Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Triflusulfuron Methyl

MRID #: 427495-01

Matrix: Soil

Analysis: HPLC/UV

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427495-01

TRADE SECRET

Study Title

Analytical Method for the Quantitation of DPX-66037 in Soil

Rec'd EPA/ECS 05/19/93

Authors

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John M. Brisbin
Oliver R. Hunt

Study Completed on

December 5, 1991

Performing Laboratory

E.I. du Pont de Nemours and Company
Du Pont Agricultural Products
Experimental Station
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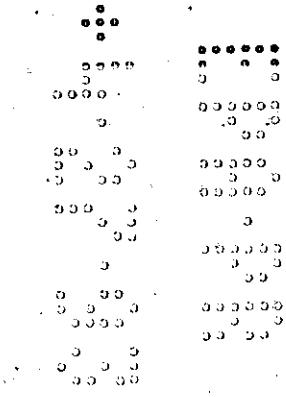
Laboratory Project ID

AMR 1965-91



5-24-93

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

Company Agent: James W. Denny (Typed Name) Date: 4/26/93

Product Registration Manager (Title) James W. Denny (Signature)

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GOOD LABORATORY PRACTICE STATEMENT

This study was conducted in the spirit of compliance with Good Laboratory Practice (GLP) requirements as specified in 40 CFR Part 160. Method validation was done in compliance with Good Laboratory Practices, except no protocol was written and no conduct audit was done. Any known deviations are documented in the study records.

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

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

Company Representative: James W. Denny — Dec. 5, 1991
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ANALYTICAL METHOD FOR THE QUANTITATION OF DPX-66037 IN SOIL

Kristin H. Milby, John M. Brisbin, and Oliver R. Hunt

SUMMARY

A method is described for the extraction and quantitation of DPX-66037 residues in soil. Soils are extracted with acetonitrile/ammonium carbonate, pH neutralized, partitioned into methylene chloride, and the methylene chloride/acetonitrile phase evaporated to dryness. After reconstitution, the samples are analyzed by column switching/eluent switching HPLC with UV detection. The chromatography system is automated to incorporate both eluent and column switching to provide significant sample cleanup prior to DPX-66037 quantitation.

The validity of both the extraction procedure and the HPLC method have been demonstrated previously in AMR 1656-90 ("Freezer Storage Stability of DPX-66037 in Soil" by Hunt and Brisbin, Reference 1) and AMR 2021-91 ("Automated Analytical Method for the Quantitation of DPX-66037 in Sugar Beets" by Major, Larochelle, Bedwell and Rossi, Reference 2), respectively.

The limit of quantitation for this method was determined to be 0.005 µg/g soil (ppm) and the limit of detection was determined to be 0.002 ppm, based upon 10-g soil samples. Recoveries from seven fortified control samples were an average of 94% with a standard deviation of 7%. Ten additional fortified control samples analyzed as part of a study (Reference 4) had an average recovery of 97% ± 6 (±SD)

I. INTRODUCTION

DPX-66037 (structure shown in Figure 1) is a member of the sulfonylurea class of herbicides. It is a weak acid with a pKa of 4.43 (Reference 3). A high degree of selectivity for this compound is achieved by making use of pH adjustments during its extraction, clean up, and analysis. In the present method, soils are taken through a relatively simple extraction, solvent partition and evaporation scheme prior to quantitation by HPLC with UV detection. The chromatography includes both eluent and column switching to provide significant sample cleanup. The method described in this report provides for extraction and quantitation of DPX-66037 from soils with a detection limit of 0.002 µg/g soil (ppm) and a quantitation limit of 0.005 ppm based on a 10.0-g sample.

The soil extraction procedure was adapted from the procedure used for AMR 1656-90, "Freezer Storage Stability of DPX-66037 in Soil" (Reference 1). DPX-66037 is extracted from soil into acetonitrile/0.1 M ammonium carbonate buffer. After centrifugation to remove solids, the pH of the supernatant is lowered to 7.5. The analyte is partitioned into methylene chloride and the solvent removed just to the point of dryness using a rotary evaporator. After the sample is reconstituted in a phosphate buffer/methanol solution, it is ready for chromatography.

The chromatography used in this method is an adaptation of AMR 2021-91 "Automated Analytical Method for the Quantitation of DPX-66037 in Sugar Beets" (Reference 2). Both the sugar beet method and this soil method use high performance liquid chromatography (HPLC) with eluent and column switching. Similar HPLC methods have been applied successfully to a variety of other sulfonylurea herbicides. The eluents have been modified slightly for the present method to achieve a lower detection limit and to adjust for the differences in background from the soil versus plant matrix. Detection of the DPX-66037 residues is by UV absorbance at 232 nm.

The sample is acidified prior to HPLC injection. Because DPX-66037 is relatively unstable under acidic conditions, samples are always kept on ice after acidification.

Two milliliters of the standard or extracted sample are pumped onto a phenyl column that retains the DPX-66037, which is uncharged under the initially acidic (pH 3.5) chromatography conditions. Polar compounds are readily eluted and do not interfere with subsequent chromatography. At a fixed time before the analyte elutes, the eluent is switched to one of higher pH (7.0) and lower methanol concentration. The analyte then becomes charged and elutes from the first column ahead of most nonpolar compounds which continue to be retained by the phenyl column. At the appropriate time, the eluent containing the entire DPX-66037 peak is transferred from the phenyl column outlet to a C18 column. Following transfer of the peak, chromatography continues on the C18 column only. Upon elution from the C18 column, the DPX-66037 is detected with a UV absorbance detector at 232 nm. Peak areas are compared to those of standards to calculate the amount detected, which is then used to calculate concentration in the sample.

II. MATERIALS

A. Equipment

Equivalent equipment or apparatus may be substituted.

Balance - Mettler model PM400 (Mettler Instrument Corporation, Hightstown, New Jersey).

Centrifuge - Du Pont Sorvall® model RC-5C refrigerated centrifuge (Du Pont Instruments, Wilmington, Delaware).

Centrifuge Rotors - Du Pont model H5-4 (Du Pont Instruments).

Centrifuge Bottles - 250-mL polypropylene, IEC Maxiforce® #2050, or 250-mL polypropylene, Nalge™ #21020-028 (VWR Scientific, Bridgeport, New Jersey).

pH Meter - Beckman model Φ-11™ (Beckman Instruments, Inc., Fullerton, California).

Filters - 0.22 µm, 47 mm Durapore® filter, Millipore® Millex®-HV, #SLHV025NB (Millipore Inc., Bedford, Massachusetts).

Liquid Chromatograph - This modular instrument is configured as shown in the Appendix using the following components. Equivalent equipment may be substituted.

Pump 1 (Main Pump) - Waters model 510 with high pressure dampening filter (Millipore Inc.).

Pump 2 (Auxiliary Pump) - Kratos Spectroflow model 400 with high efficiency filter (ABI Analytical, Foster City, California).

Detector - Kratos Spectroflow model 783G with 12 µL flow cell and gradient control option (ABI Analytical).

Column Oven and Controller - Waters oven model WAT038039, and Waters Temperature Control Module (Millipore®).

Valves 1 and 2 - Rheodyne model 7000P high pressure six-port two-position valve (Rheodyne Inc., Cotati, California).

Valve 3 - Rheodyne model 5011P low pressure six-position rotary valve (Rheodyne Inc.).

