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SEP 29 1994

MEMORANDUM

SUBJECT: Environmental Chemistry Method Lab Evaluation Report for ECM #0016S1-Triflusaluron Methyl (DPX66037) in Soil (MRID 427495-01)

FROM: Aubry E. Dupuy, Jr., Section Chief *Aubry E. Dupuy, Jr.*
 BEAD/ACB/Environmental Chemistry Section

THRU: Donald A. Marlow, Branch Chief ~~_____~~
 BEAD/ACB/Environmental Chemistry Section (7503W)

TO: Henry M. Jacoby, Branch Chief
 EFED/Environmental Fate and Ground Water Branch (7507C)

The Environmental Fate and Ground Water Branch has requested an Environmental Chemistry Method Lab Evaluation on Triflusaluron Methyl (DPX66037) in soil. The analytical method, entitled "Analytical Method for the Quantitation of DPX-66037 in Soil," was submitted by DuPont Company (MRID 427495-01).

As requested, the fortification levels were the same as in the submitted method at 0.005 and 0.02 parts per million (ppm) in soil. Four replicate analyses and a sample blank were performed at each level. The attached Analysis Report contains a summary, analytical results and experimental details, including representative chromatograms, calibration curves and examples of calculations.

If you have any questions concerning this report, please contact Han Tai (601) 688-3252) or me (601-688-3212).

cc: Danny McDaniel, QA Officer, ECS
 Han Tai, Chemist, ECS



Environmental Chemistry Method Validation Report

Triflusulfuron Methyl (DPX66037) in Soil

ECM #0016S1

Environmental Chemistry Section

Analytical Chemistry Branch

Biological and Economic Analysis Division

Prepared by: Han Tai

Date: 9/30/94

Reviewed by: *Danny McDonald*

Approved by: *Aubrey E. Dupuy, Jr*

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Part I

Summary and Conclusions

An Environmental Chemistry Method Lab Evaluation has been performed for the analysis of Triflusulfuron Methyl in soil. The analytical method is described in DuPont Report Number AMR 1965-91 (MRID 427495-01), "Analytical Method for the Quantitation of DPX-66037 in Soil." DPX-66037 is DuPont's code for Triflusulfuron Methyl.

The method employs a relatively simple procedure of extraction and solvent partitioning. After the clean-up of the extracts, the quantitation of the analyte DPX-66037 is performed by High Performance Liquid Chromatography (HPLC), using two columns and four eluents. The extracts are first loaded on a phenyl column to concentrate the analyte and to remove the interfering compounds by eluents of phosphate buffers at pH 3.5 and 7.0. The analyte is then transferred onto a C-18 column for quantitation. The HPLC system is automated through the use of high pressure valves and a programmable HPLC pump to handle the elaborate steps of eluent and column switching. The present instrumentation requires about 85 minutes for each HPLC run.

Fortification levels were 0.005 and 0.020 parts per million (ppm) for the soil samples. Four replicate analyses have been done for each fortification level. The mean percent recovery values for the 0.005 ppm fortification level was 75.0% with an RSD of 6.35% and a range of 72.0-81.0%. The 0.02 ppm fortification level had a mean percent recovery of 84.8% with an RSD of 7.00% and a range of 76.0-88.7%.

The results appear to be acceptable. Considering the HPLC run time for each sample or standard at about 85 minutes, we agree with the statement in the method (p.13) that no more than three samples should be run between standards. A set of four standards and three samples would require a working day of at least 10 hours.

Part II

Method: "Analytical method for the Quantitation of DPX66037 in Soil," by Kristin Milby, John M. Brisbin and Oliver R. Hund, Dec. 5, 1991. DuPont Report Number AMR 1965-51 (MRID Number 427495-01)

Results:

<u>ppm added</u>	<u>ppm Found(1)</u>	<u>% Recovery</u>	<u>χ(2)</u>	<u>SD</u>	<u>%RSD</u>
0.02	0.0176	88.0			
0.02	0.0152	76.0			
0.02	0.0173	86.5			
0.02	0.0177	88.7	84.8	5.94	7.00
0.005	0.0041	82.0			
0.005	0.0036	72.0			
0.005	0.0037	74.0			
0.005	0.0036	72.0	75.0	4.76	6.35
0.000 (Blank)	ND(3)	--			
0.000 (Blank)	ND	--			
0.000 (Blank)	ND	--			
0.000 (Blank)	ND	--			

Note: (1) Values are results of four replicates, including extraction and HPLC

(2) χ = Mean; SD = Standard Deviation;; %RSD = Relative Standard Deviation (100SD/X)

(3) ND = Not Detected. For the present procedure and sample size of 10 grams Minimum Detection Limit (MDL) is 0.002 ppm (3 x noise). Limit of Quantitation is 0.003 ppm (5 x noise).

Part III
Experimental

General Description of Methods

A. Extraction and solvent partition:

Soil sample, 10.0 gm, is extracted, on a wrist-action shaker, with 40 ml of extraction solution (3/1 acetonitrile/0.1 M ammonium carbonate). Centrifuge and decant extract. Repeat above procedure with another 40 ml of extraction solution and combine extracts. Partition with 50 ml of methylene chloride in a separatory funnel. Repeat once with an additional 35 ml. of methylene chloride. Combine extracts and evaporate to dryness on rotary evaporator at about 35°C. Dissolve in 10.0 ml. dilution solution (phosphate buffer in methanol, pH 6.8). Filter through 0.2 um syringe filter into a 15 ml centrifuge tube. Keep at 0°C (ice bath).

