Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Phosphamidon

MRID #: 414690-06

Matrix: Soil

Analysis: GC/NPD

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Biospherics Study No. 88005-05
Envirosphere Study No. CIBA 100-003

STUDY DIRECTOR CHANGE

Biospherics Study No.: 88-005-08

Client Study No.: none provided

Client: CIBA-GEIGY Corporation
P.O. Box 18300
Greensboro, North Carolina 27417

Study Title: Analysis of Petri Dishes for Phosphamidon

Original Study Director: Annette Troy

New Study Director: Tom Koontz

Rationale: Ms. Troy has resigned from Biospherics, Inc.

Effective Date: October 26, 1989. This form is issued retroactively, contrary to Biospherics policy. The analytical portion of the study had been completed at the time of the Study Director change, and this documentation was overlooked.

Reviewed and Accepted:

Biospherics Facility Management:

H. Markus Gudnason
Laboratory Manager
Biospherics Incorporated

1/18/90
Date

Analytical Study Director: Tom Koontz Date: 2/22/90
Tom Koontz
Project Leader

Appendix G

January 16, 1989

DETERMINATION OF PHOSPHAMIDON AND
ITS C5518 METABOLITE RESIDUES IN SOIL
(This is a correction of the method submitted
1-12-89, dated 1-4-89.)

Prepared By: _____ Date: _____
Annette Troy
Project Leader

Approved By: _____ Date: 1/13/89
Christine Olinger
Section Leader

_____ Date: 1/17/89
Marcella Saynuk
Assistant Director
Laboratory Division

PRINCIPLE:

Soil samples are extracted with 9:1 acetonitrile:deionized water and the filtered extracts are evaporated until only water remains. The water extract is split precisely, each half undergoing separate partitions. The first half is partitioned with 50% dichloromethane (DCM) in hexane and concentrated by rotary evaporation at no more than 30°C, after addition of methyl ethyl ketone (MEK). The extract is analyzed for phosphamidon by gas chromatography using flame photometric detection, and quantitation by an external standard technique. The second half is partitioned with 15% DCM in hexane and concentrated by rotary evaporation at no more than 25°C, after addition of MEK. The C5518 metabolite is analyzed using gas chromatography with nitrogen phosphorus detection and quantitated using an external standard technique. The limit of quantitation of the extracts is 0.3 µg/mL for both analytes. A flow diagram for the method is shown in Figure 1.

MATERIALS:

1000 mL glass bottles with teflon-lined caps
Whatman #4 filters
Glass Microfibre filters
10, 100, 500 mL graduated cylinders
250 mL separatory funnels
15 mL centrifuge tubes
500 mL erlenmeyer flasks

MATERIALS:—cont'd

500 mL round bottom flasks
50, 100, 250, 500 uL syringes
25 mL Class A volumetric pipettes
2, 10, 50, 100 mL volumetric flasks
Shaker
Rotary vacuum evaporator
Nitrogen evaporator
Hobart food processor
7 ¼" electric mitre saw

REAGENTS:

Deionized water
Dry ice
Acetonitrile, Mallinckrodt nanograde or equivalent
Dichloromethane, Mallinckrodt nanograde or equivalent
Methyl ethyl ketone, "Baker Analyzed" reagent or equivalent
Hexane, Mallinckrodt nanograde or equivalent
Sodium chloride, J.T. Baker or equivalent
Sodium sulfate, J.T. Baker or equivalent
Phosphamidon standard of known purity, CIBA-GEIGY Corporation
N,N-diethyl-2-chloroacetoacetamide (phosphamidon metabolite CS518) standard of known purity, CIBA-GEIGY Corporation

SAMPLE PREPARATION:

Soil samples obtained during the first month post-application are in two parts, representing the 0-6" and the 6-48" depth interval. After the first month, a single auger core is collected to represent the entire 0-48" depth. The sampling process involves the use of an auger tube whose opening is narrower than the diameter of the acetate tube (sleeve, liner) into which the sample is collected. Hence, soil cores which represent 6-48" depth will not necessarily be 42" long. Soil height will also vary based on moisture and soil consistency at a given location. Therefore, each tube will be measured for total length and a ratio will be established such that representative 6 or 12" intervals will be cut from the tube for homogenization and analysis. For example, if a tube covering 6-48" measures 35", 35:42 gives a ratio of 0.833. To obtain an interval representing 6", a 5" 0.833 x 6 = 4.98". In this fashion, the five replicate tubes will be cut to obtain 0-6", 6-12", 12-18", 18-24", 24-36" and 36-48" depth intervals (0-6" where applicable).

