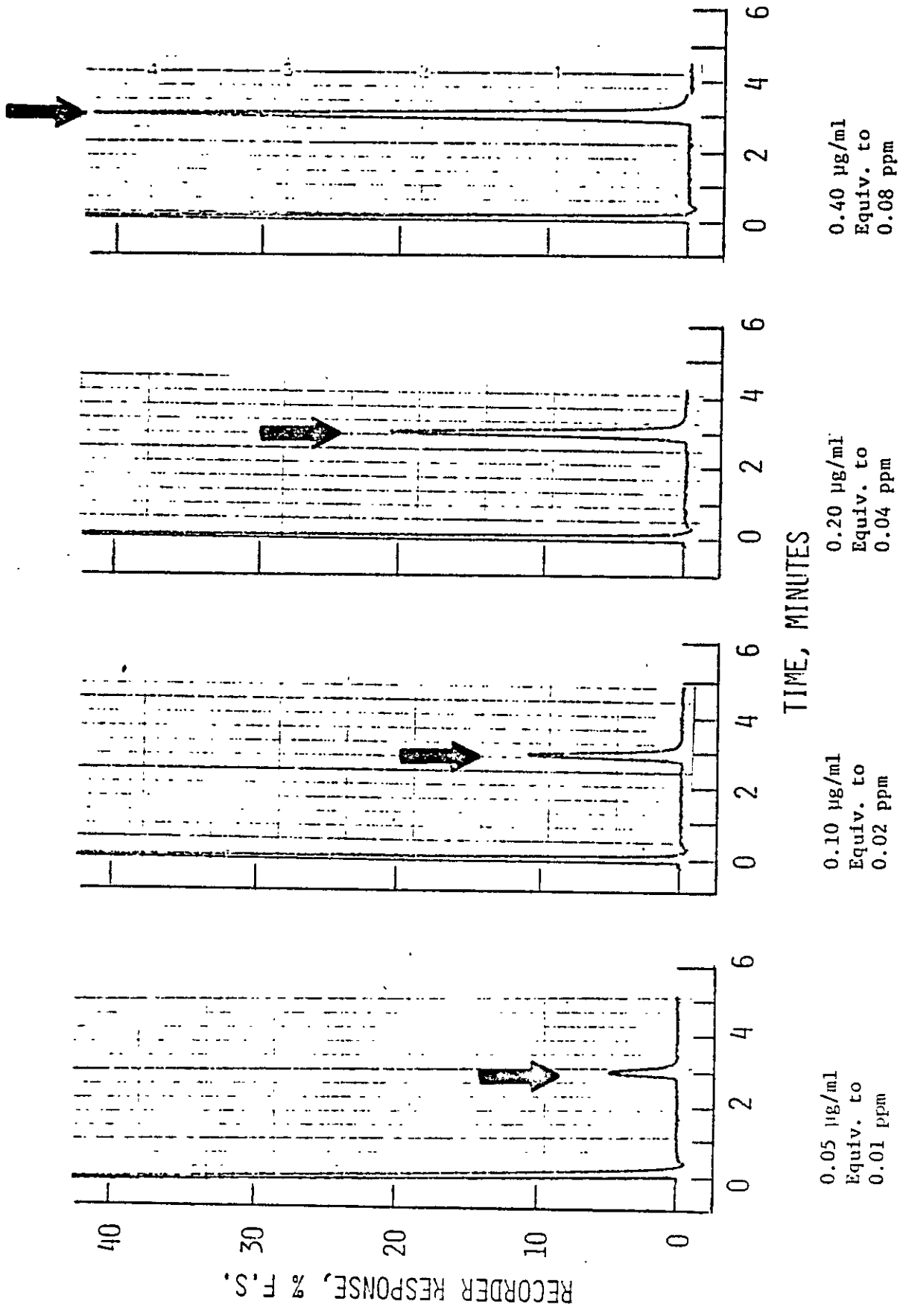