B. High performance Liquid Chromatography (HPLC):

- a. Waters Automated Valve Station (WAVS) Connections ("plumbing")-as shown in Figure 1
- b. Waters Model 590 Programable Solvent Delivery System (Pump A)- Six "Event Out" terminals are used for eluent and column switching. The 590 Events have 11 Time Segments.

HPLC Instrument Parameters:

- Columns: (1) Zorbax phenyl, Reliance Cartridge (#820682-942) with End fitting (#820669-901)
(2) Zorbax RX-C18, 4.6 x 150 mm, (#880967-902, Serial #CU 3653)

Mobile Phases: Flow rate 1.3 ml/min; Isocratic; 3 Eluents of 0.01 M phosphate buffer, as shown below

<u>Eluent</u>	<u>pH</u>	<u>% MeOH</u>	<u>purpose</u>
1	3.5	54	Retain DPX on phenyl; Flush ionic compounds
4	7.0	34	Transfer DPX to C18; Retain non-ionic compounds on phenyl
5	7.0	40	Chromatography DPX on C18
6	(90/10 MeOH/Water) Back flush 2 columns		

Detector: Waters Model 490 Multi-Wavelength Detector
Absorbance, UV 232 nm; 0.01 AUFS (Absorbance units full scale)
time constant 5.0

Temperature: Waters Temperature Control System
2 Columns in the same oven; Temp Control Module (TCM) set at
40.0°C

Pump: (1) Waters Model 590 Programmable Pump
(2) Model 510 pump

Recorder: Kipp-Zonen Model BD 41 - Full scale 10mv; chart speed 0.5 cun/min

Retention Time:
Phenyl column, eluent 1 - about 23.0 minutes (Time t, Method p. 12)
Phenyl column, eluent 4 - about 37.0 minutes (Time T, Method p. 13)
C-18 column, eluent 5 - about 61.5 minutes for DPX 66037
Solvent front travel - about 11.10 minutes (Time E4, Method p. 12)
(from 0 time to phenyl column)

Modification-of-Method (major or minor):

None. Equivalent instruments are used.

Brief description of HPLC operation:

1. The samples and standards to be run are placed in an ice bath before injection.
2. The eluent front travel time E4 from valve outlet to halfway through phenyl column can be calculated from the flow rate and the dimensions of the transport tubings and the phenyl column.
3. Determine Eluent Switch Time (t-E4) (Method p.12):
 - a. Set Model 590 Event Segments (seg) as in Table 1. Set Seg 06 at 48.00 minutes, Seg 07 at 52 minutes and Seg 08 at 55 minutes. Set flow rate at 1.3 ml/min.
 - b. Acidify one centrifuge tube. 10 ml of standard 0.02 ug/ml with 20 ul 85% phosphoric acid (Eppendorf pipet), and mix well (vortex mixer). Avoid air bubbles in the inlet line.
 - c. Start run, from Seg 01 and time 0.
 - d. Stop run after the emergence of DPX 66037 peak. Record retention time t.
 - e. Change Seg 06 Time from 48 min to (t-E4) minutes. Re-set 590 to Seg 01.
4. Determine Column Switch Time (T - 2.50; T + 3.50):
 - a. Repeat step 3a, 3b, and 3c.
 - b. Stop run after the emergence of DPX 66037 peak. Record retention time T.
 - c. Change Seg 07 Time from 55 minutes to (T - 2.50) minutes. Change Seg. 08 Time from 55 minutes to (T + 3.50) minutes. Reset 590 to Seg. 01
5. For the present HPLC set-up: E4 is 11.10 minutes, t is about 25 minutes and T is about 37 minutes.
6. Run standard or sample:
 - a. Acidify one centrifuge tube of standard or sample as in 3b.
 - b. Reset to Seg. 01. Time 0. Start run, The analytical peak emerges at about 62 minutes.
 - c. Stop run at about 85 minutes. Reset to Seg. 01, Time 0 for next run.

Source of Analytical Reference Standard:

DPX 66037. F-1017. Lot AHZ-1. Purity 98.8% Date 03 May 1993
Obtained from: Pesticides Repository US-EPA (MD-8)
c/o Man Tech Environmental Technology, Inc.
P.O. 12313, 2 Triangle Drive
Research Triangle Park, NC 27709

Source of Sample Matrix:

ECS Field Soil from Ms. Flynt's home, Pearl River County, MS,
sieved through 5.6 mm. sieve, stored in freezer. Characteriza-
tion of this soil is listed in Appendix 2.

Comments:

1. Dr. Kristin H. Milby, Senior Research Chemist, DuPont Agriculture Products, Wilmington, DL 19880, Phone 302-695-1372 was contacted by phone, on July 9, 1993, concerning some details of the analytical procedures, including a flow diagram of the eluent/column switching. Dr. Milby's reply was received Sept. 7, 1993 and attached as Appendix 1. The reply answered all the questions with clarification. This analyst acknowledges Dr. Milby's effort and cooperation.
2. The HPLC valves and switch controls were assembled using the equipment presently available in this lab, i.e., the waters Model 590 programmable pump. The flow pattern and the time segments for eluent and column switching were identical to that described in the DuPont Method. A sample inlet line from valve 2 was manually placed in the sample or standard tube at the start of each run. Repeating this manual step once every 85 minutes or so did not cause any problem in the operation. This step could also be automated as described in the DuPont method, by using a multiple port valve with switching control from one of the unused Event Out terminals of the Model 590.
3. For the present HPLC system set-up, the run time for each standard or sample was about 85 minutes. A set of four standards and three samples would require about 10 hours. The initial determination of times t_r and T required about one hour. However, the operation of the instrument was mostly automated and not physically demanding. Under the present operation, a set of six or seven runs could be completed in ten hours. Sample extraction could also be performed while the HPLC was running.

