II. OBJECTIVE

Valent U.S.A. Corporation contracted Central California Research Laboratories, Inc. (CCRL) to perform validation trials for the residue analysis of S-1812, S-1812-DPMe, S-1812-DP and HTFP in soil using Valent Analytical Method RM-38-S2 entitled "Determination of Residues of S-1812 and Metabolites in Soil" dated May 10, 2001, according to the specifications of EPA Ecological Effects Test Guidelines, OPPTS 850.7100, Data Reporting for Environmental Chemistry Methods (EPA Draft Guideline for the Independent Laboratory Validation). The study was initiated on June 13, 2001, when the Study Director signed CCRL protocol 990087 (Appendix A).

III. MATERIAL AND METHODS

A. Test and Reference Materials

The analytical reference standards were received in good condition on March 24, 2001, from:

Valent U.S.A. Corporation
6560 Trinity Court
Dublin. California 94568-2628

The following standards along with Certificates of Analyses and MSDS' were received and stored frozen for the duration of the study:

Analytical Standards	Description	Lot #	Purity (%)	Expiration Date
S-1812 CAS# 179101-81-6	clear liquid	AS 1817c	99.7	04/17/02
S-1812-DPMe CAS# Not Assigned	clear liquid	AS 1939a	96.0	08/14/02
S-1812-DP CAS# Not Assigned	white crystalline solid	AS 1922a	98.0	08/15/02
HTFP CAS# Not Assigned	off-white powder	AS 1938b	97.4	01/03/03

Upon receipt, the neat S-1812, S-1812-DPMe, S-1812-DP and HTFP standards were stored in a freezer maintained at <0°C. As per the analytical method, solutions of S-1812, S-1812-DPMe, S-1812-DP and HTFP were prepared in acetone to serve as the stock solutions. Subsequent dilutions of the stock solutions were prepared in acetone for use as the spiking solution

and in toluene for use as GC reference standards for S-1812, S-1812-DPMe and S-1812-DP. Subsequent dilutions of the HTFP stock solution were prepared in acetonitrile:0.01% acetic acid in water (20%:80%) for use as HPLC reference standards. The spiking solution contained all four compounds. One set of GC reference standards contained S-1812 and S-1812-DPMe and the second set of GC reference standards contained only S-1812-DP. One set of HPLC reference standards contained only HTFP. Preparation and dilution data forms pertaining to the stock and working solutions are located in the raw data. All stock and working solutions were stored refrigerated (~4°C ± 5°C) when not in use.

B. Reagents and Equipment

See Appendix A – Study Protocol and Analytical Method for a list of Reagents and Equipment used for this study.

C. Untreated Control (UTC) Material

Valent U.S.A. Corporation provided a frozen soil sample suitable for use as untreated control material. This soil sample was a composite sample prepared on May 21, 2001 by Mr. John Stearns, Valent U.S.A. Corporation, and was created using 400 g of each of the following untreated samples:

V-21021-A-2ul
V-21021-A-3ul
V-21021-A-7ul
V-21021-A-11ul
V-21021-A-17ul

The composite soil sample was received on May 23, 2001 and had been previously processed to a homogenous state. Upon receipt, the sample was stored frozen (<0°C) pending analysis.

D. Processing Procedures

No homogenization of the untreated control soil sample was required. Dates pertaining to the receipt, storage, and sample preparation of the sample are listed in the raw data.

E. Analysis Method

1. Method Summary: Soil

All samples for the independent lab validation were analyzed according to Valent Analytical Method RM-38-S2 entitled "Determination of Residues of S-1812 and Metabolites in Soil" dated

May 10, 2001 (Appendix A). Minor glassware and equipment substitutions were made in the procedure. For example, a Tekmar Tissuemizer® was also used in the extraction step and a 125-ml flat-bottom flask was used in the Silica SPE cleanup. The procedure is summarized as follows:

EXTRACTION (ALL COMPOUNDS): Approximately 50 grams of homogenous sample were weighed into a one-pint Mason jar. Control samples were fortified with S-1812, S-1812-DPMe, S-1812-DP and HTFP at appropriate levels (i.e. 0.0200 µg/g or 0.200 µg/g). A 100-ml portion of extraction solvent (acetonitrile / 1% HCl / water, 180 / 0.5 / 20, v / v / v) was added to the jar and the sample was blended for ten minutes using a Tekmar Tissuemizer® or an Omni-Mixer. After blending, approximately 50 mls of Celite was added and the sample extract was filtered into a 500-ml side-arm flask through a Büchner funnel containing a 9 cm Whatman GF/A filter paper, pre-wetted with acetonitrile, using vacuum. The sample and filter were transferred back to the sample jar. An additional 100-ml portion of the extraction solvent was added to the sample and blended again for ten minutes. The sample extract was filtered through the same Büchner funnel, combining the sample extracts in the 500-ml flask. The blender jar and filter cake were rinsed with 2 x 10 ml portions of acetonitrile and added to the filtrates. The combined filtrates were transferred to a 250-ml graduated mixing cylinder and the final volume was adjusted to 250 ml with acetonitrile.

