Assay of Dacthal Formulation

Formulation	Batch Number	DCPA	HCB
Dacthal W-75	LSLOLUM	74.8	0.087

SAMPLE AND ASSAY IDENTIFICATION

Prior to assay, samples were assigned a laboratory code for identification. Upon initiation of the analytical procedure, the sample to be assayed was assigned an identifying laboratory sample reference number.

The laboratory code, sample code and corresponding sample description were recorded on Operations and Data Collection Forms.

Gas chromatographic data were identified by the laboratory sample reference number.

VALIDATION OF ASSAY PROCEDURE

Untreated soil was amended prior to extraction by the addition of separate standard solutions of DCPA, SDS-1449, SDS-954 and HCB. Some of the soil used as amended and unamended check, labeled 87-860, was locally obtained and not related to the field test conducted near Gilroy, CA. Soil samples were amended at concentrations within the range of 0.03 ppm to 20.0 ppm for DCPA, 0.03 ppm to 10.0 ppm for SDS-1449 and SDS-954 and 0.01 ppm to 0.05 ppm for HCB. The amended samples were processed through the described analytical procedure to evaluate the validity of the assay procedure.

The assay procedure was validated prior to initiation and during sample assay.

ASSAY PROCEDURE

Residues of DCPA, SDS-1449, SDS-954 and HCB were extracted from the soil and selectively partitioned into an organic solvent. The residues of DCPA and HCB were separated by column chromatography prior to subsequent quantitation by electron capture gas chromatography. The residue of SDS-1449 was derivatized to its methyl propyl ester prior to quantitation. The residue of SDS-954 was derivatized to its dipropyl ester prior to quantitation. The residues of derivatized SDS-1449 and SDS-954 were cleaned up by column chromatography prior to quantitation.

MATERIALS

Chemicals

Acetic acid, glacial, A.C.S. certified

Acetone, A.C.S. certified

Acetonitrile, A.C.S. certified

Acid/Acetone Extraction Solvent: 5% 10 N H_2SO_4 and 95% acetone (v/v).

Alumina (aluminum oxide W200 acid), activated at 115°C ± 5°C at least 24 hours before use, manufactured by Woelm Pharma of West Germany, available from ICN Nutritional Biochemicals, 26201 Miles Road, Cleveland, Ohio 44128.

Diazopropane solution, propylating reagent; prepared (just prior to use) as follows: A 2.3 g portion of potassium hydroxide was dissolved in 2.3 ml of deionized water, cooled in an ice bath and overlaid with 50 ml of diethyl ether. A 1.5 g portion of the precursor (N'-nitro-N-nitroso-N-propylguanidine) was weighed to the nearest 0.1 g and slowly added to the cooled caustic/ether solution in 0.1-0.2 g increments. Each portion of the precursor was added only after all signs of reaction from the previous addition had ceased. This procedure was followed until all reagent

had been added and the ether phase turned yellow. The diazopropane, residing in the ether phase, was decanted into an Erlenmeyer flask (which was cooled in the ice bath). The caustic solution remaining and any unused reagent was neutralized/destroyed by adding excess glacial acetic acid. EXTREME CARE WAS TAKEN IN HANDLING THE REAGENTS DURING ALL OPERATIONS. THE PRECURSOR IS A CANCER SUSPECT AGENT, EXTREMELY TOXIC AND POTENTIALLY EXPLOSIVE. THE REACTION PRODUCTS ARE ALSO POTENTIALLY CARCINOGENIC AND EXTREMELY TOXIC. A FULL COMPLEMENT OF PERSONAL PROTECTIVE EQUIPMENT (LAB COAT AND GLOVES, AND WORKING IN A FUME HOOD BEHIND A SAFETY SHIELD) WAS USED BY LAB PERSONNEL HANDLING THE REAGENTS AND NO SCRATCHED, CHIPPED OR GLASS-STOPPERED GLASSWARE WAS USED.