The Zorbax column in the Dupont method produced a shorter retention time than the Zorbax column we used for the present work (52 minutes vs 62 minutes). A column that would allow a shorter retention time than that produced in this method with adequate separation of DPX66037 from interferences would reduce the overall HPLC run time and, consequently, increase the sample output per working day. This would be more time efficient in applying this method to routine analytical work.

Chromatograms:

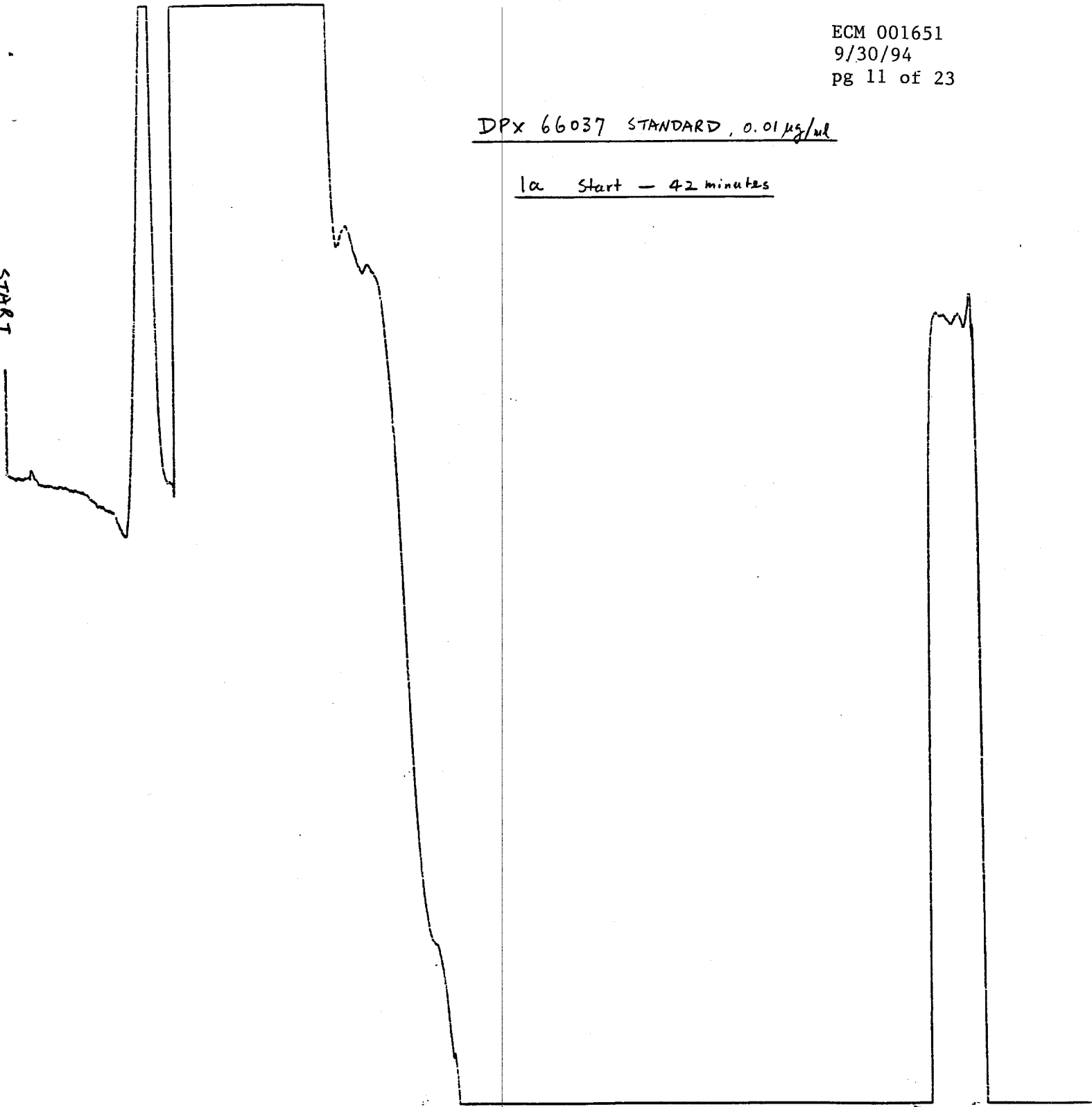
1. DPX 66037 standard 0.01 ug/ml
 - 1a. Start - 42 minutes
 - 1b. 42 - 82 minutes
2. DPX 66037 standard, 0.02 ug/ml
3. DPX 66037 standard, 0.005 ug/ml
4. DPX 66037 standard 0.002 ug/ml
5. Soil fortified DPX 66037 0.02 ppm
6. Soil fortified DPX 66037 0.005 ppm
7. Soil, blank
8. Calibration curve

Note: On the chromatograms, the response of the detector to the eluent/column switching is also recorded. The recorder tracings between start and 50 minutes and 70 to 80 minutes indicate such response. These patterns are essentially identical for all the runs, regardless of the sample or a standard. Chromatogram 1 (1a and 1b) is an example of a full run for a standard 0.01 ug/ml. Other representative chromatograms, 2 to 7, are copies of a segment between 50 minutes and 70 minutes where the analytical peak of DPX 66037 is located.