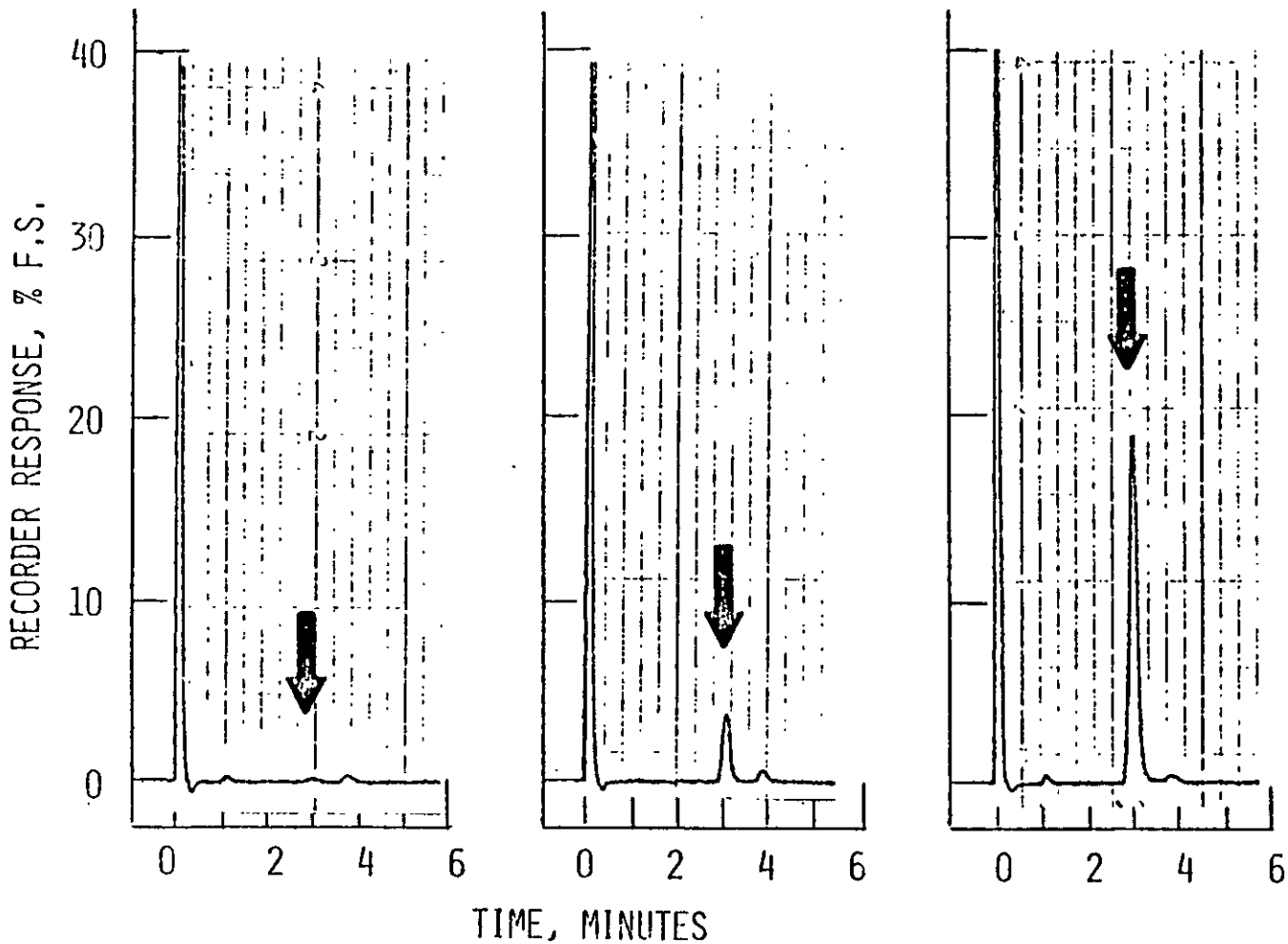