To homogenize the five replicates of a given depth interval, soil will be removed from the acetate tubes, placed in a Hobart food processor, and homogenized for 10 minutes. Dry ice will be used as needed to prevent clumping or simply to increase sample volume to render the food processor a more efficient mixer. Stones will be removed and discarded. A volume of up to one liter of homogenized soil will be retained for analysis. Any soil in excess of one liter volume will be discarded.

PROCEDURE:

Extraction: Weigh 50 g of homogenized soil (prepared as described above) into a 1 L glass bottle with teflon-lined cap. Add 500 mL 9:1 acetonitrile:deionized water and shake sample on a mechanical shaker for one hour. Vacuum filter using a Whatman #4 filter paper and a glass microfiber filter. Rotary evaporate sample (water bath 25°C) until all acetonitrile has been removed leaving about 25 mL aqueous solution. Raise to volume in a 50 mL volumetric flask using deionized water. Split sample into two 25 mL aliquots using a 25 mL class A volumetric pipette.

Partition #1: Transfer the first 25 mL aliquot of the aqueous solution to a 250 mL separatory funnel and add 10 mL saturated aqueous sodium chloride and 100 mL deionized water. Partition with 80 mL of 50% DCM in hexane and drain into a 500 mL erlenmeyer flask. Repeat partition twice with 80 mL aliquots of 50% DCM in hexane, draining into the same erlenmeyer flask: add 40 g Na_2SO_4 to the erlenmeyer flask, swirl and decant solvent into a 500 mL round bottom flask. Add 50 mL methyl ethyl ketone MEK) keeper into the round bottom flask. Rotary evaporate combined solvent layers to about 10 mL in a 30°C water bath. Transfer solution to a 15 mL centrifuge tube with 1 mL MEK rinses. Concentrate the solution to just below 2 mL under a stream of N_2 at no more than 27°C. Raise to volume in a 2 mL volumetric flask with MEK. Analyze by GC-FPD for the parent phosphamidon.

Partition #2: Transfer the remaining 25 mL aliquot of the aqueous solution to a 250 mL separatory funnel and add 10 mL saturated aqueous sodium chloride and 100 mL deionized water. Partition with 80 mL 15% DCM in hexane and drain into a 500 mL erlenmeyer flask. Repeat partition twice with 80 mL aliquots of 15% DCM in hexane, draining into the same erlenmeyer flask: add 40 g Na_2SO_4 to the erlenmeyer flask, swirl, and decant solvent into a 500 mL round bottom flask. Add 50 mL MEK keeper into the round bottom flask. Rotary evaporate combined solvent layers to about 10 mL in a 25°C water bath. Transfer solution to a 15 mL centrifuge tube with 1 mL MEK rinses. Concentrate the solution to just below 2 mL under a stream of N_2 at no more than 27°C. Raise to volume in a 2 mL volumetric flask with MEK. Analyze by GC-NPD for the metabolite CGS18.

GAS CHROMATOGRAPHY INSTRUMENTATION:

Two Hewlett Packard Model 5890A or equivalent gas chromatographs must be available, each with multiramp temperature programming capability and a capillary injection port: an automatic sampler is recommended. Data reduction using a stand-aline integrator (HP 3396) or computerized data system is recommended.

The first gas chromatograph should be equipped with a flame photometric detector (FPD), and the second should be equipped with a nitrogen-phosphorus detector (NPD). Conditions are listed in Tables I and II.

CALIBRATION

Samples will be interspersed through each set of GC calibration standards, with no more than seven samples per set of calibration solutions. Each set as described above will start and end with a calibration standard.

PREPARATION OF STOCK SOLUTIONS

Phosphamidon 1000 µg/mL: Prepare by quantitatively transferring 0.0511 g phosphamidon standard into a 50 mL volumetric flask. Bring to volume with methyl ethyl ketone. Store refrigerated in a brown bottle.