DPX 66037 STANDARD, 0.01 $\mu\text{g}/\text{ml}$

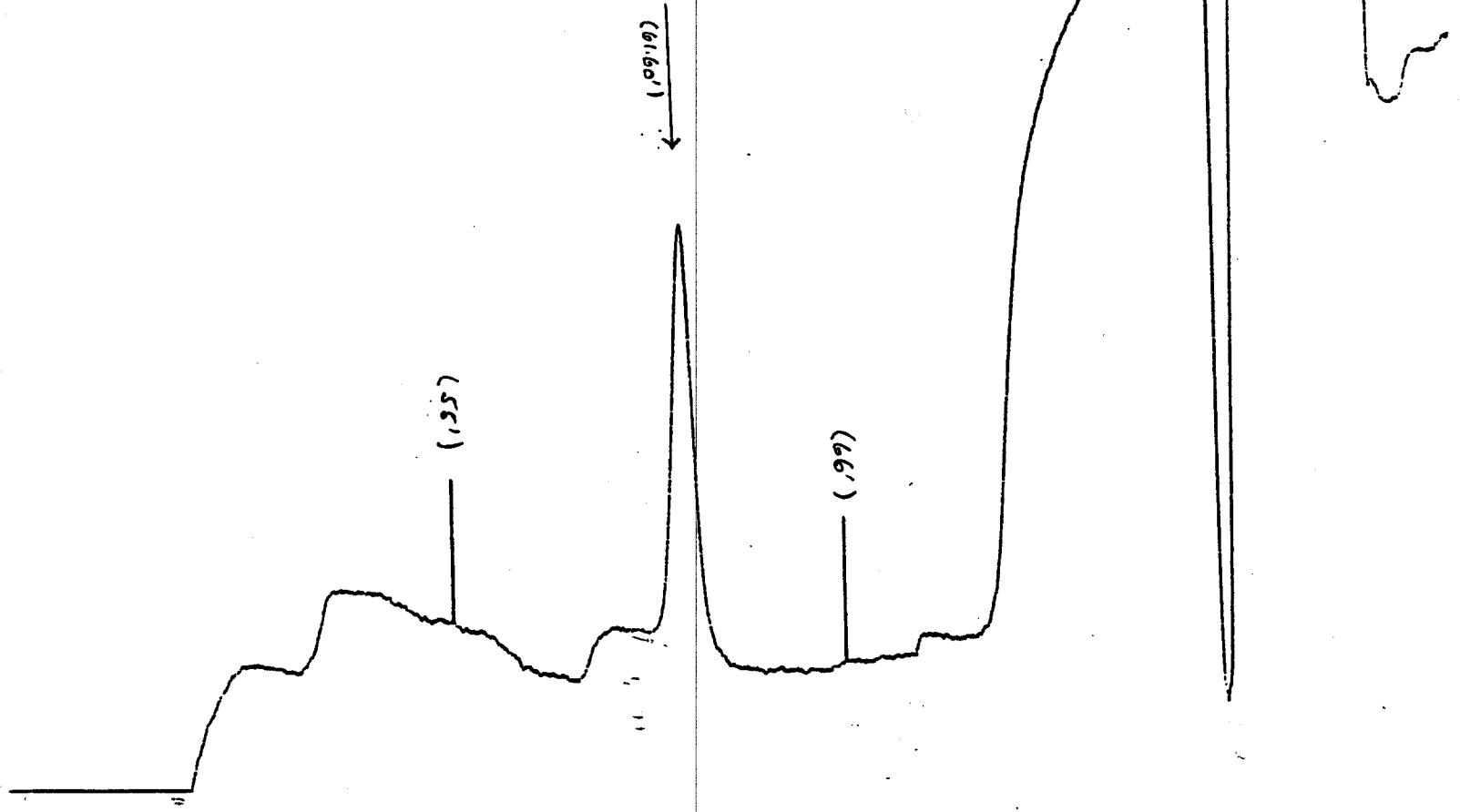
1a Start - 42 minutes

START



DPX STANDARD .001 µg/ml

1b. 42-82 minutes

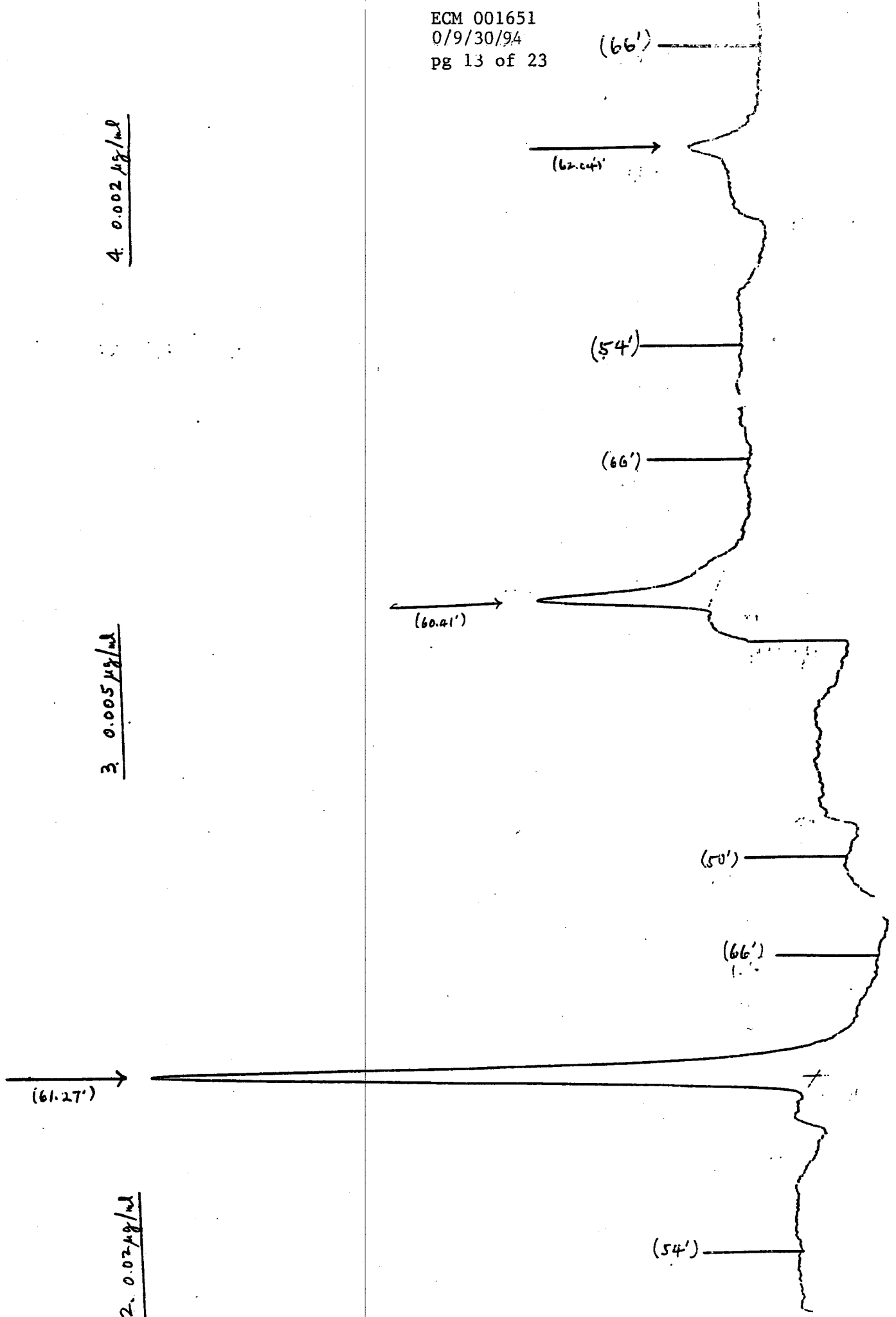