Diethyl ether, anhydrous, A.C.S. reagent grade

Dimethyl tetrachloroterephthalate (DCPA, SDS-893-0402), 99.2% pure

Eluant A, consisting of 20% methylene chloride and 80% hexane (v/v)

Eluant B, consisting of 50% methylene chloride, 49.5% hexane and 0.5% acetonitrile (v/v/v)

Ethyl acetate, A.C.S. certified

Florisil, 60/100 mesh PR, activated at 115°C ± 5°C for 12 hours before use, available from Floridin Company, Berkeley Springs, West Virginia 25441.

Hexachlorobenzene (HCB, SDS-1497-0202), 100% pure

Hexane, A.C.S. certified

Hydrochloric acid (HCl), concentrated, A.C.S. reagent grade

"Keeper" solution, consisting of 2 ml light mineral oil in 100 ml petroleum ether

Methylene chloride, A.C.S. certified

Monomethyl tetrachloroterephthalate (SDS-1449-0401), 98.7% pure

N_2, high purity

N'-nitro-N-nitroso-N-propylguanidine (catalog number 14,319-7, Aldrich Chemical Company)

n-Propanol, A.C.S. certified

Paraffin oil, technical grade

Petroleum ether, pesticide grade

Potassium hydroxide (KOH), A.C.S. certified

Sep-Pak silica cartridges, available from Waters Associates, Milford, Massachusetts 01757

Sodium bicarbonate ($NaHCO_3$), A.C.S. certified

Sodium hydroxide (NaOH), A.C.S. certified

Sodium sulfate (Na_2SO_4), anhydrous

Sulfuric acid (H_2SO_4), 10N, A.C.S. reagent grade

Tetrachloroterephthalic acid (SDS-954-0201), 99% pure

Toluene, A.C.S. reagent grade

Equipment

Bottles, 2 oz., 4 oz. and 8 oz., with polyseal lined caps, Fisher Scientific Company, Cat. Nos. 03-326,5B,5C and 5D

Culture tubes, 16 mm x 125 mm, with polypropylene screw caps, Fisher Scientific Company, Cat. No. 14-962-26G

Residues were quantitated using a Varian Model 3700 or Model 6000/6500 gas chromatograph equipped with a Varian Model 8000 autosampler, a ^{63}Ni electron capture detector, and a Varian Vista 402 data system. Quantitation was also done on a Hewlett Packard Model 5880A GC equipped with a Model 7673A automatic sampler and a ^{63}Ni electron capture detector.

Operating parameters for the gas chromatograph were:

Column 1: 6′ x 1/4" o.d. x 2 mm i.d. glass column filled with 3% OV-7 on 100/120 mesh Supelcoport

Column 2: 6′ x 1/4" o.d. x 2 mm i.d. glass column filled with 3-5% OV-210 on 80/100 mesh Supelcoport (available from Supelco, Inc., Bellefonte, PA 16823)

Column 3: 30 m DB-5 capillary column, 0.25 mm i.d., 1 µm film thickness

Temperature:

Column 1:	190-200°C for DCPA	
	200-215°C for derivatized SDS-1449 and SDS-954	
	180°C for HCB	
Column 2:	180°C for DCPA	
	140°C for HCB	
Column 3:	250°C for DCPA	
	270°C for derivatized SDS-1449 and SDS-954	
	230°C for HCB	

Injection Port Temperature: 270-300°C

Detector Temperature: 300°C

Carrier Gas: Column 1 & 2: High purity nitrogen or ultra high purity 10% methane in argon, 30-40 ml/min.

Column 3: High purity helium

pH Meter equipped with standard combination electrode

Chromatographic glass columns 200 mm x 9 mm, Kontes Co., Cat. No. K420100-23

Standard laboratory glassware: beakers, flasks, separatory funnels, etc.

Water bath, maintained at a maximum temperature of 37°C.