Metabolite CS518 1000 µg/mL: Prepare by quantitatively transferring 0.0500 g CS518 standard into a 50 mL volumetric flask. Bring to volume with toluene. Store refrigerated in a brown bottle.

Stock solutions may be stored for one year before disposal.

PREPARATION OF FORTIFICATION STANDARDS

Phosphamidon 100 µg/mL: Prepare by transferring 10 mL phosphamidon stock solution into a 100 mL volumetric flask. Bring to volume with methyl ethyl ketone. Store refrigerated in a brown bottle.

Metabolite CS518 100 µg/mL: Prepare by transferring 10 mL CS518 stock solution into a 100 mL volumetric flask. Bring to volume with methyl ethyl ketone. Store refrigerated in brown bottle.

Fortification solutions may be stored for one year before disposal.

PREPARATION OF PHOSPHAMIDON CALIBRATION SOLUTIONS

0.3 µg/mL Phosphamidon Calibration Solution: Transfer 30 µL phosphamidon stock solution to a 100 mL volumetric flask and bring to volume with methyl ethyl ketone. Store refrigerated in a brown bottle.

0.5 µg/mL Phosphamidon Calibration Solution: Transfer 50 µL phosphamidon stock solution to a 100 mL volumetric flask and bring to volume with methyl ethyl ketone. Store refrigerated in a brown bottle.

1.0 µg/mL Phosphamidon Calibration Solution: Transfer 100 µL phosphamidon stock solution to a 100 mL volumetric flask and bring to volume with methyl ethyl ketone. Store refrigerated in a brown bottle.

3.0 µg/mL Phosphamidon Calibration Solution: Transfer 300 µL phosphamidon stock solution to a 100 mL volumetric flask and bring to volume with methyl ethyl ketone. Store refrigerated in a brown bottle.

Calibration solutions may be stored for three months before disposal.

PREPARATION OF CS518 CALIBRATION SOLUTIONS

0.3 µg/mL CS518 Calibration Solution: Transfer 30 µL CS518 stock solution to a 100 mL volumetric flask and bring to volume with methyl ethyl ketone. Store refrigerated in a brown bottle.

0.5 µg/mL CS518 Calibration Solution: Transfer 50 µL CS518 stock solution to a 100 mL volumetric flask and bring to volume with methyl ethyl ketone. Store refrigerated in a brown bottle.

1.0 µg/mL C5518 Calibration Solution: Transfer 100 µL C5518 stock solution to a 100 mL volumetric flask and bring to volume with methyl ethyl ketone. Store refrigerated in a brown bottle.

3.0 µg/mL C5518 Calibration Solution: Transfer 300 µL C5518 stock solution to a 100 mL volumetric flask and bring to volume with methyl ethyl ketone. Store refrigerated in a brown bottle.

Calibration solutions may be stored for three months before disposal.

CALIBRATION OF GAS CHROMATOGRAPH

Only Phosphamidon Isomer II is quantitated to determine the Phosphamidon concentration. A calibration curve is prepared for phosphamidon Isomer II and for metabolite C5518 from the results of the calibration solution analyses. Assure linearity of phosphamidon Isomer II and metabolite C5518 by checking the linear regression correlation for each analyte. Typical chromatograms of the calibration solutions, in-house fortifications, and treated samples are shown below Tables I and II.

DETERMINATION OF SAMPLE RESIDUES

The concentration of each phosphamidon isomer and metabolite C5518 in the final extract is determined by comparing peak heights of unknown samples with results of the least squares calculations of calibration solutions.

Concentration of analytes in soil is calculated on a dry weight basis, corrected for procedural recovery, using the following equation:

$$ppm\ dry = \frac{Vol_{final} \times C_{extract} \times A \times DF}{(1.00-M) \times R \times W}$$

where:

Vol_{final} = final volume of extract
$C_{extract}$ = concentration of analyte in final extract
A = molecular weight ratio of metabolite to parent
M = decimal % moisture
W = weight of soil sample extracted
R = decimal % recovery of analyte in procedural recovery sample
DF = dilution factor