HEXANE/ACETONITRILE PARTITIONING (S-1812, S-1812-DPMe AND S-1812-DP): A 50-ml aliquot (equivalent to 10 grams of soil) of the sample extract was transferred to a 500-ml separatory funnel. A 50-ml portion of 1% (w/v) sodium chloride solution and a 100-ml portion of hexane saturated with acetonitrile were added to the separatory funnel.

The separatory funnel was shaken for ~ 1 minute and after phase separation, the lower aqueous phase was drained into a temporary container. The hexane was transferred to a 500-ml flat-bottom flask. The retained aqueous phase was transferred back to the separatory funnel and partitioned two more times with 100-ml portions of hexane saturated with acetonitrile and the hexane fractions were combined.

The combined hexane fractions were evaporated to approximately 5 ml using a rotary evaporator with a water bath at ~35°C. Approximately 20 ml of ethyl acetate was added and the rotary evaporation continued just to dryness. Hexane was added to remove residual ethyl acetate and evaporated again to dryness. The dried

residues were dissolved in a 5-ml portion of hexane and sonicated to ensure the sample was completely dissolved.

SILICA SOLID PHASE EXTRACTION CLEANUP (S-1812, S-1812-DPMe, and S-1812-DP): A 2 gram MegaBond Silica Solid Phase Extraction (SPE) Tube was conditioned by passing 2 x 5 ml portions of Eluant 1 (95/5, hexane/ethyl acetate, v/v) followed by 2 x 5 ml portions of hexane through the column, using gravity to elute the solvents. The flow was discontinued when the solvent level reached the top of the packing. The silica packing was not allowed to go dry during or after conditioning. The conditioning solvents were discarded.

The sample extract was transferred to the column, and rinsed with 3 x 5 ml portions of hexane (the hexane was used to rinse the flat bottom flask prior to transfer the column). The hexane rinses were discarded.

FRACTION A (S-1812 AND S-1812-DPMe): A 125-ml flat-bottom evaporation flask was placed under the Silica SPE column and S-1812 and S-1812-DPMe were eluted with a 20-ml portion of Eluant 1 (95/5, hexane/ethyl acetate, v/v) that was used to rinse the 500-ml flat-bottom evaporation flask prior to transferring to the column. When the elution solvent reached the top of the Silica bed, elution was ceased.

FRACTION B (S-1812-DP): A 125-ml flat-bottom evaporation flask was placed under the Silica SPE column and S-1812-DP was eluted with a 15-ml portion of Eluant 2 (85/15, hexane/ethyl acetate, v/v) that was used to rinse the 500-ml flat-bottom evaporation flask prior to transferring to the column. When the elution solvent reached the top of the Silica bed, elution was ceased.

CONCENTRATION (S-1812, S-1812-DPMe, and S-1812-DP): The sample was evaporated just to dryness using a rotary evaporator with a water bath at ~35°C.

The sample was re-dissolved in 1.0 ml of toluene, sonicated to ensure the sample was completely dissolved. The sample was transferred to a chromatography vial and submitted for analysis by GC with NPD detection.

CONCENTRATION (HTFP): A 50-ml aliquot (equivalent to 10 grams of soil) of the sample extract was transferred to a 250-ml flat-bottom flask. A 1-ml portion of the keeper solution (1% propylene glycol in acetonitrile) was added. The sample was evaporated to

approximately 5 ml with a water bath set at ~40°C. Approximately 50 ml of ethyl acetate was added to the sample and evaporation was continued until just dryness. Where ethyl acetate persisted, hexane was added and again evaporated to dryness. A 10-ml portion of 10/1, hexane/acetone, v/v, was immediately added and the sample was sonicated to ensure the residues were completely dissolved.

SILICA SOLID PHASE EXTRACTION CLEANUP (HTFP): A 2 gram MegaBond Silica Solid Phase Extraction (SPE) Tube was conditioned by passing 2 x 5 ml portions of 10/1, hexane/acetone, v/v, followed by 2 x 5 ml portions of hexane through the column, using gravity to elute the solvents. The flow was discontinued when the solvent level reached the top of the packing. The silica packing was not allowed to go dry during or after conditioning. The conditioning solvents were discarded.

The sample extract was transferred to the column, and rinsed with 3 x 5 ml portions of hexane (the hexane was used to rinse the flat bottom flask prior to transfer the column). The hexane rinses were discarded.

A 125-ml flat-bottom evaporation flask was placed under the Silica SPE column and HTFP was eluted with a 25-ml portion of Eluant 3 (2/3, hexane/acetone, v/v) that was used to rinse the 250-ml flat-bottom evaporation flask prior to transferring to the column. The solvent was completely eluted from the cartridge.