Valve 4 - Valco model ECSD12P-HC high pressure 12-position rotary valve with electric actuator (Valco Instrument Co., Houston, Texas).

Solenoids - Rheodyne model 7163 air actuated, set for four-way operation.

Data Collection and Integration - HP3396 series II Integrator (Hewlett Packard Co., Mountain View, California).

Data Storage - HP 9114B External Disk Drive (Hewlett Packard Co.)

Phenyl HPLC Columns (Column 1) - Zorbax® Phenyl, 4.0 mm x 80 mm, 5 micron, Reliance series cartridge #820662-942 and column end fittings #820669-001 (MAC-MOD Analytical Inc., Chadds Ford, Pennsylvania).

C18 HPLC Columns (Column 2) -Zorbax® Rx-C18™, 4.6 mm x 250 mm, 5 micron, analytical column #880967-902 (MAC-MOD Analytical Inc.).

B. Reagents and Standards

Equivalent reagents may be substituted.

Water - Deionized water passed through a Milli-Q® Water Purification System (Millipore, Corp.).

Methanol - EM R Omnisolve® #MX0488-1 (EM Scientific).

Acetonitrile - Fisher HPLC-grade #NA1648 (Fisher Scientific, Fair Lawn, New Jersey).

K_2HPO_4 - "Baker Analyzed"® Reagent #3252-01 (J. T. Baker Chemical Co., Phillipsburg, New Jersey).

KH_2PO_4 - EM® low absorbance grade #PX 1566-2 (EM Scientific).

H_3PO_4 - Fisher o-phosphoric acid, 85%, HPLC grade (Fisher Scientific).

$(NH_4)_2CO_3$ - "Baker Analyzed"® Reagent #0642-01 (J. T. Baker Chemical Co.)

DPX-66037 - Primary Reference Standard DPX-66037-12, 97.2% pure (Du Pont Agricultural Products, E.I. du Pont de Nemours and Company, Wilmington, Delaware).

C. Preparation of Solutions

1 M KH_2PO_4 - Dissolve 136 g of KH_2PO_4 in approximately 800 mL of water and dilute to 1 L. Filter through a 0.22 micron filter.

0.1 M KH_2PO_4 - Mix 100 mL of 1 M KH_2PO_4 with 900 mL of water.

1 M K_2HPO_4 - Dissolve 174 g of K_2HPO_4 in approximately 800 mL of water and dilute to 1 L. Filter as above.

0.1 M K_2HPO_4 - Mix 100 mL of 1 M K_2HPO_4 with 900 mL of water.

1 M $(NH_4)_2CO_3$ - Dissolve 96.09 g $(NH_4)_2CO_3$ in approximately 800 mL of water and dilute to 1 L. Filter through a 0.22 micron filter.

0.1 M $(NH_4)_2CO_3$ - Mix 100 mL of 1 M $(NH_4)_2CO_3$ with 900 mL of water.

Extraction Solution - acetonitrile / 0.1 M $(NH_4)_2CO_3$ buffer (3/1 by volume). Mix 750 mL of acetonitrile with 250 mL of 0.1 M $(NH_4)_2CO_3$.

Solution A - 35% 0.1 M Potassium Phosphate, pH 6.8, 65% methanol. Combine 400 ± 5 mL of 0.1 M KH_2PO_4 with 400 ± 5 mL of 0.1 M K_2HPO_4 solutions. While monitoring the pH of the mixture, add additional K_2HPO_4 of KH_2PO_4 solution as needed to reach a pH of 6.8. Mix 700 mL of this 0.1 M solution with 1300 mL of methanol.

Dilution Solution - 40% Solution A, 60% water. Mix 800 mL of Solution A with 1200 mL of water.

Chromatographic Eluents:

Eluent 1 - 0.01 M Potassium Phosphate, 54% methanol, pH 3.5. Add 20 mL of 1 M KH_2PO_4 to 920-mL water. Add 1100 mL of methanol and mix. Adjust the pH to 3.5 (as measured by a calibrated pH meter) by addition of 85% H_3PO_4. Sparge about 5 minutes with helium to degas.

Eluent 4 - 0.01 M Potassium Phosphate in 34% methanol, pH 7.0. Add 10 mL of 1 M K_2HPO_4 to 650-mL water. Add 340-mL methanol and mix. Adjust the pH to 7.0 (as measured by a calibrated pH meter) by addition of 85% H_3PO_4. Sparge as above.

Eluent 5 - 0.01 M Potassium Phosphate in 42% methanol, pH 7.0. Add 20 mL of 1 M K_2HPO_4 to 1100 mL water. Add 840 mL methanol and mix. Adjust the pH of to 7.0 ± 0.02 (as measured by a calibrated pH meter) by addition of 85% H_3PO_4. Sparge as above.

Eluent 6 - 90% methanol. Mix 1800-mL methanol with 200-mL water. Sparge as above.

Standards:

Stock Standard Solution - Approximately 20 mg of DPX-66037 Primary Reference Standard is accurately weighed and brought to 50-mL volume with acetonitrile to make a stock standard solution at about 400 µg/mL. An intermediate dilution from the stock standard to 10.0 µg/mL in acetonitrile is made and used for fortification of samples and preparation of chromatographic standards. The stock standard solutions are kept in the freezer for storage. The purity of the DPX-66037 primary reference standard is taken into account for all calculations.

Chromatographic Standard Solutions - Prepare chromatographic standards ranging from 0.0020 to 0.050 µg/mL in Dilution Solution from the 10 µg/mL standard solution above. The concentration of acetonitrile in these final dilutions is kept below 2%. Keep all chromatographic standards at or below 4°C following preparation. These standards are stable for several days. Before chromatography, acidify aliquots of these standards to a pH of 2.5 to 3, as detailed in Section III. Once acidified, the stability of the standards is greatly reduced; the acidified solutions should be kept on ice at all times and chromatographed within the next 18 hours.

D. Soils

Method development was carried out using Fargo-Ryan silty clay soil and untreated soils from AMR 1653-90, "The Magnitude of Residues of DPX-66037 Herbicide in Sugar Beets Grown in France in 1990" (Reference 4). Method validation was carried out with untreated soils from two test sites of AMR 1653-90.

III. METHODS

A. Preparation of Soil

Soil samples are homogenized in the presence of dry ice using a Hobart food chopper as per AMR 525-86, Section 3.

B. Fortification

Weigh 10.0-g samples of untreated soil into a 250-mL centrifuge bottle. Record the weights of the soil to 0.01 g. Fortify at levels from 0.002 through 0.05-µg DPX-66037/g soil (ppm) using an intermediate stock standard solution in acetonitrile. Allow the acetonitrile to evaporate.

C. Extraction

Accurately weigh 10.0 g of homogenized soil into a 250-mL centrifuge bottle. Record the weights of the soil samples to 0.01 g. Fortify as in step B if required. Add 40-mL Extraction Solution (acetonitrile/ammonium carbonate, 3:1 by volume) and mix. Shake with wrist-action shaker for 10 minutes. Centrifuge the mixture in the Sorvall centrifuge for 10 minutes at approximately 2800 rpm and 0°C. Decant liquid into a labeled 100-mL beaker. Add 40-mL more of Extraction Solution to the soil and break up the pellet. Shake for 10 minutes on the wrist-action shaker. Centrifuge as above. Decant liquid into the same beaker as before.