FIGURE 3. TYPICAL CHROMATOGRAMS FROM STANDARD SOLUTIONS OF CHLORPYRIFOS-METHYL.

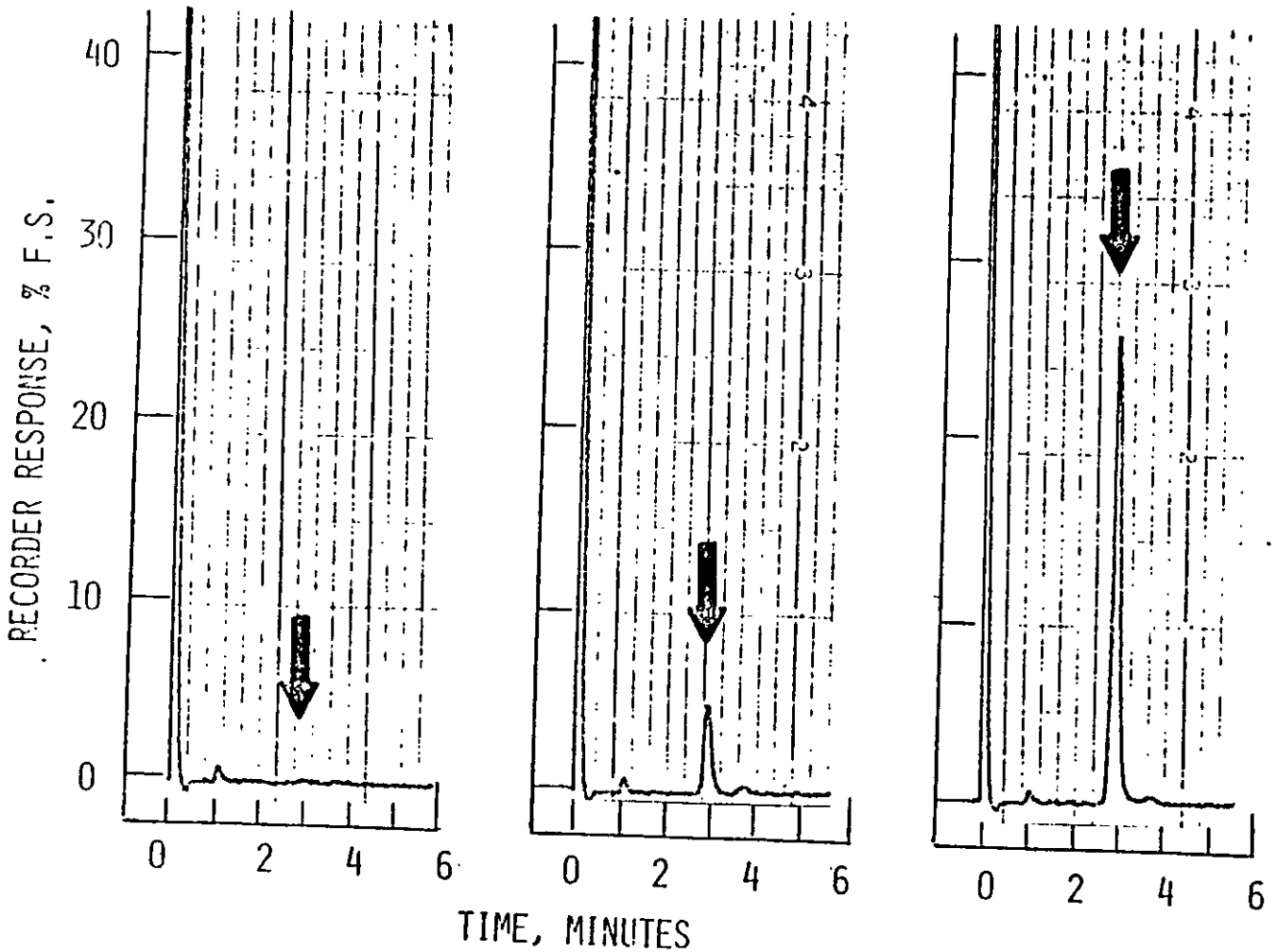


Control
Sample No. 143910
Blank = 0.00 ppm

Sample No. 143909
Fortified with
0.01 ppm
84% Recovery

Sample No. 143909
Fortified with
0.05 ppm
91% Recovery

FIGURE 4. TYPICAL CHROMATOGRAMS FROM THE ANALYSIS OF BOVINE MUSCLE FORTIFIED WITH CHLORPYRIFOS-METHYL.