Table 1

Chromatographic conditions for the Determination of Phosphamidon

Column:	J&W Scientific DB-5 (5% phenyl) 30m x 0.53 mm i.d., 1.0 um film
Carrier:	Helium, 99.995 + %
Head Pressure:	60 kPa
Oven Temperature Parameters	
Initial Temperature:	175°C isothermal
Bake Out Program Rate:	20°C per minute
Bake out Temperature:	245°C, hold 2 minutes
Equilibration Time:	2 minutes
Injection Mode:	Splitless
Injection Volume:	2 uL
Injection Temperature:	200°C
Detector Temperature:	225°C

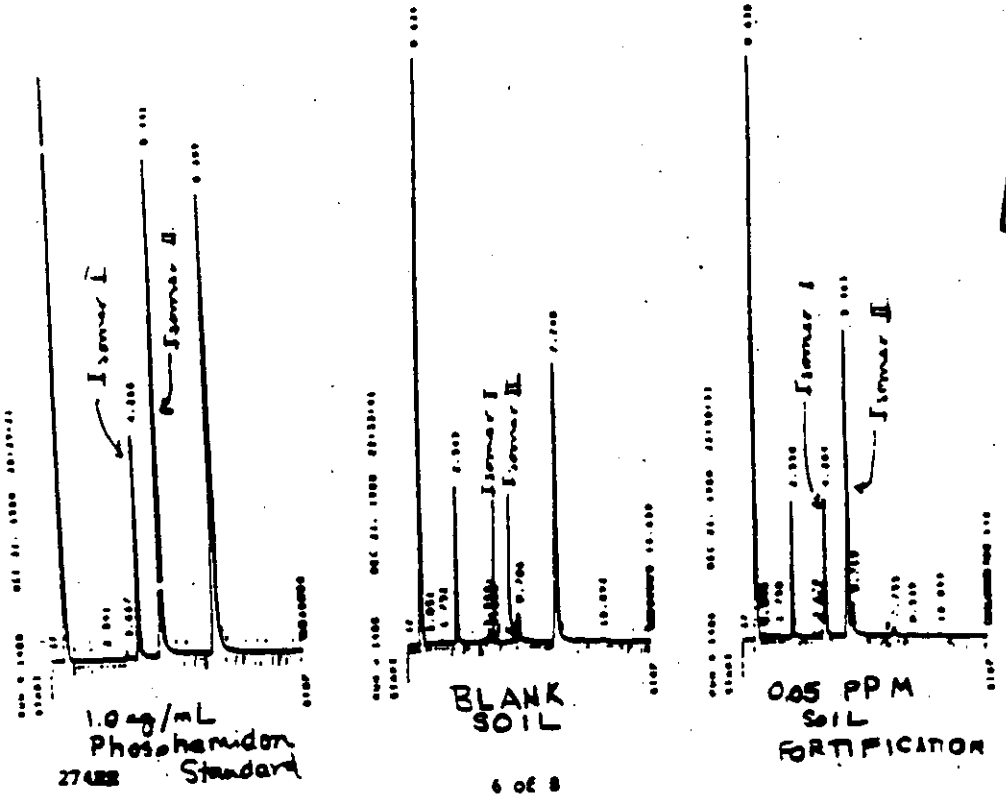
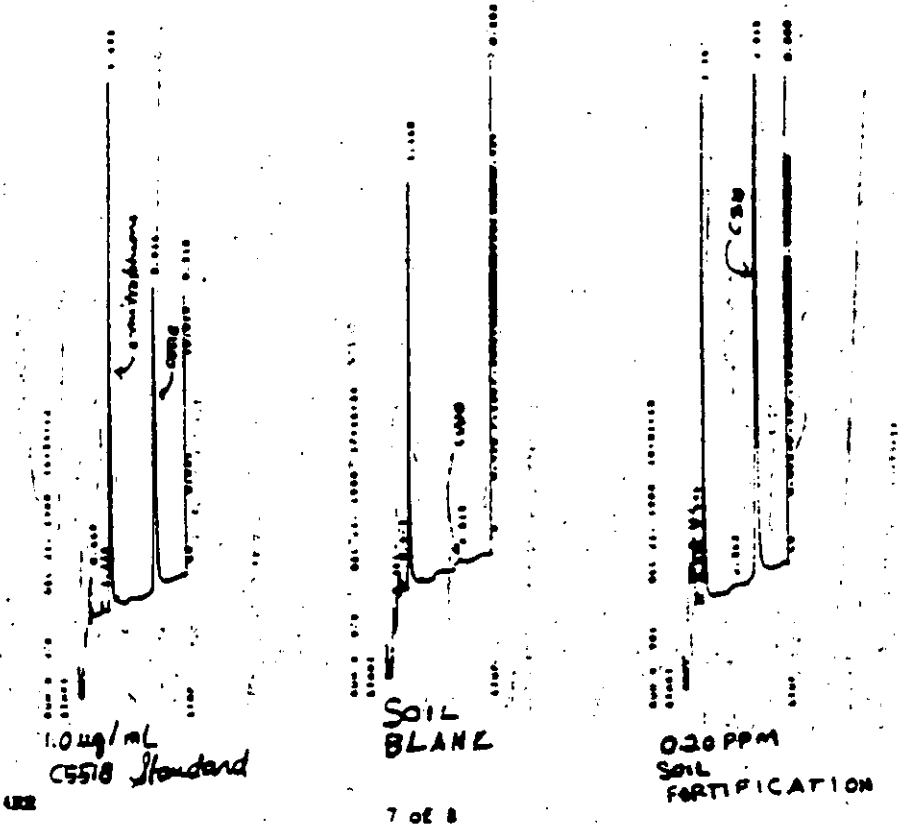