PREPARATION OF STANDARD SOLUTIONS

DCPA and HCB

A standard solution of DCPA was prepared by weighing 0.1 g DCPA to the nearest 0.1 mg into a tared weighing pan. The DCPA was quantitatively transferred to a 100 ml volumetric flask using toluene. The contents of the volumetric flask were diluted to volume with toluene to produce a stock-solution of 1000 ug DCPA per ml. This stock solution was serially diluted with toluene to result in a "working standard" of 0.03 ug per ml or 0.05 µg/ml for the quantitation of DCPA. Separate 1000 ug/ml stock and "working standard" solutions of HCB (0.01 ug/ml to 0.03 ug/ml) were prepared in a similar manner as described for DCPA. Serial dilutions of all compounds were used to amend check samples for recoveries.

SDS-1449 and SDS-954

All pipets and volumetrics used to prepare or transfer SDS-1449 or SDS-954 standards were acid washed by rinsing with 1N H_2SO_4, deionized water and acetone and drying prior to use. Individual stock solutions

were prepared to contain 1000 μg/ml SDS-1449 and SDS-954 in acetone in the manner described above. A 10 ml aliquot of both 1000 μg/ml solutions was quantitatively transferred to an acid washed 100 ml volumetric flask and brought to volume with acetone to form a 100 μg/ml combined standard. Serial dilutions of the 100 μg/ml combined standard were done in acetone to prepare 10.0 and 1.0 μg/ml combined standards. One drop of concentrated HCl was added to each solution. These solutions were used to amend samples for recoveries.

A 1.0 ml aliquot of the 10 μg/ml SDS-1449/954 combined standard was transferred to an acid washed 4 oz. disposable bottle. The solvent was evaporated to dryness in a water bath with a dry nitrogen stream. The standard was derivatized as described below. The propylated SDS-1449/-SDS-954 was then dissolved in 100 ml of toluene.

A 0.01 μg/ml or 0.03 μg/ml dilution was prepared in toluene and used as a standard for G.C. quantitation of the DCPA soil metabolites.

PREPARATION OF FIELD SAMPLES

The field soil sample was removed from the freezer and allowed to sufficiently thaw until the sample could be thoroughly mixed by hand to produce a composite field sample. From this point each sample was treated in duplicate (as sample size permitted). Residue determinations are based upon "as received" sample weights. In addition, appropriate portions of each sample were removed and the soil moisture content determined. Final analytical results are reported on a dry weight basis.

EXTRACTION OF RESIDUES

A 20 g portion of soil was transferred to an 8 oz. bottle equipped with a Polyseal cap. A 100 ml portion of the acid/acetone extraction solvent was added, and the bottle sealed. The sample was shaken on a reciprocating shaker for 2 hours. After the solids settled, a measured and recorded portion of the filtrate equivalent to approximately 6 g of the subsample was transferred to an appropriately sized beaker. A 5 ml portion of "Keeper" solution and 20 ml of water were added to the filtrate. The

filtrate was evaporated free of acetone by placing the beaker in the water bath with a stream of dry nitrogen impinging on the solvent surface or by allowing the filtrate to stand overnight in an operating fume hood.

SELECTIVE PARTITIONING

DCPA and HCB

After the acetone had evaporated, a 50 ml portion of 0.8M $NaHCO_3$ was added to the beaker. The pH of the contents of the beaker was adjusted to 10 with the aid of a pH meter using either 1N NaOH or 10N H_2SO_4. The aqueous solution at pH 10 was quantitatively transferred to an appropriately sized separatory funnel using 50 ml petroleum ether. The separatory funnel was vigorously shaken manually for two minutes, the phases were allowed to separate, and the lower aqueous phase was drained into the beaker. The ether phase was poured from the top of the separatory funnel into an acetone rinsed 4 oz. bottle. Care was taken so that no water was transferred with the ether. The aqueous phase was quantitatively transferred to the separatory funnel using an additional 50 ml portion of petroleum ether. The partitioning was repeated once again as previously described. The petroleum ether extracts were combined and 0.4 ml of "Keeper" solution was added. The ether was concentrated in the water bath to approximately 2 ml. The remaining solvent was evaporated to dryness with caution to prevent loss of DCPA and HCB using a gentle stream of clean, dry nitrogen only. The residue was dissolved in 6 ml of Eluant A and transferred to a culture tube which had been rinsed with Eluant A and air dried. The culture tube was sealed using a polypropylene screw cap, appropriately labeled with the laboratory sample reference number and reserved for cleanup and residue separation by column chromatography.