DPX 66037 STANDARDS

4. 0.002 µg/mL

3. 0.005 µg/mL

2. 0.002 µg/mL

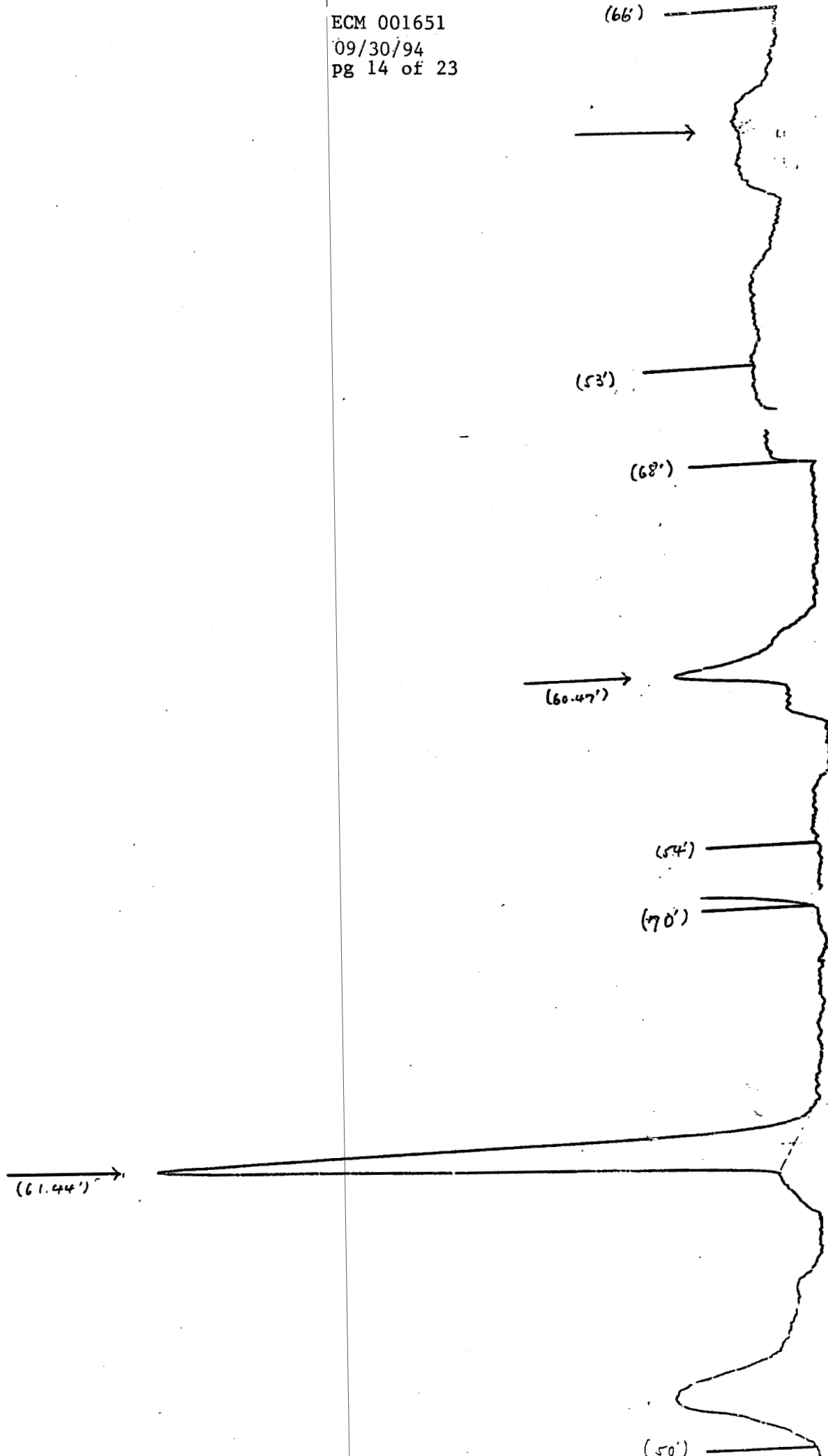


SOIL SAMPLES (10.0 gm)

7. BLANK - NO FORTIFICATION