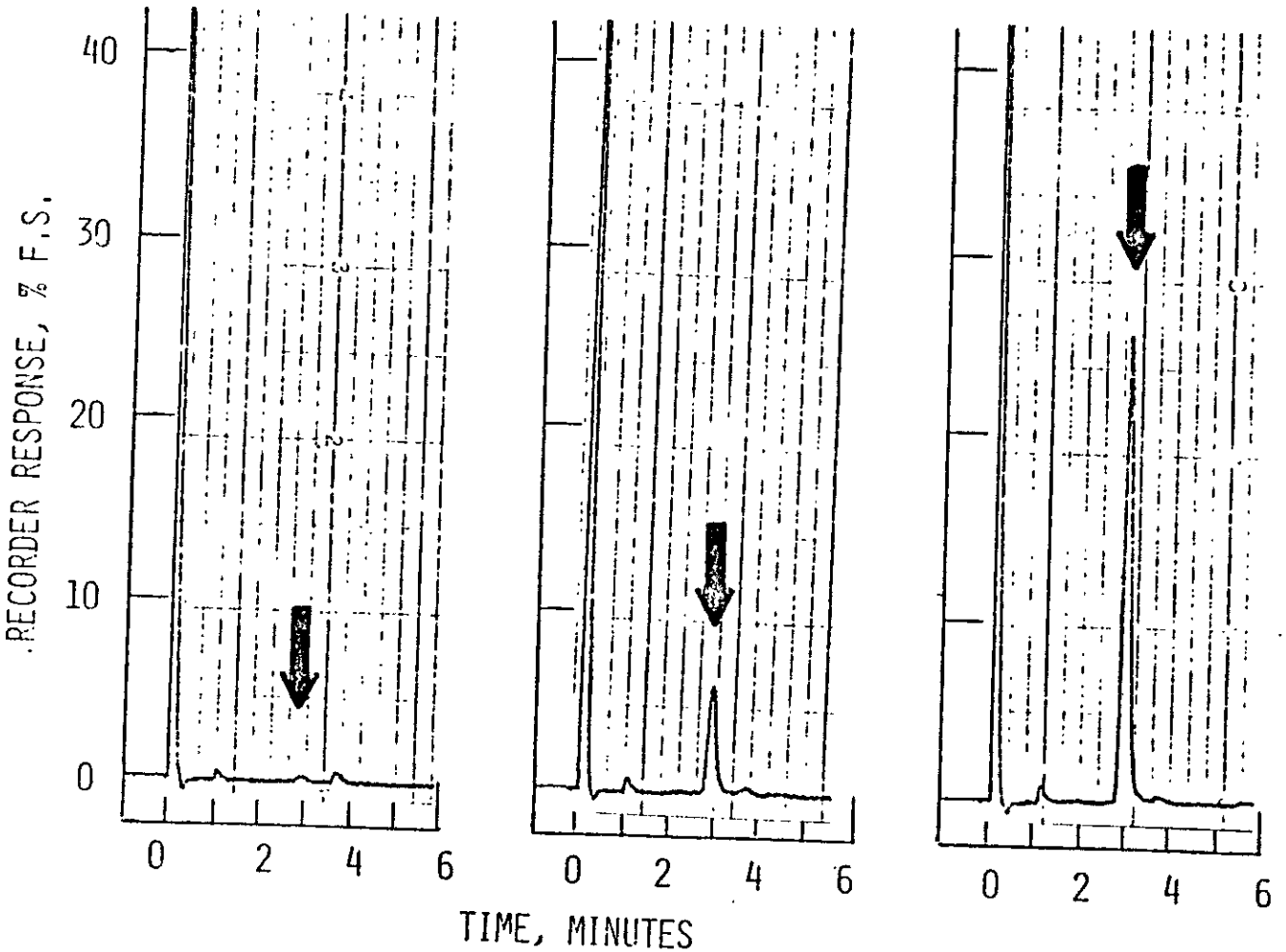


Control
Sample No. 143932
Blank = 0.00 ppm

Sample No. 143932
Fortified with
0.01 ppm
87% Recovery

Sample No. 143932
Fortified with
0.05 ppm
86% Recovery

FIGURE 5. TYPICAL CHROMATOGRAMS FROM THE ANALYSIS OF BOVINE LIVER FORTIFIED WITH CHLORPYRIFOS-METHYL.