Adjust the pH of the decanted extraction solution to 7.5 using concentrated HCl initially, then dilute HCl.

Transfer sample into a labeled 250-mL separatory funnel and add approximately 50-mL methylene chloride. Shake for approximately 20 seconds. Vent as necessary to release pressure. Collect bottom (methylene chloride) layer into

a labeled 250-mL round bottom flask. Add approximately 35-mL methylene chloride to the separatory funnel. Again shake with venting for approximately 20 seconds. Collect the bottom (methylene chloride) layer into the same 250-mL round bottom flask.

Evaporate the sample to between 5 and 10 mL on a rotary evaporator using appropriate vacuum and temperature to control the process. Continue to evaporate just to the point of dryness, then remove flask from evaporator.

To reconstitute the sample, use a pasteur pipet to rinse the flask with 3 to 5 mL of Dilution Solution and transfer the liquid to a labeled 10-mL volumetric flask. Adjust the volume to 10.0 mL with Dilution Solution. Note that the 10.0-mL final volume represents the entire 10.0-g soil sample. Filter through a 0.45-µ filter prior to chromatography.

At this point, samples may be capped and stored in the refrigerator for several days, if necessary. When ready to chromatograph, allow samples to equilibrate with the injector ice bath temperature and acidify by adding 20 µL of 85% H_3PO_4.

D. Chromatography

1. Establish Switching Times

The times for the eluent and column switching are determined each day during initialization to adjust for variations in retention times. Such variations are to be expected for several reasons: fresh eluents which are slightly different from the previous eluent, fouling of the phenyl column which is being used for sample clean-up, and column aging.

Evaluate the phenyl column and determine eluent switch time: A 0.02 µg/mL DPX-66037 standard is chromatographed on the phenyl column only using Eluent 1 only. For the equipment in this study, this was accomplished using Program 2 in the Appendix. If the peak is noticeably tailed or broadened (i.e. if the peak width at half-height was greater than 1.3 min) it is time to replace the phenyl column. If the peak shape is acceptable, the retention time (t) is noted. The eluent switching time is this retention time (t) minus a time, E4, chosen so that the peak of interest is approximately half-way through the phenyl column. For the equipment

used in the present study E4 is eleven minutes. When alternate equipment is used, E4 must be determined as discussed in AMR 2021-91 (Reference 1).

Determine column switching time window: Using the phenyl column only, chromatograph a 0.02 µg/mL DPX-66037 standard using Eluent 1 and switching to Eluent 4 at the eluent switching time determined above (t-E4). Refer to Program 6 in the Appendix. Determine the retention time (T). The column switching time window begins 2.5 minutes prior to this retention time, and ends 3.5 minutes after this retention time. This provides a six-minute window during which the phenyl column effluent is quantitatively transferred directly to the C18 column inlet.

After these initial runs are made, standards and samples can be run using the updated eluent and column switching program (Program 4 for both the detector and gradient, see Appendix).

2. Calibration Standards

The 10 µg/mL DPX-66037 stock solution is prepared as described in the Materials section above and stored in a freezer. Chromatographic standards are prepared by serial dilution of this stock solution using the Dilution Solution. Five levels in the range 0.0020 to 0.050 µg/mL are used. Standards are stored in a refrigerator and may be used for four days. Prior to chromatography, standards are acidified by adding 20 µL of 85% H_3PO_4 to adjust the pH to between 2 and 3.

Note that when using the pump for injections, as done here, sufficient sample volume is required to flush the pump and tubing with sample prior to pumping it onto the column. The equipment described in this report required 9-mL sample. Because volume is temperature dependent, allow the standards to equilibrate with the injector ice bath temperature prior to injection. No more than three samples were run between standards.

3. Analysis

When a sample or standard is acidified and temperature equilibrated in the injector ice bath, a sample inlet tube from valve 4 is placed in the sample or standard and the chromatographic method initiated. The steps below describe in

general terms the events which occur during the chromatography. The specific program used by the instrument in this study is included as Program 4 in the Appendix and can be adapted for other equipment.

Initial conditions. The phenyl column is in-line with the main pump, Eluent 1 is selected.

Sample is pumped through the main pump, bypassing both columns. This flushes the pump and tubing with the sample.

The phenyl column is put in-line with the main pump so that sample is pumped onto the column. The timing of this step is chosen to allow 2.0-mL sample to be pumped onto column 1.

The main pump begins pumping Eluent 1, bypassing both columns to flush the pump and tubing with Eluent 1.

The phenyl column is placed in-line and chromatography with Eluent 1 begins.

Eluent switching. Chromatography with Eluent 4 through the phenyl column begins. The timing of this step is determined each day as outlined in Section D1 above so that it occurs when the peak of interest is approximately half-way through the phenyl column.

Column switching. The C18 column is put in-line so that the eluent from the phenyl column is transferred to the inlet of the C18 column. The timing for this step is determined each day as outlined in Section D1 above so that the entire peak of interest is transferred.

After the column switching, the phenyl column is taken off-line and backflushed for 31 minutes with Eluent 6 using the auxiliary pump. Chromatography on the C18 column continues with Eluent 5 until after the peak of interest has eluted.

Clean the C18 column using the main pump and Eluent 6.

Equilibrate the C18 column with Eluent 1.

Take the C18 column off-line and place the phenyl column in-line. Allow the phenyl column to equilibrate with Eluent 1.

The UV absorbance signal from the detector was sent to the integrator which measured peak retention times (minutes) and peak areas (microvolt-

seconds). Alternatively, a stripchart recorder could be used to obtain retention times and peak heights.

E. Calculations

A Response Factor is calculated using the peak areas (or heights) from the analysis of standards. A plot of peak area versus concentration of DPX-66037 in the standards can be used to construct a standard curve via a least squares fit program. This curve is determined to be linear over the range of interest with a near-zero intercept (see section IV). Therefore, the Response Factor can be obtained simply by calculating:

$$\text{Response Factor} = \frac{\text{peak area}}{\text{DPX-66037 } \mu g/mL}$$

The Response Factor used to calculated a sample concentration was the average of Response Factors for the standards analyzed the same day.

The extraction procedure results in a 10.0-mL volume of sample for HPLC analysis which represents the entire 10.0 g of soil sample. The HPLC analysis, (injection volume, integration parameters, etc.) is equivalent for standards and samples. Therefore, the area of the sample peak of interest and the Response Factor are used in a straightforward equation to calculate the concentration in µg/mL of the sample as injected, which is equivalent to the ppm concentration of DPX-66037 in the soil sample.

$$\frac{\text{sample peak area}}{\text{Response Factor}} = \text{DPX-66037 } \mu g/mL \text{ sample} = \mu g \text{ DPX-66037/g soil (ppm)}$$

F. Time Required for Analysis

Typically six soil samples at a time are prepared for HPLC analysis, which requires four to five hours. During that time, the HPLC system is initialized and standards are run. For the equipment used in this study, chromatography required 73 minutes per sample or standard.

IV. RESULTS AND DISCUSSION

Figure 2 displays a graph of peak area versus concentration in µg/mL for DPX-66037 chromatography standards. The relationship is shown to be linear over the tested range of 0.0020 to 0.050 µg/mL and was approximated by a least squares fit line with the equation

$$\text{area} = 47{,}620{,}000 \times \text{concentration} - 25660$$

where the slope is the Response Factor. The correlation coefficient for this fit was 0.9966. The standard curves were very consistent throughout the method development and validation. The y-intercept is at an area equal to -0.0005 ppm and can be considered as zero.