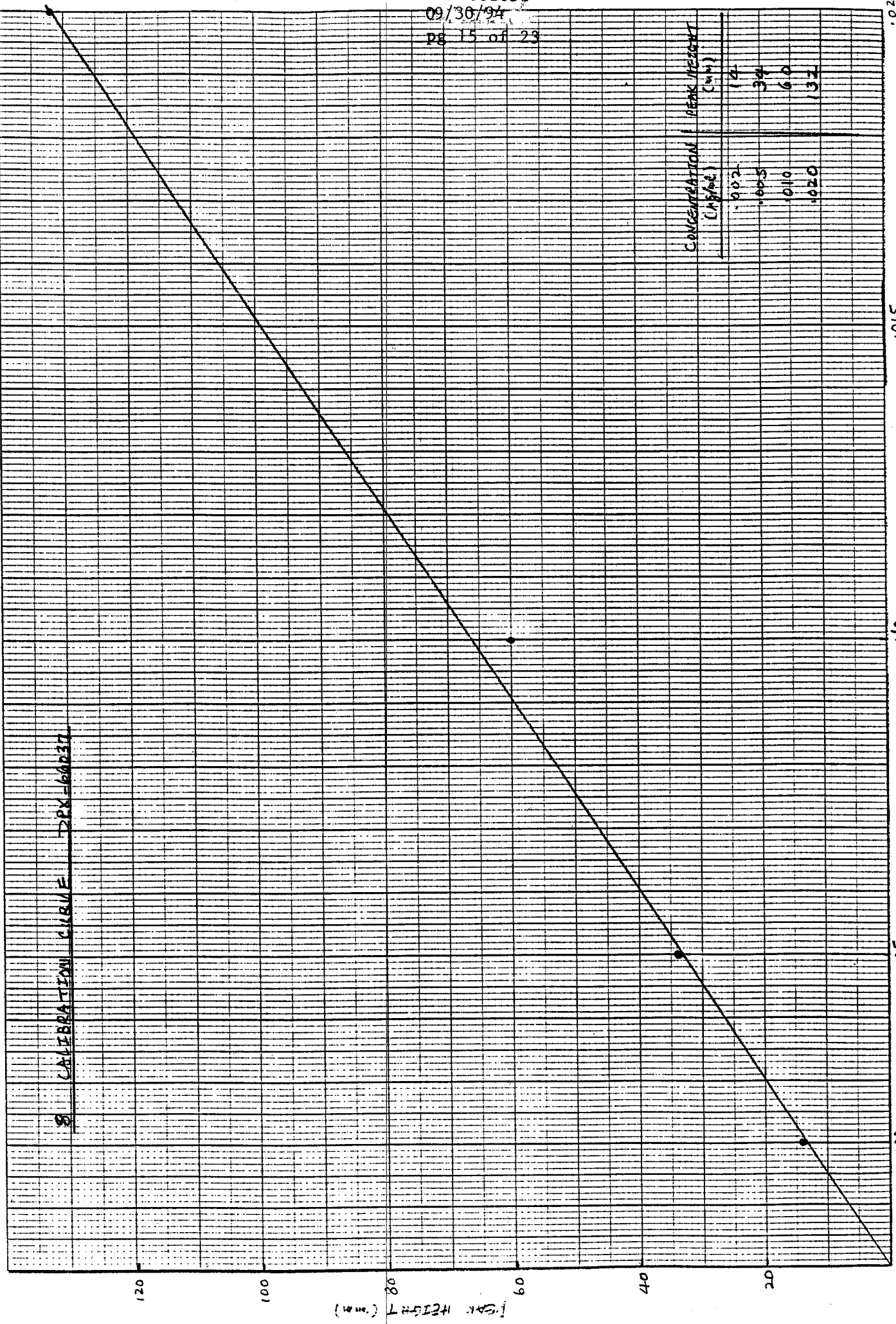
6. DPX 66037 FORTIFIED 0.005 PPM

5. DPX 66037 FORTIFIED 0.02 PPM



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8 CALIBRATION CURVE DPK-64037