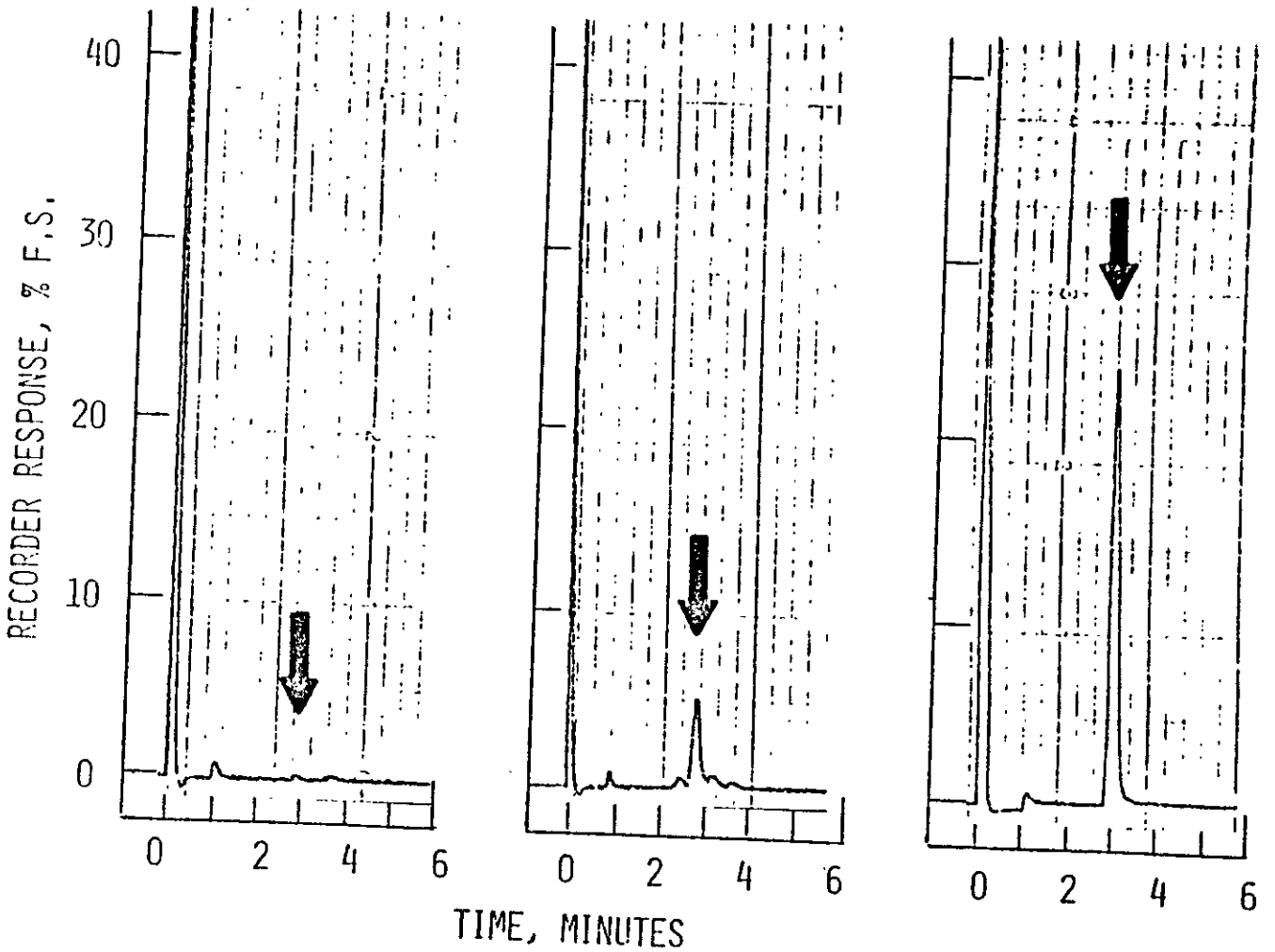


Control
Sample No. 143957
Blank = 0.00 ppm

Sample No. 143956
Fortified with
0.01 ppm
106% Recovery

Sample No. 143956
Fortified with
0.05 ppm
95% Recovery

FIGURE 6. TYPICAL CHROMATOGRAMS FROM THE ANALYSIS OF BOVINE KIDNEY FORTIFIED WITH CHLORPYRIFOS-METHYL.



Control
Sample No. 143981
Blank = 0.00 ppm

Sample No. 143980
Fortified with
0.01 ppm
89% Recovery

Sample No. 143980
Fortified with
0.05 ppm
88% Recovery

FIGURE 7. TYPICAL CHROMATOGRAMS FROM THE ANALYSIS OF BOVINE FAT FORTIFIED WITH CHLORPYRIFOS-METHYL.

November 9, 1978 ACR 77.6.S1

DETERMINATION OF RESIDUES OF CHLORPYRIFOS-METHYL IN MILK AND CREAM

milk

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Purpose:

This is a modification of method ACR 77.6(1) which has been designed for the determination of chlorpyrifos-methyl in milk and cream and has been validated for residues as low as 0.01 ppm.

Reagents and Materials:

a. Methanol, Analytical Reagent Grade, Mallinckrodt, St. Louis, Missouri 63147.

b. Sodium chloride, ACS, J. T. Baker Chemical Co., Phillipsburg, New Jersey 08865.

Recovery of Chlorpyrifos-Methyl From Fortified Milk and Cream

1. Warm milk or cream sample to 45°C and shake by hand to mix.

2. Weigh a 10 g of sample into a 12-dram vial.

3. Fortify the sample at the desired concentration by adding an appropriate aliquot of the working standard.