The recovery of DPX-66037 from freshly spiked soils was determined to be 94% ± 7 (± SD). Untreated soils from study AMR 1653-90, "The Magnitude of Residues of DPX-66037 Herbicide in Sugar Beets Grown in France in 1990" (Reference 4), were fortified and used for recovery measurements. Representative chromatograms of a standard, an unfortified soil sample, and a fortified soil sample are given in Figures 3 through 5. Results from these experiments are given in Table I. Recovery values from samples fortified below the level of quantitation (0.005 ppm) are reported, but are not used in the calculation of average recoveries. There is one unusually low recovery (41%) from a 0.0050-ppm fortified sample. Using the Q-test (a statistical method of assessing suspect measurements), this value can be rejected as an outlier from the data set with 95% confidence. With the seven remaining values, the mean and standard deviation are 94% ± 7. The range of recoveries was 82 to 105%.

Additional fortified soil samples were analyzed as part of AMR 1653-90, "The Magnitude of Residues of DPX-66037 Herbicide in Sugar Beets Grown in France in 1990" (Reference 4). Recoveries are given in Table II. The average ± SD for these ten samples is 97% ± 6. Combining the data from method validation and AMR 1653-90 fortified samples and setting a confidence limit of 0.95, Figure 6 shows that the data support a limit of detection of 0.0020 ppm and a limit of quantitation of 0.0050 ppm. The criteria which control the limits are matrix effects or small amounts of interferences as shown in Figure 4.

The validation of this extraction procedure and the freezer stability of DPX-66037 in soils has been shown in AMR 1656-90, "Freezer Storage Stability of DPX-66037 in Soil" (Reference 1) which at the time of this report has been completed through nine months with no significant loss of recovery.

CONCLUSION

This method is suitable for the quantitation of DPX-66037 in soils down to 0.005 ppm. The quantitation limit and recovery efficiencies are adequate for the analysis of DPX-66037 residues in soils.

CERTIFICATION

ANALYTICAL METHOD FOR THE QUANTITATION OF DPX-66037 IN 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:

Kristin H. Milby
Study Director — Date: 6 Dec. 1991

John M. Brisbin
Laboratory Technician — Date: Dec 6, 1991

Oliver R. Hunt
Senior Research Chemist — Date: 12/6/91

Approved by:

Ronald D. Collins
Research Supervisor — Date: 12/6/91

Date Study Initiated: September 9, 1991

Date Study Completed: December 5, 1991

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Wilmington, Delaware 19880-0870

Sponsor:
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Du Pont Agricultural Products
Research and Development Division
Experimental Station
Wilmington, Delaware 19880-0402

TABLE I

METHOD VALIDATION RECOVERIES

Fortification/ Extracted	Sample Number	Date HPLC'd	Peak Area	Measured ppm	Fortification ppm	Recovery %
10-SEP-91	S73589#3	10-SEP-91	173783	0.0042	0.0050	84
10-SEP-91	S73589#4	10-SEP-91	407840	0.0091	0.0100	91
10-SEP-91	S73589#5	10-SEP-91	827729	0.0179	0.0200	90
10-SEP-91	S73589#2	11-SEP-91	101606	0.0027	0.0020	134*
10-SEP-91	S73589#6	11-SEP-91	954920	0.0206	0.0500	(41)**
10-SEP-91	S73589#1	11-SEP-91	no peak	<0.002	0.0000	
12-SEP-91	S73719#5	12-SEP-91	861757	0.0186	0.0200	93
12-SEP-91	S73719#3	12-SEP-91	225001	0.0053	0.0050	105
12-SEP-91	S73719#4	12-SEP-91	450271	0.0100	0.0100	100
12-SEP-91	S73719#1	13-SEP-91	no peak	<0.002	0.0000	
12-SEP-91	S73719#2	13-SEP-91	52736	0.0016	0.0020	82*
12-SEP-91	S73719#6	13-SEP-91	2215414	0.0471	0.0500	94
					mean	94
					SD	7
					N	7

* Recoveries from fortification levels below the level of quantitation were not included in the calculations of the mean and standard deviation.

** Rejected via the Q-test. See text.

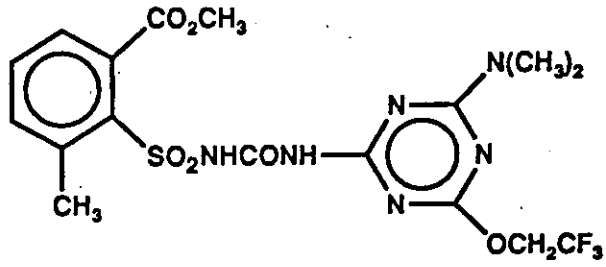
TABLE II

RECOVERY OF DPX-66037 FROM FORTIFIED SOILS FOR AMR 1653-90 "THE MAGNITUDE OF RESIDUES OF DPX-66037 HERBICIDE IN SUGAR BEETS GROWN IN FRANCE IN 1990"

Fortified ppm	Measured ppm	Recovery %
0.02000	0.01930	97
0.02000	0.02000	100
0.02000	0.02010	101
0.02000	0.02160	108
0.02000	0.01920	96
0.00500	0.00482	96
0.00500	0.00476	95
0.00500	0.00484	97
0.00500	0.00419	84
0.00500	0.00463	92
	average	97
	SD	6
	N	10

FIGURE 1

STRUCTURE AND CHEMICAL NAME FOR DPX-66037



DPX-66037

Methyl 2-[[[[[4-(dimethylamino)-6-(2,2,2-trifluoroethoxy)-1,3,5-triazin-2-yl]amino]carbonyl]amino]sulfonyl]-3-methylbenzoate