SDS-1449, SDS-954

After the parent partitioning, the pH of the aqueous phase containing SDS-1449 and SDS-954 was adjusted to less than 1 by the addition of 40 ml of 10N H_2SO_4. The resulting solution was quantitatively transferred to the separatory funnel using approximately 10 ml water and 50 ml of a 1:1

mixture of petroleum ether/ethyl ether (v/v). The separatory funnel was vigorously shaken manually for two minutes, the phases were allowed to separate, and the lower aqueous phase was drained into the beaker. The diethyl ether phase was poured from the top of the separatory funnel into a 4 oz. bottle which had been rinsed with acid water (pH <2), deionized water, acetone and allowed to dry before use. Care was taken so that no water was transferred with the diethyl ether. The partitioning was repeated. Both ether extracts were combined, concentrated and evaporated as previously described. The residue containing SDS-1449 and SDS-954 was reserved for derivatization using the procedure described below.

DERIVATIZATION OF SDS-1449 AND SDS-954

Diazopropane Solution

The SDS-1449 and SDS-954 were converted to the propyl and dipropyl ester derivatives, respectively by the addition of 10 µl of 1:3 concentrated HCl:n-propanol (v/v) and 3 ml of the diazopropane solution to the dried residue remaining in the flask after evaporation. EXTREME CARE WAS TAKEN IN HANDLING THE REAGENTS DURING ALL OPERATIONS. THE PRECURSOR IS A CANCER SUSPECT AGENT, EXTREMELY TOXIC AND POTENTIALLY EXPLOSIVE. THE REACTION PRODUCTS ARE ALSO POTENTIALLY CARCINOGENIC AND EXTREMELY TOXIC. A FULL COMPLEMENT OF PERSONAL PROTECTIVE EQUIPMENT (LAB COAT AND GLOVES, AND WORKING IN A FUME HOOD BEHIND A SAFETY SHIELD) WAS USED BY LAB PERSONNEL HANDLING THE REAGENTS AND NO SCRATCHED, CHIPPED OR GLASS-STOPPERED GLASSWARE WAS USED. The reaction mixture was allowed to set in an operating fume hood at ambient temperature for 30 minutes, after which time the solvent was evaporated with caution to prevent loss using a gentle stream of dry nitrogen. To insure complete derivatization, an additional 1 ml of the diazopropane solution was added and the mixture was allowed to set for 10 minutes. The solvent was evaporated using a gentle stream of clean, dry nitrogen. The residue was dissolved in 6 ml of methylene chloride and transferred to a culture tube which was previously rinsed with acetone and air dried. The culture tube was sealed with a polypropylene screw cap, appropriately labeled with the laboratory sample reference number and reserved for separation and cleanup by column chromatography prior to quantitation by gas chromatography.

SAMPLE CLEANUP OF SDS-1449 AND SDS-954

A glass chromatographic column, 200 mm x 9 mm, was packed from bottom to top with 0.5 cm bed of glass wool, 3 g of the activated alumina and 1 cm anhydrous sodium sulfate. The column was washed with 10 ml methylene chloride. The sample (2 gram equivalent) in methylene chloride was quantitatively transferred to the column. Immediately upon the addition of the sample to the column, a 2 oz. bottle (acetone rinsed) was placed under the column to collect the eluant. After the sample had moved into the column bed, the derivatized SDS-1449/SDS-954 was eluted from the column with 40 ml methylene chloride.