CONCENTRATION (ug/ml)	PEAK HEIGHT (mm)
0.002	15
0.005	35
0.010	60
0.020	130

0.020
 0.015
 0.010
 0.005
 0.002

PEAK HEIGHT (mm)

CONCENTRATION (ug/ml)

Examples of Calculation (Method p. 15)

1. Peak Intensity:

The peak intensity is expressed in terms of peak height. The peak height, in millimeters (mm), is measured manually from the apex of the analytical peak to the baseline drawn across the base of the peak. Injection volume for all standards and samples was 2.0 ml on the Phenyl column, therefore, no injection volume adjustment is necessary for calculation.

2. Calculation Formulas:

a. Response Factor (F):

$$F = \frac{\text{Peak height (mm)}}{\text{Concentration of Standard (ug/ml)}}$$

F is the average of Response Factors for the standards analyzed in the same day.

b. Sample Concentration of DPX-66037 (C):

$$C = \frac{\text{Peak height (mm)}}{F \text{ (mm/ug/ml)}} = C \text{ ug/ml}$$

c. Parts per Million (ppm):

$$\text{ppm} = \frac{C \text{ (ug/ml)}}{\text{Sample matrix concentration (gm/ml)}}$$

For the present procedure, sample matrix concentration is 10.0 gm/10.0 ml or 1.0 gm/ml. Therefore:

$$\text{ppm} = \frac{C}{1} = C$$

d. Percent Recovery:

$$\% \text{ Recover} = \frac{\text{ppm found}}{\text{ppm added}} \times 100$$

3. Example:

a. Standards and Response Factors:

Concentration ug/ml	Peak Height mm	Response Factor F	Chromatogram No.
0.020	132	6600	2
0.010	60	6000	1b
0.005	34	6800	3
0.002	14	7000	4
		Average	
		6600	

Regrssion Line: $Y \text{ (mm)} = 6522X \text{ (ug/ml)} - 0.33$
 Correlation coefficient = 0.9960

b. Fortification at 0.020 ppm: (Chromatogram 5)

Peak height = 116 mm; F = 6600

$C = \text{mm}/F = 116/6600 = 0.0176 \text{ ug/ml} = 0.0176 \text{ ppm}$

$\% \text{ Recovery} = (0.0176/0.020) \times 100 = 88.0$

c. Fortification at 0.005 ppm: (Chromatogram 6)

Peak height = 27 mm; F = 6600

$C = \text{mm}/F = 27/6600 = 0.0041 \text{ ug/ml} = 0.0041 \text{ ppm}$

$\% \text{ Recovery} = (0.0041/0.005) \times 100 = 82.0$

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Appendix 1: Letter, Kristin H Milby to Han Tai, dated August 27, 1993

AGRICULTURAL PRODUCTS
Experimental Station
P.O. Box 80402
Wilmington, Delaware 19880-0402

August 27, 1993

Dr. Han Tai
EPA Environmental Chemistry Section
Stennis Space Center, MS 39529

Dear Dr. Tai:

Thank you for taking the time and trouble to contact me directly with your questions concerning the report, "Analytical Method for the Quantitation of DPX-66037 in Soil," DuPont Agricultural Products Document No. AMR 1965-91. I apologize for the delay in my response. At last I have some time to refresh my memory of this work and answer the questions you gave me over the telephone on July 9. I will just follow my notes from that conversation and answer each question/concern. If I have misinterpreted my notes, misunderstood your questions, or otherwise failed to clarify something, please do not hesitate to call again.

General questions

1. More detailed schematic and flow sheets to see how this method could be adapted for other equipment.

The attached Figure 1 is a modification of the "Schematic of the Chromatograph" included in the method report. I have added some further details to make the connections clearer. To present more detail of how the system worked, I have included flow diagrams for each step along with verbal explanations taken from Page 14 and the times for each step. Figures 2A through 2E show the program to determine t , the first eluent switching time. Figures 3A through 3F show the programs to determine T , the column switching time. Figures 4A through 4K show the full column/eluent switching program. For our system, $E4$ was 11 minutes, t was approximately 21.5 minutes and T was approximately 35 minutes.

Others in DuPont Agricultural Products have run similar column/eluent switching methods with a variety of hardware configurations. Besides modular systems such as the one as used for this work, integrated HPLC systems from Hewlett Packard and Waters have been used by adding an auxiliary switching valve. When a 2.0-mL sample loop is used to apply the sample (either manually or via an autosampler), the run time and sample volume required are decreased.

Knowing that this type of equipment is not always readily available, we have also submitted a split HPLC method as an enforcement method for sugarbeet tops and roots. The peak of interest is captured from a clean-up HPLC system and

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then injected onto an analytical HPLC system. This is DuPont Agricultural Products Document Number AMR 1930-91. I can send you a copy if you are interested.