FIGURE 2

STANDARD CURVE FOR AMR 1965-91 METHOD VALIDATION

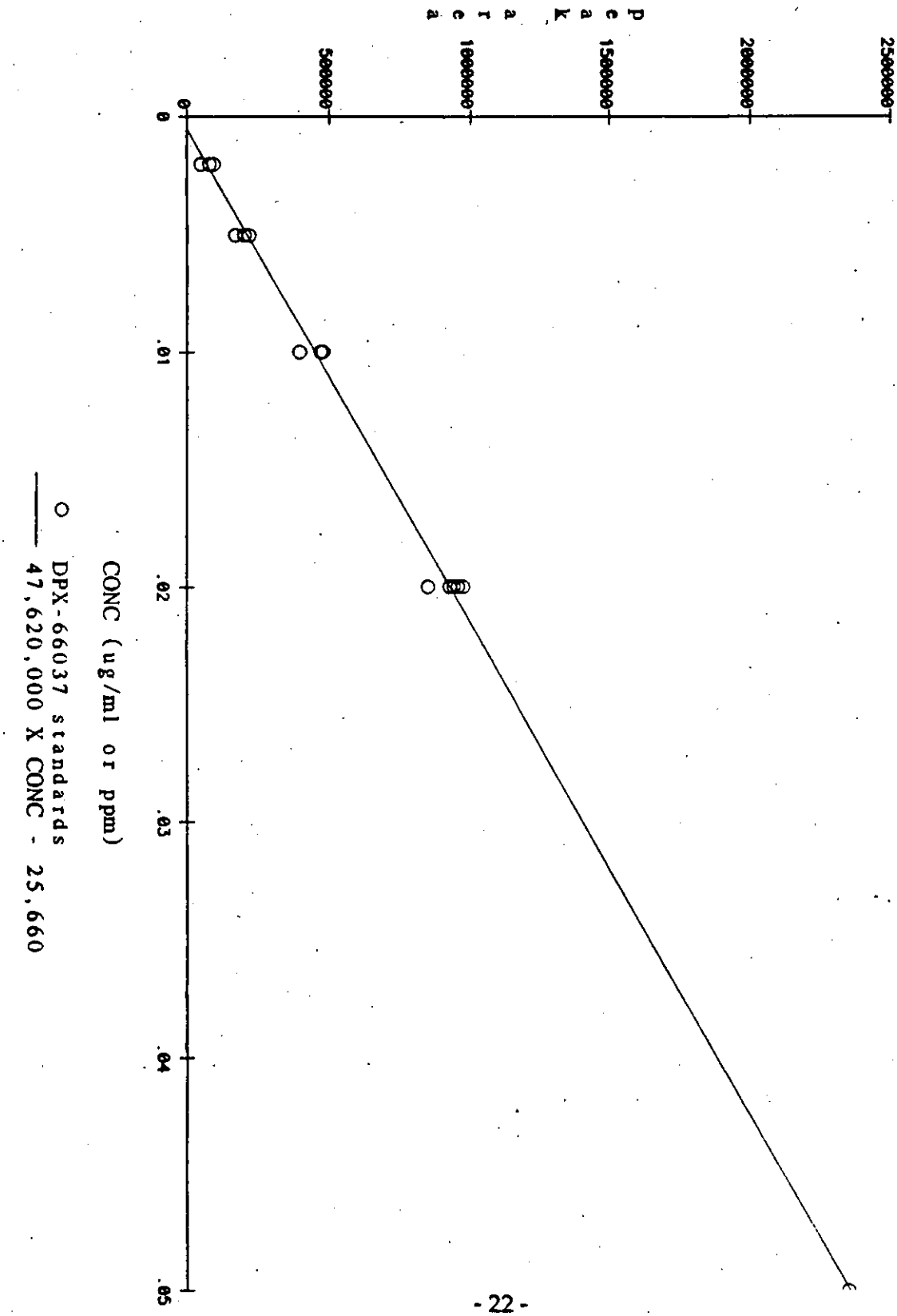


FIGURE 3

DPX-66037 0.0050 µg/mL STANDARD

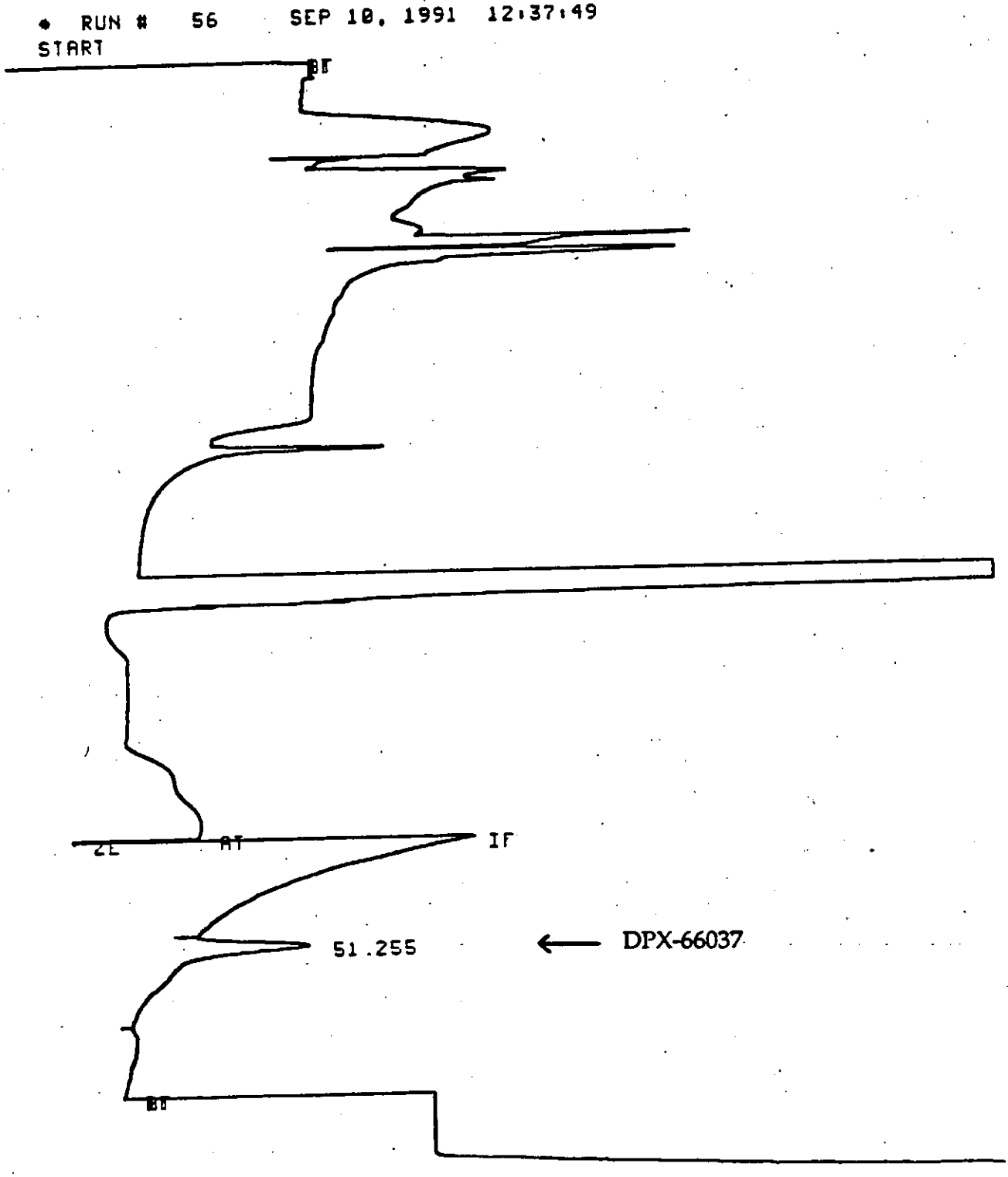


FIGURE 4

UNTREATED SOIL SAMPLE - NO FORTIFICATION

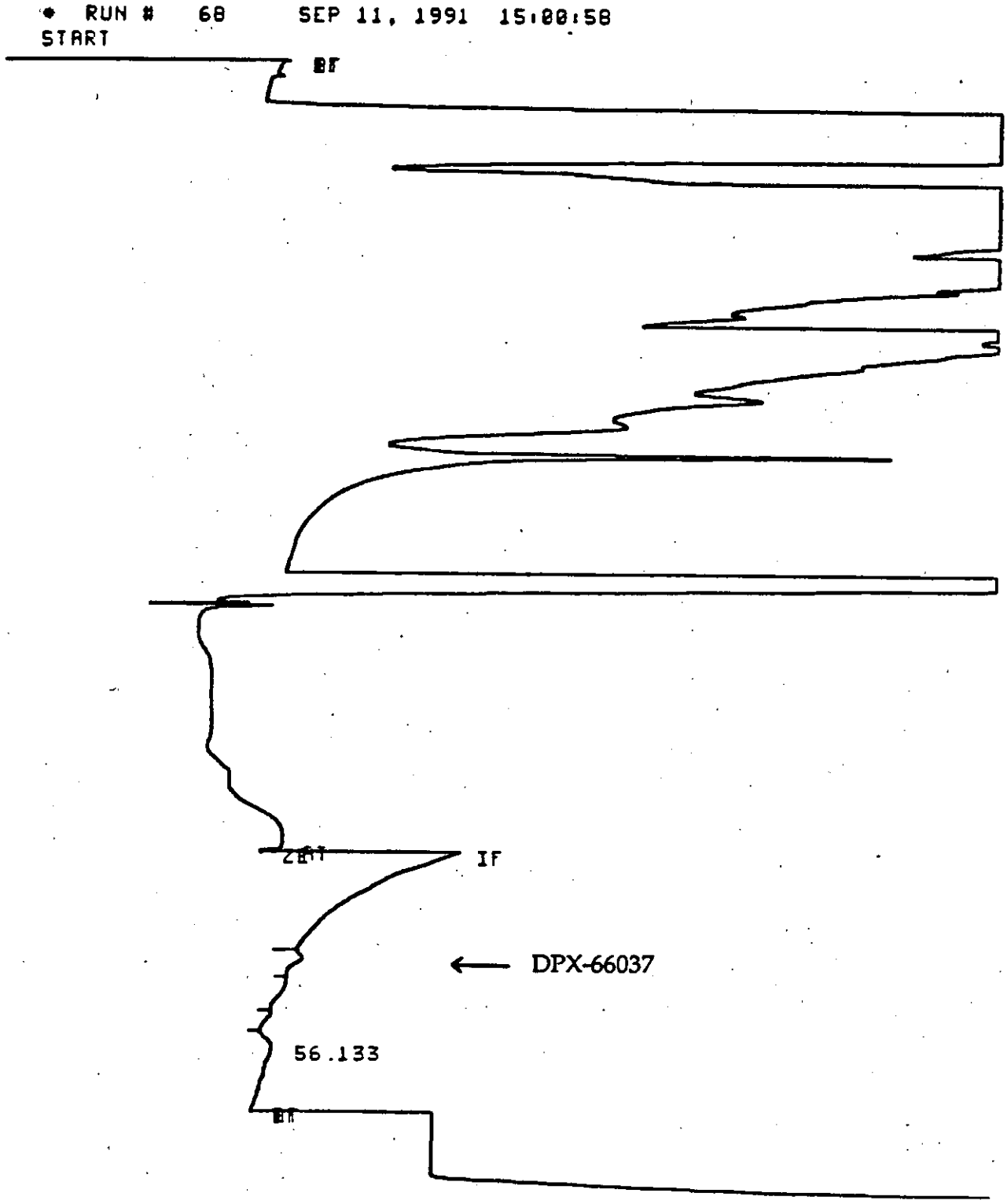


FIGURE 5

UNTREATED SOIL SAMPLE 0.005 ppm FORTIFICATION

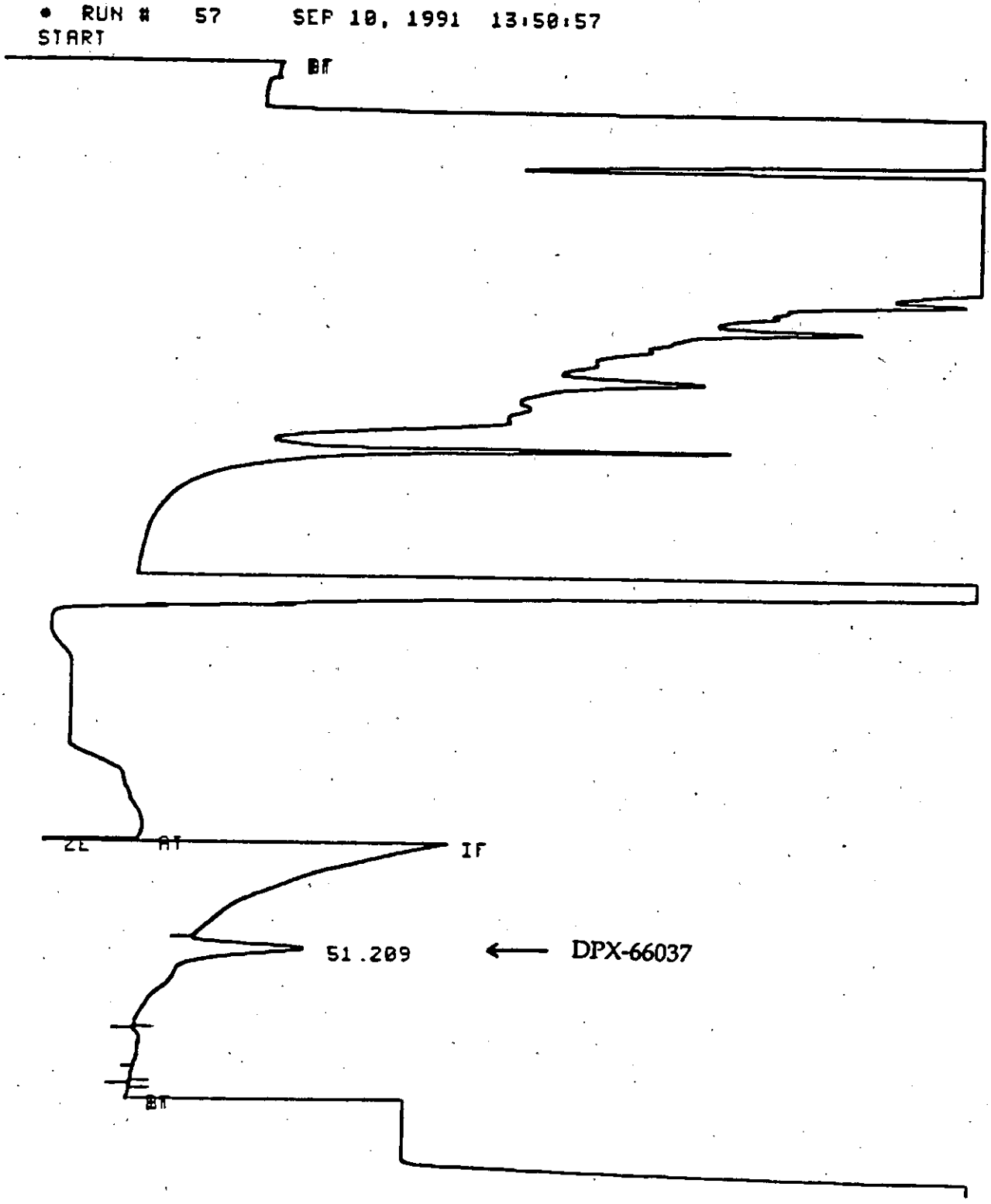


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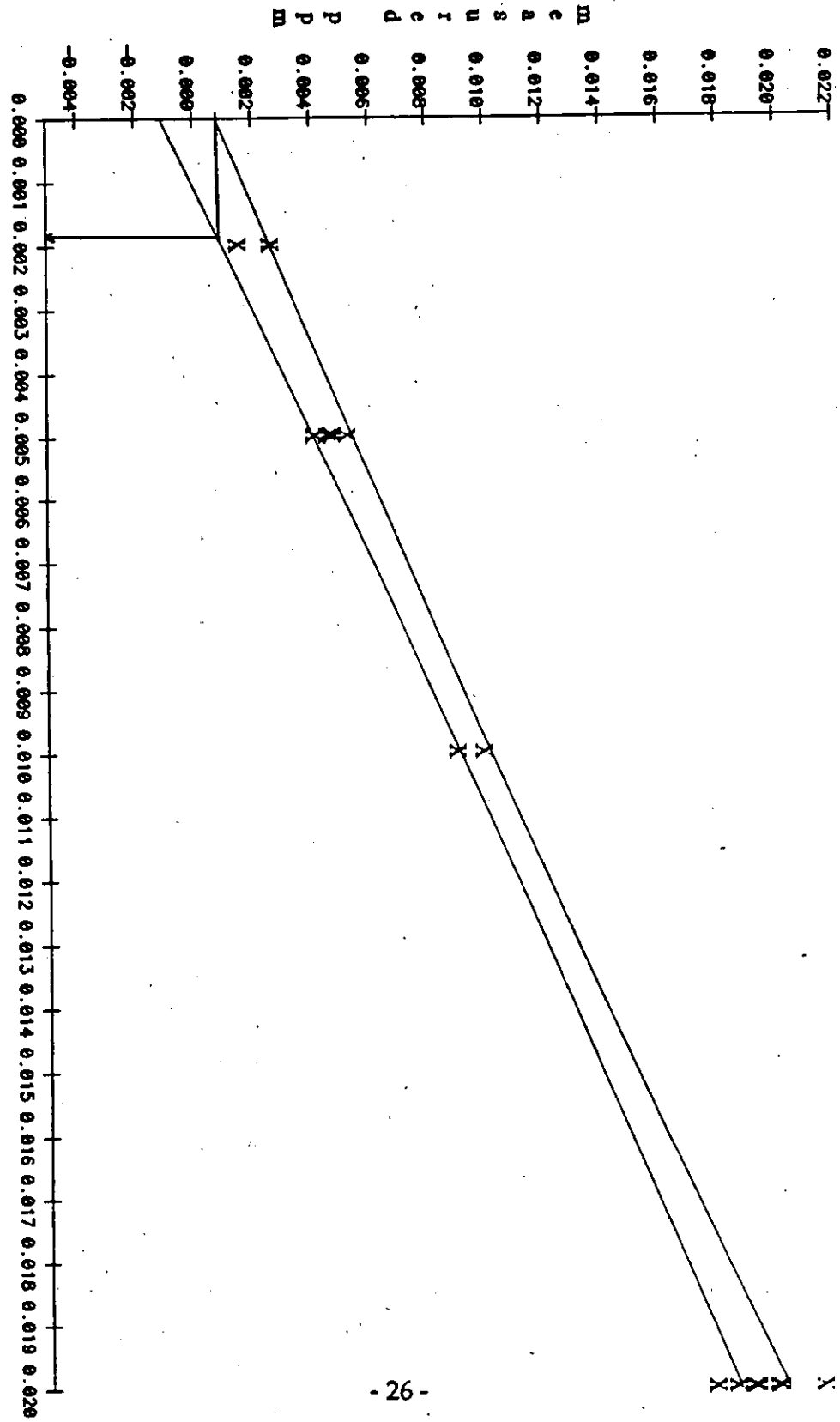
FIGURE 6

RECOVERY DATA FROM METHOD VALIDATION
AND AMR 1653-90 FORTIFIED SAMPLES



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1. Major, L. J., Larochelle, J. H, Bedwell, L. L. , Rossi, P. G, "Automated Analytical Method for the Quantitation of DPX-66037 in Sugar Beets", Du Pont Report No. AMR 2021-90, Revision No. 1, Du Pont Agricultural Products.

2. Hunt, O. R., Brisbin, J. M., "Freezer Storage Stability of DPX-66037 in Soil", Du Pont Report No. AMR 1656-90, in process.

3. Rhodes, B. C., "Determination of Dissociation Constant of DPX-66037", Du Pont Report No. AMR 1983-91, Du Pont Agricultural Products.

4. Slates, R. V., Brisbin, J. M., Devine, P. G. Major L. J., and Milby, K. H., "The Magnitude of Residues of DPX-66037 Herbicide in Sugar Beets Grown in France in 1990", Du Pont Report No. AMR 1653-90, in process.

5. McIntosh, C. L., "Laboratory Analysis of Pesticide Residues in Plant, Animal, and Soil Samples - Guidelines and Procedures", Du Pont Report No. AMR 524-86, Agricultural Products Department, Du Pont Company,.