Table II

Chromatographic conditions for the Determination of Phosphamidon Metabolite C5518

Column:	J&W Scientific DB-17 (50% phenyl) 15m x 0.53 mm I.d., 1.0 um film
Carrier:	Helium, 99.995 + %
Head Pressure:	35 kPa
Oven Temperature Parameters	
Initial Temperature:	110°C hold 1 minute
Program Rate:	5°C per minute
Final Temperature:	130°C hold 0.5 minutes
Bake Out Program Rate:	20°C per minute
Bake out Temperature:	245°C, hold 4 minutes
Equilibration Time:	6 minutes
Injection Mode:	Splitless
Injection Volume:	2 uL
Injection Temperature:	225°C
Detector Temperature:	260°C



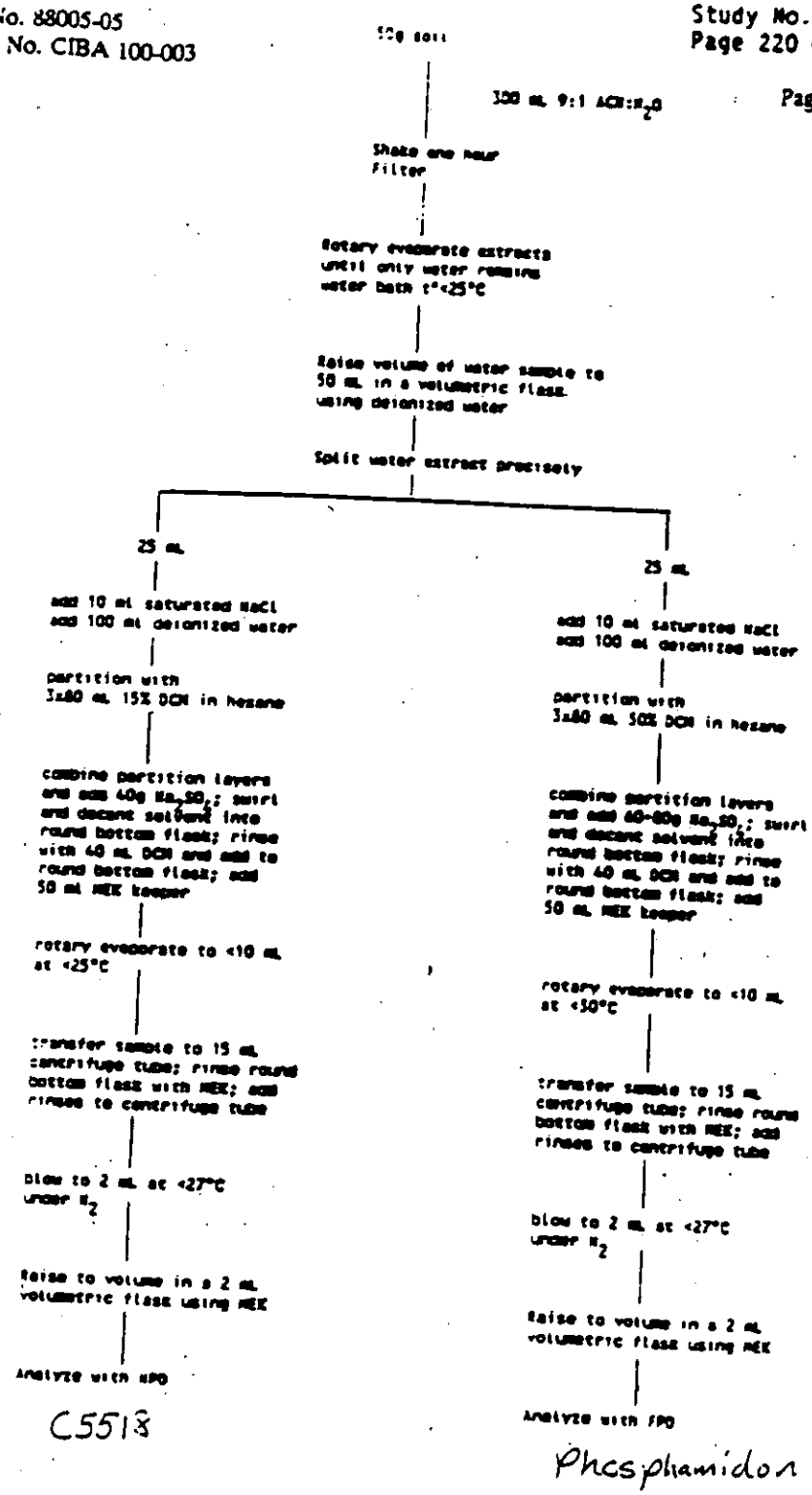


FIGURE 1

METHOD MODIFICATION

Project: Phosphamidon Soil Dissipation

Study Number: 88-005

Method: Determination of Phosphamidon and Its C5518 Metabolite Residues in Soil (January 16, 1989)

Modification No: One

Date Effective: January 16, 1989

Issued by: Annette Troy Date 3-16-89
Annette Troy
Project Leader

Approved by: Christine L. Olinger Date 3-16-89
Christine L. Olinger
Section Leader

Client Principal Investigator: Dr. Richard A. McLaughlin Date 4-12-89
~~Dr. R. Date~~
CIBA GEIGY

Item 1
Section: Extraction

Original Method:

Vacuum filter using a Whatman #4 filter paper and a glass microfiber filter.

Modified Method:

Vacuum filter using a microfiber filter overlaying a Whatman #4 filter paper.

Justification:

Clarification

327RR

Item 2