2. Determination of E4

E4 is a time allowance for the sample to be pumped from the sample tube to approximately halfway through the first column. This is best determined experimentally by measuring the time it takes unretained components (the solvent front) to move from a sample tube through the first column with no eluent switching. If necessary, an adjustment of a minute or two is made to assure that the column switching window does not include the solvent peak when the full program is used. The value of E4 only needs to be determined once for a hardware system. For further details on the determination of E4, AMR 2021-91 was referenced. The relevant section of this report is Attachment 1.

3. Need for a refrigerated centrifuge?

Section IIA lists a refrigerated centrifuge and Section IIIC calls for centrifugation at 0°C. The refrigerated centrifuge was used due to convenience in our laboratory. In reality, since the sample is not kept cold before or after centrifugation and the centrifugation time is only ten minutes, the refrigeration does not add to the stability of the analyte and is not necessary for the method.

4. Description of injection ice bath

The ice bath was simply an ice-filled Styrofoam container just big enough to hold a test tube rack with the sample tubes. Standards and samples were always allowed to thermally equilibrate in the ice bath prior to injection.

5. How many runs per day with 73 minutes/run?

Each morning the programs to determine t and T were run, then standards were run through the full program. During that time, a set of six samples (including controls and fortified controls) was prepared as described in the report. By the time the samples were ready, two or three standards had been run using the full program. The samples were then run with standards included in the middle and end of the set. So a total of 10 or 11 full runs plus the preliminary runs were made each day. In our case, one person started the process early in the morning and another finished it in the early evening. Although these long days would not be suitable for enforcement purposes, this method was developed to obtain data on a relatively small number of soil samples and was sufficient to meet that goal. We did not choose to do so, but we had the option of allowing the instrument to run automatically unattended which would have allowed one person to carry out the work each day and leave the sample set running overnight.

Specific questions

1. How many standards were prepared (see Pages 10 and 22)?

The standards used routinely were 0.0020, 0.0050, 0.010, and 0.020 $\mu\text{g}/\text{mL}$. During a day's run as described above, these four would be run and sometimes one or more run twice. The higher level, 0.050 $\mu\text{g}/\text{mL}$, shown on Page 22 was prepared as part of the serial dilutions but was only run as a further verification of the linearity of the HPLC method.

2. What kind of pipette was used on Page 12?

Gilson Microman® adjustable volume positive displacement pipettes were used throughout this work for the transfer of microliter volumes. These should have been included in the equipment list.

3. Were 2-mL injections used to establish the switching times?

Yes, the injection volumes were always the same.

4. Why is 10 mL needed for injection (2nd parag. of pt 2, Page 13)?

The samples and standards are passing through the pump and tubing from the sample tubes to the top of the column. Approximately 7 mL is required to flush this volume. Then the 2.0 mL of sample volume is pumped onto the column.

5. Column temperature is held at 40°C, but ice-temperature samples are being pumped onto the column?

The column oven was used to help maintain consistency in the day-to-day operation of the system. It was not intended to mean that the sample reached 40°C. All samples and standards were thermally equilibrated to the ice bath and traveled the same distance at the same flow rate from the ice bath to the column. If consistent operation is achieved by other means, the column oven is not necessary.

6. How critical is the timing? Could manual switching be used?

Again consistency of operation is the key factor. Certain steps have critical timing: pumping of the 2.0 mL sample onto the column and timing of the eluent and column switches. In the case of sample application, a 10% timing error would translate directly to a 10% error in quantitation. Other times, such as flushing lines and equilibrating columns, have a minimum but no maximum. I would not recommend using this method if it had to be done with manual switching. Rather the split column method mentioned under the answer to General Question No. 1 would be more appropriate.

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7. Page 31 typo?

The valves are shown on Page 30, not 28 as stated in the first sentence.

8. Page 27 typos?

References 1 and 2 are reversed in the Reference list on Page 27.

The report given as Reference No. 1 (which should be number 2) has an incorrect AMR number. It should be AMR 2021-91 rather than -90.

I hope this has taken care of your questions and provided you with enough information to allow you to complete your work on this report. If I can help you with anything else, please do not hesitate to call me at 302-695-1372.

Sincerely,



Kristin H. Milby, Ph.D.
Senior Research Chemist

KHM/mc
Att.

MECHANICAL (TEXTURE) ANALYSIS
Mississippi Cooperative Extension Service, Soil Testing Lab
P.O. Box 9610
Mississippi State, MS 39762

U.S. EPA / E. Flynt

NAME:
Building 1105, Stennis Space, MS 39529

ADDRESS:
November 15, 1993

DATE:
UNCL

COUNTY:

<u>LAB#</u>	<u>FIELD#</u>	<u>%CLAY</u>	<u>%SILT</u>	<u>%SAND</u>	<u>TEXTURE</u>
<u>EPA1</u>	<u>1</u>	<u>15.5</u>	<u>20.3</u>	<u>64.2</u>	<u>SILT LOAM</u>
<u>EPA2</u>	<u>1</u>	<u>14.8</u>	<u>22.0</u>	<u>63.2</u>	<u>SILT LOAM</u>
<u>(Average)</u>	<u>1</u>	<u>15.2</u>	<u>21.2</u>	<u>63.7</u>	<u>SILT LOAM</u>

pH - 5.2

% Organic Matter - 2.76%

Mississippi State University does not discriminate on the basis of race, color, religion, national origin,
sex, age, handicap/disability or veteran status.