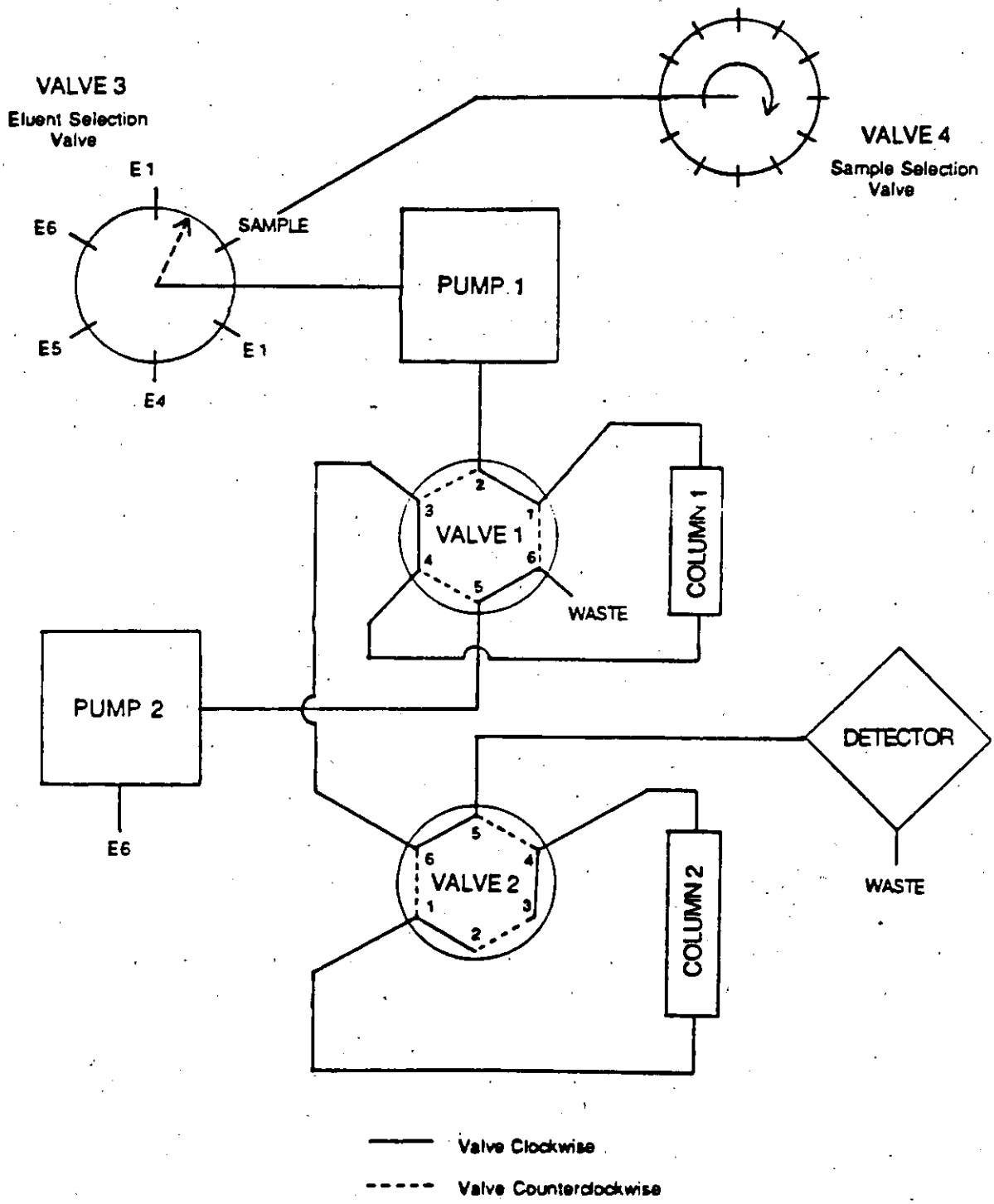
APPENDIX

INSTRUMENT CONFIGURATION AND OPERATION

Summary of Chromatographic Conditions

Column:	1	Zorbax® Phenyl 4.0 mm x 80 mm, 5 micron Reliance series cartridge
	2	Zorbax® Rx-C18™ 4.6 mm x 250 mm, 5 micron analytical
Column Temperature:		40° C
Pump 1 Eluent Flow Rate:		1.3 mL/min.
Eluent:	1	10 mM potassium phosphate in 54% methanol, pH 3.5
	4	10 mM potassium phosphate in 34% methanol, pH 7.0
	5	10 mM potassium phosphate in 42% methanol, pH 7.00
	6	90% methanol / 10% water
Sample Volume:		2.0 mL
Detector:	Wavelength	232 nm
	Sensitivity	1 volt = 1 AUFS (Absorbance Unit Full Scale)
		5 to 10 mV full scale should provide adequate sensitivity

Schematic of the Chromatograph



Detector Programming

The valves shown on page 28 are controlled through programming of the Kratos Spectroflow detector. Detector Programs 2, 6 and 4 follow. These programs control four internal relays, three of which switch 12-volt power supplies to activate solenoid air valves which, in turn, activate chromatographic valves 1, 2 and 3. Relay 1 controls addition and removal of the phenyl column through Valve 1; relay 2, addition or removal of the C18 column through Valve 2; relay 3, eluent selection through Valve 3, and relay 4, sample selection through Valve 4 (electrically actuated). (Refer to the figure on page 28 for the instrument configuration.) "Pneumatic valve-switching stations" have been constructed which permit the option of manual override of the detector.

Time: The time (in minutes) after the run begins at which each event occurs.

Relay: Each relay may be in one of two positions, 1 = on and 0 = off. Relays 1 through 4 actuate the following:

Relay 1 - Valve 1. 1 = valve counterclockwise for phenyl column backflushing by Pump 2 and removed from series with Pump 1.

Relay 2 - Valve 2. 1 = valve counterclockwise to place the Rx-C18™ column in-line with Pump 1.

Relay 3 - Valve 3. 1 = valve steps to the next position, selecting between eluents. 0 = valve resets.
Valve 3 is rotated clockwise by a ratchet mechanism. Thus, the actuator must be reset before the valve can advance to the next position.

Relay 4 - Valve 4. 1 = valve steps to next position to select next sample.

Detector Program #2

Chromatographic program for t-E4 determination: one column, one eluent

STEP	TIME	RELAY 1234	EVENTS
1	0.01	0000	start, valve 3 position = 1
2	0.83	1010	phenyl column out, valve 3 position = 2 flush lines
3	1.43	1000	reset valve 3
4	5.33	0000	phenyl column in, sample loaded onto column
5	6.87	1010	phenyl column out, valve 3 position = 3 flush lines
6	10.00	0000	phenyl column in, reset valve 3
7	44.00	1010	phenyl column out, valve 3 position = 4
8	44.10	1000	reset valve 3
9	44.20	1010	valve 3 position = 5
10	44.30	1000	reset valve 3
11	44.40	1010	valve 3 position = 6
12	44.50	1000	reset valve 3
13	44.60	1010	valve 1 position = 3
14	48.00	0000	reset conditions: return phenyl column; system reequilibrates

Note: Program may be aborted after step 6 and valve 3 may be manually reset to position 1 after the DPX-66037 peak has eluted.

Detector Program #6

Chromatographic program for column-switching time determination: one column, two eluents.

STEP	TIME	RELAY 1 2 3 4	EVENTS
1	0.01	0 0 0 0	start: valve 3 position = 1
2	0.83	1 0 1 0	phenyl column out, valve 1 position = 2 flush lines
3	1.43	1 0 0 0	reset valve 3
4	5.33	0 0 0 0	phenyl column in, sample loaded onto column
5	6.87	1 0 1 0	phenyl column out, valve 3 position = 3 flush line
6	10.00	0 0 0 0	phenyl column in & reset valve 3 chromatography begins
7	t-E4	0 0 1 0	valve 3 position = 4
8	44.80	1 0 0 0	phenyl column out
9	45.00	1 0 1 0	valve 3 position = 6
10	45.10	1 0 0 0	reset valve 3
11	45.20	1 0 1 0	valve 3 position = 6
12	45.30	1 0 0 0	reset valve 3
13	45.40	1 0 1 0	valve 3 position = 1
14	45.50	0 0 0 0	reset conditions: return phenyl column; system reequilibrates

Note: Program may be aborted after step 7 and valve 3 may be manually reset to position 1 after the DPX-66037 peak has eluted.

Detector Program #4

Complete chromatographic program, including eluent- and column- switching.

STEP	TIME	RELAY 1 2 3 4	EVENTS
1	0.01	0 0 0 0	start: valve 3 position = 1
2	0.83	1 0 1 0	phenyl column out, valve 3 position = 2 flush lines
3	1.43	1 0 0 0	reset valve 3
4	5.33	0 0 0 0	phenyl column in, sample loads onto column
5	6.87	1 0 1 0	phenyl column out, valve 3 position = 3, flush lines
6	10.00	0 0 0 0	phenyl column in & reset valve 3 chromatography begins
7	t-E4	0 0 1 0	valve 3 position = 4
8	24.00	0 0 0 0	reset valve 3
9	T-2.5	0 1 0 0	add C18 column
10	T+3.5	1 1 1 0	valve 3 position = 5, remove phenyl column backflush of phenyl column begins
11	40.00	1 1 0 1	reset valve 3; move to next sample position
12	60.00	1 1 1 0	valve 3 position = 6
13	60.50	1 1 0 0	reset valve 3
14	65.00	1 1 1 0	valve 3 position = 1, equilibrate C18 column
15	66.00	1 1 0 0	reset valve 3
16	68.00	0 0 0 0	reset conditions: remove C18 column, return phenyl column; system reequilibrates

Gradient Program #4

Backflush of phenyl column after the column switching

Segment	Start Time (min.)	Length (min.)	Pump Flow (mL/min.)
0	0	28	0
1	28	1	0
2	29	30	1
3	59	1	1
4	60	15	0

Turns Pump 2 flow on at 1.0 mL/min. from 29 to 60 minutes.