4. Add 10 ml methanol, 3 g sodium chloride and 10 ml hexane. Place on a shaker at 190 excursions per minute for 5 minutes. Centrifuge for 2 minutes at 2000 rpm. (See note).

5. Transfer the hexane to a 125-ml separatory funnel. Repeat the extraction with two additional 10-ml aliquots of hexane.

b. Continue with Step g, paragraph 7 of ACR 77.6

Note:

After centrifugation of milk or cream samples, if the phases are not well resolved, add 1 ml methanol slowly without shaking and recentrifuge.

References:

1. Kuper, A. W. 1977. Determination of Residues of Chlorpyrifos-Methyl in Bovine Tissues. ACR 77.6, unpublished report of Dow Chemical U.S.A.

TABLE I. RECOVERY OF CHLORPYRIFOS-METHYL FROM MILK AND CREAM FORTIFIED WITH KNOWN AMOUNTS.

Substrate	Chlorpyrifos-methyl, ppm Added	Found	% Recovery
Milk	0.01	0.0084	84
		0.0090	90
		0.0084	84
		0.0092	92
		0.0085	85
	0.02	0.0166	83
		0.0168	84
		0.0169	84
		0.0178	89
		0.0172	86
	0.05	0.048	96
	0.10	0.084	84
		0.086	86
	1.0[1]	0.938	94
			87±2[2]
Cream	0.01	0.0085	85
		0.0099	99
	0.02	0.0157	78
		0.0162	81
		0.0154	77
	0.05	0.039	78
		0.043	86
	0.10	0.085	85
	0.50	0.416	83
		0.433	87
	1.0[1]	0.876	88
			84±4[2]

[1] Fortified on 12/13/77 and stored at -20°C until analyzed on 11/6/78.

[2] Mean and 95% confidence limit for the mean.