Section: Partition #1

Original Method:

Repeat partition twice with 80 mL aliquots of 50% DCM in hexane, draining into the same erlenmeyer flask; add 40 g Na_2SO_4 to the erlenmeyer flask, swirl and decant solvent into a 500 mL round bottom flask. Add 50 mL methyl ethyl ketone MEK keeper into the round bottom flask.

Modified Method:

Repeat partition twice with 80 mL aliquots of 50% DCM in hexane, draining into the same erlenmeyer flask; add 40 g Na_2SO_4 to the erlenmeyer flask, swirl an decant solvent into a 500 mL round bottom flask. Rinse Na_2SO_4 with 40 mL 100% DCM and add this to the round bottom flask. Add 50 mL methyl ethyl ketone MEK keeper into the round bottom flask.

Justification:

Clarification

Item 3

Section: Partition #2

Original Method:

Repeat partition twice with 80 mL aliquots of 15% DCM in hexane, draining into the same erlenmeyer flask; add 40 g Na_2SO_4 to the erlenmeyer flask, swirl, and decant solvent into a 500 mL round bottom flask. Add 50 mL MEK keeper into the round bottom flask.

Modified Method:

Repeat partition twice with 80 mL aliquots of 15% DCM in hexane, draining into the same erlenmeyer flask, add 40 g Na_2SO_4 to the erlenmeyer flask, swirl, and decant solvent into a 500 mL round bottom flask. Rinse Na_2SO_4 with 40 mL 100% DCM and add this to the round bottom flask. Add 50 mL MEK keeper into the round bottom flask.