ADDITIONAL COLUMN CLEANUP FOR PROPYLATED SDS-1449/954

A 0.4 ml portion of "Keeper" solution was added to each bottle. The bottle was placed in the water bath and the solvent was concentrated to approximately 0.5 ml using a gentle stream of dry nitrogen directed on the surface of the solvent to aid evaporation. The bottle was removed from the water bath and the contents were evaporated to dryness using a stream of dry nitrogen. The residue was dissolved in 2 ml of petroleum ether. A SEP-PAK® cartridge was attached to a 10 ml syringe with Luer end fitting. The ether was pumped through the SEP-PAK® cartridge and discarded. The cartridge was then flushed two times with 10 ml of petroleum ether. A 10 ml portion of 5% ethyl acetate in petroleum ether (v/v) was then used to elute the sample from the cartridge into an appropriate sized bottle.

SAMPLE CLEANUP AND SEPARATION OF DCPA AND HCB

Column Preparation

A glass chromatographic column, 200 mm x 9 mm, was packed bottom to top with 0.5 cm bed of glass wool, 2 g of Florisil and 0.2 cm of anhydrous sodium sulfate. The column was packed using Florisil directly from the oven and vibrated to settle and compact the Florisil. The column was washed with 10 ml hexane. After the hexane entered the sodium sulfate layer, a 2 oz. bottle was placed under the column.

Elution of HCB and DCPA

A 2 g equivalent portion of the sample extract, dissolved in Eluant A, was transferred to a separately prepared column. After the sample extract migrated into the sodium sulfate layer, HCB was eluted from the column with 23 ml of Eluant A. A total of 25 ml of Eluant A was collected in the 2 oz. bottle. A separate 2 oz. bottle was placed under the column. The DCPA was eluted from the column with 30 ml Eluant B.

PREPARATION FOR QUANTITATION

A 0.4 ml portion of "Keeper" solution was added to each respective eluant. The bottle was placed in the water bath and the solvent was concentrated to approximately 0.5 ml using a gentle stream of dry nitrogen directed on the surface of the solvent to aid evaporation. The bottle was removed from the water bath and the contents were evaporated to dryness using a stream of dry nitrogen. The residue was dissolved in an appropriate volume of toluene for quantitation of DCPA, propyl SDS-1449, dipropyl SDS-954, and HCB by gas chromatography.

QUANTITATION

Sample Assay by Autosampler Injection

Residues of DCPA, propyl SDS-1449, dipropyl SDS-954, and HCB were determined by electron capture gas chromatography (^{63}Ni) using equipment which allowed automated injection and automatic data reduction.

Portions of the sample extract (approximately 1 ml) were sealed in separate vials designed for use with the autosampler. This included all samples, the "working standard," and other standards in the concentration range of interest to demonstrate linearity. The detection limit was established by the lowest standard.

The instrument was calibrated with the "working standard" (0.03 ug/ml or 0.05 ug/ml DCPA, 0.03 ug/ml or 0.01 ug/ml propylated SDS-1449/954 0.03 ug/ml or 0.01 ug/ml HCB). The sample weight, sample volume, concentration of the "working standard" and retention time of the compound of interest were entered into the system. Using these parameters, the instrument reduced the data to ppm values (ug of DCPA, SDS-1449, SDS-954, or HCB per gram of sample) by external standard calibration.

$$\text{Calibration Factor} = \frac{\text{Standard Concentration (ug/ml)}}{\text{Standard Area (or Peak Height)}}$$

$$\text{Assay Weight} = \frac{\text{Sample Weight} \times \text{Aliquot} \times \text{Dilution to Cleanup}}{\text{Extraction Volume}}$$

$$\text{ppm residue} = \frac{\text{Calibration Factor} \times \text{Vol. for G.C.} \times \text{Sample Area (or Peak Height)}}{\text{Assay Weight}}$$