CONCENTRATION (HTFP): A 1-ml portion of the keeper solution (1% propylene glycol in acetonitrile) was added and the sample was evaporated just to dryness using a rotary evaporator with a water bath at ~35°C.

The sample was re-dissolved in 1.0 ml of HPLC Mobile Phase (20%ACN : 80% 0.01% acetic acid) and sonicated to ensure the sample was completely dissolved. The sample was transferred to a chromatography vial and submitted for analysis by HPLC with UV/Vis detection.

2. Instrument Parameters (S-1812 and S-1812-DPMe)

Instrument:	Hewlett Packard Model 5890 Series II GC equipped with a Nitrogen Phosphorus Detector (NPD) and a 7673 autosampler
GC Column:	Restek Rtx-1 (30 m x 0.53 mm id x 0.25 µm film) Cat. #10125

Data System:	ChromPerfect® Spirit Chromatography Software, Revision 5.0.0, Justice Innovations, Inc.
Injector Liner:	Bottom-tapered silanized liner with fused silica
Injector Temperature:	250°C
Detector Temperature:	280°C

Gas Flow Rates:

Helium (column)	20 ml/min
Helium (makeup)	20 ml/min
Air (makeup)	110 ml/min
Hydrogen	4 ml/min
Septum Purge	2.5 ml/min
Purge Valve on at	1.0 min

Temperature Program:

Initial Oven Temperature	200°C
Initial Time	2.0 min
Rate	15°C/min
Final Temperature	275°C
Final Time	3.0 min

Run Time: 10.0 min

Retention Time:

S-1812-DPMe	~3.9 min
S-1812	~6.7 min

Injection Volume: 2 µl

GC conditions varied slightly when instrument and column maintenance were performed. The instrument parameters were optimized for analyte sensitivity and resolution prior to each chromatographic run, and the exact parameters were documented with each data set.

3. Instrument Parameters (S-1812-DP)

Instrument:	Hewlett Packard Model 5890 Series II GC equipped with a Nitrogen Phosphorus Detector (NPD) and a 7673 autosampler
GC Column:	Agilent HP-5 (30 m x 0.53 mm id x 1.5 µm film) Cat. # 19095J-323

Data System:	ChromPerfect® Spirit Chromatography Software, Revision 5.0.0, Justice Innovations, Inc.
Injector Liner:	Bottom-tapered silanized inlet liner with fused silica
Injector Temperature:	250°C
Detector Temperature:	280°C

Gas Flow Rates:

Helium (column)	~20 ml/min
Helium (makeup)	~10 ml/min
Air (makeup)	~110 ml/min
Hydrogen	~4 ml/min
Septum Purge	~2.5 ml/min
Purge Valve on at	1.0 min

Temperature Program:

Initial Oven Temperature	250°C
Initial Time	4.0 min
Rate	20°C/min
Final Temperature	290°C
Final Time	4.0 min

Run Time: 10.0 min

Retention Time:

S-1812-DP	~5.7 min

Injection Volume: 2 µl

GC conditions varied slightly when instrument and column maintenance were performed. The instrument parameters were optimized for analyte sensitivity and resolution prior to each chromatographic run, and the exact parameters were documented with each data set.

4. Instrument Parameters (HTFP)

Instrument: Hewlett-Packard Model 1050 Series HPLC equipped with an UV/Vis Detector and autosampler.

HPLC Column:	Phenomenex Prodigy 5 micron ODS (3) (250 mm x 4.60 mm id) Cat. #00G-4097-E0
Data System:	ChromPerfect® Spirit Chromatography Software, Revision 5.0.0, Justice Innovations, Inc.
Column Temperature:	35°C
Wavelength:	235 nm
Mobile Phase A:	20% Acetonitrile:80% (0.01% Acetic Acid in Water)
Mobile Phase B:	100% Acetonitrile
Mobile Phase Program:	
(0.0 – 11.0 min)	100% Mobile Phase A
(11.0 – 13.0 min)	50% Mobile Phase A : 50% Mobile Phase B
(13.0 – 20.0 min)	100% Mobile Phase A
Flow Rate:	1.0 ml/min
Run Time:	20 min
Retention Time:	~10.5 min
Injection Volume:	50 µl

Note: Due to differences in instrumentation, 100% mobile phase A was used as the initial mobile phase composition and was held at 100% mobile phase A until the elution of HTFP. After HTFP eluted, a gradient was used to wash off the column before the next sample injection. The mobile phase program used in this study was modified from that described in the method resulting in a retention time of ~10.5 minutes rather than 4.9 minutes as stated in the method. HPLC conditions varied slightly when instrument and column maintenance were performed. The instrument parameters were optimized for analyte sensitivity and resolution prior to each chromatographic run, and the exact parameters were documented with each data set.