Justification:

Clarification

327RR

APPENDIX I

SAMPLE CALCULATIONS

A. Petri dish

sample 88-06-108-21A

1) $\mu g/mL$ in extract analyzed = $\frac{\text{(Peak Height Isomer X) - (Y- intercept, Isomer X)}}{\text{Slope, Isomer X}}$

where X is I or II

Peak Height Isomer II : 138 Y-intercept Isomer II : 5.63 Slope Isomer II : 74.9

$\mu g/mL$ Isomer II = $\frac{138 - 5.63}{74.9}$ = 1.77 $\mu g/mL$

2) Total μg/plate = ($\mu g/mL$ in extract) (dilution factor) (100 mL final volume)

Total μg/plate = (1.77 $\mu g/mL$) (20) (100mL) = 3540 μg/plate

3) $\mu g/cm^2$ = $\frac{\text{Total } \mu g\text{/plate}}{78.5\ cm^2\text{/plate}}$

$\mu g/cm^2$ = $\frac{3540\ \mu g\text{/plate}}{78.5\ cm^2}$ = 45.1 $\mu g/cm^2$

Biospherics Study No. 88005-05
Envirosphere Study No. CIBA 100-003

B. Soil sample

Sample: 88-08-118-13A

1) $\mu g/mL$ in extract analyzed = $\frac{\text{(Peak Height analyte) - (Y- intercept, analyte)}}{\text{Slope, analyte}}$

where analyte is Isomer I or II or C5518

Peak Height C5518 : 53376 Y-intercept C5518: -1435 Slope C5518 : 39974

$\mu g/mL$ C5518 = $\frac{53376 + 1435}{39974}$ = 1.37 $\mu g/mL$

2) μg analyte = (final volume) x (extract concentration) x (dilution factor)

μg C5518 = (2 mL) (1.37 $\mu g/mL$) (1) = 2.74 μg

3) ppm (wet) = $\frac{\text{Weight residue } (\mu g) \times \text{(M.W. parent/M.W. analyte)} \times 2}{\text{Weight sample (g) x recovery (decimal)}}$

ppm (wet) = $\frac{(2.74\ \mu g)\ (300/224)\ (2)}{(50.0\ g)\ (0.80)}$ = 0.183

4) ppm (dry) = $\frac{\text{ppm (wet)}}{\text{1.00 - (decimal moisture fraction)}}$

ppm (dry) = $\frac{0.183}{(1 - 0.180)}$ = 0.22

Appendix J
Spreadsheets of Residue Calculations

NOTES: EXPLANATIONS TO BE USED FOR ALL SPREADSHEETS OF RESIDUE CALCULATIONS

Abbreviations:

N.I.	not integrated
N.A.	not applicable: measurement not taken; residue of this analyte determined in a previous analytical set where only one fortification recovery was not acceptable.
N.D.	not detected
C	control sample
S	spike = control fortified
T	field treated sample
I	interference

Units of columns:

Weights of soil and/or dishes :	g
Phos., or CS518, Final Volume :	mL
Isomer II, or CS518, Spike Levels:	ppm
Total Residue as Isomer II:	ppm

Explanations of headings:

Isomer II, or CS518, Found PPM Wet:	no corrections applied
CS518 PPM Wet Corr.:	corrected for Molecular Weight and % recovery
Isomer II Wet Corr.:	corrected for % recovery
Isomer II, or CS518, PPM Dry:	corrected for % moisture only
CS518 PPM Dry Corr.:	corrected for Molecular Weight, % recovery and % moisture
Isomer II Dry Corr.:	corrected for % recovery and % moisture
Total Residue as Isomer II:	CS518 (corrected for Molecular Weight) + Isomer II, corrected for % recovery and % moisture

Calculations for the fortified samples ("S") should be considered only through % Recovery.

Note:
Peculiarities in the spreadsheet program did not always print the entries "<0.05", "<0.08", when the cell entry directed it to; further calculations were then continued automatically. Hence, discrete values appear at times, even though they are below the detection limit. Changes were made only in cells where calculations with the correction factors brought the values above the detection limit, and were therefore misleading. These changes were done with a pen, so as to not erase the formula in the spreadsheet cells.

End of Pages to be Scanned