F. Quantitation Procedures

ChromPerfect® Spirit Chromatography Software, a product of Justice Innovations, was used to acquire, integrate and calculate the concentrations of S-1812, S-1812-DPMe, S-1812-DP and HTFP in soil using ChromPerfect® Spirit's quadratic regression function. For the regression calculations, concentration was designated as the independent variable and

plotted on the x-axis. Peak response was designated as the dependent variable and plotted on the y-axis. From this regression curve, a slope, y-intercept and correlation coefficient of the standard curve run with each analytical set were calculated. The correlation coefficients were all greater than 0.990. S-1812, S-1812-DPMe, S-1812-DP and HTFP calibration standards were injected every one to three sample injections, as well as at the beginning and ending of the injection sequence. Five different standard concentrations ranging from 0.100 to 2.50 µg/ml were injected with each analysis set. The analyte concentrations in the sample extracts were extrapolated from the curve equation. The concentration, as µg/g, of residue found in the samples were then calculated by ChromPerfect® Spirit using the following equation:

$$\mu g/g = \frac{(\mu g/ml \text{ from curve}) \times (\text{Aliquot Factor}) \times (\text{Final Vol. in ml}) \times (\text{Dilution Factor})}{(\text{sample weight in grams})}$$

and,

$$\text{Aliquot Factor} = \frac{\text{Extraction volume in ml}}{\text{Aliquot volume in ml}}$$

$$\text{Dilution Factor} = \frac{\text{final volume after dilution (ml)}}{\text{initial volume (ml)}}$$

Recovery of S-1812, S-1812-DPMe, S-1812-DP and HTFP from fortified samples was calculated as follows:

$$\%\text{Recovery} = \frac{(\text{Measured Concentration, } \mu g/g)}{(\text{Theoretical Concentration, } \mu g/g)} \times 100$$

An example calculation for a low level S-1812 laboratory fortification in set 087ILV01F2, sample 087ILV01-5, Low Fort 2 at 0.0200 µg/g, is as follows:

standard curve equation: $y = -511.4055 (x^2) + 20243.58 (x) - 172.5209$
where x *= S-1812 concentration in µg/ml and*
y *= peak response = 3927.3*
S-1812 concentration from the curve = 0.204 µg/ml
dilution factor = (1 ml / 1 ml) = 1

$$\mu g/g = \frac{(0.204 \ \mu g/ml \text{ S-1812}) (5) (1 \text{ ml}) (1)}{(50 \text{ grams})} = 0.0204 \ \mu g/g$$

$$\%\text{ recovery} = \frac{0.0204 \ \mu g/g}{0.0200 \ \mu g/g} \times 100 = 102\%$$

Fortification samples were not corrected for residues detected in control samples. All unfortified control samples were reported as <MQL.

B. Problems Encountered

No problems with the method as written were encountered. During the first method trial, an error was made during the preparation of the 0.01% Acetic Acid solution used in the Mobile Phase A solution which resulted in co-elution of an interference peak (from Celite) with HTFP. Prior to starting Trial 2, modifications to the Mobile Phase A were made (changing the amount of acetonitrile in the solution), which provided separation between the HTFP peak and the interference peak. During the second Method Trial, the mobile phase preparation error was discovered. Once this error was corrected, the peak separation and general chromatography for HTFP was similar to the examples presented in the method.

C. Critical Steps

1) A good stopping point is after the samples have been extracted, filtered and brought to 250-ml volume. Samples can be refrigerated overnight and the analysis can be continued the next day.

D. Time Requirement

Twelve hours are required for one person to prepare an analysis set from the time samples are weighed to GC and HPLC analysis. Automated GC and HPLC analysis can be performed overnight. An additional 1.5 hours may be spent on data calculation and tabulation the following day.

E. Description of Contact

The initial contact on July 3, 2001, with the Sponsor Representative involved a discussion regarding the amount of Celite to add to the samples. The method stated "50 ml", however, Celite is a solid and there was some concern to as whether or not this was a typo. The Sponsor Representative explained that the Celite was to be measured in a beaker to the 50-ml mark. Therefore, the method was correct as stated.

Upon completion of the first validation trial, an interference was observed near the elution time of HTFP during the HPLC analysis. After review of the data and discussions with the study monitor on July 5, 2001, modifications to the HPLC Mobile Phase were made to achieve separation of the HTFP from the interference. Once adequate separation was achieved, the sponsor monitor recommended a second method trial to be conducted for HTFP only. After the analysis of the second method trial, a mobile phase preparation error was discovered. The sponsor monitor was notified of this error on August 2, 2001. The correct Mobile Phase A was prepared and the samples from the second method trial were reanalyzed. Chromatographic separation similar to that found in the method was obtained and the HTFP portion of the method was successfully validated.