CHLOROTHALONIL
415 C48-30

ASSAY PROCEDURE

Residues of chlorothalonil, SDS-3701, SDS-47525, SDS-46851, SDS-19921, SDS-47523/47524, HCB and PCBN were extracted from the soil and selectively partitioned into an organic solvent. The residues of chlorothalonil, HCB and PCBN were separated by column chromatography prior to subsequent quantitation by electron capture gas chromatography. The fraction containing residues of metabolites was separated into two cuts. One cut was

derivatized; the other cut was quantitated directly without cleanup. In some cases, each cut was cleaned up by column chromatography prior to quantitation by electron capture gas chromatography.

MATERIALS

Chemicals

Acetic acid, glacial, A.C.S. reagent grade

Acetone, A.C.S. certified

Acetonitrile, A.C.S. certified

Alumina (aluminum oxide W200 acid), activated at 120°C ± 5°C for 12 hours before use, manufactured by Woelm Pharma of West Germany, available from ICN Nutritional Biochemicals, 26201 Miles Road, Cleveland, Ohio 44128.

3-Carboxy-2,5,6-trichlorobenzamide (SDS-46851), batch 0203, 99% pure

3-Cyano-2,4,5,6-tetrachlorobenzamide (SDS-19221) batch 0104, 99% pure

3-Cyano-2,4,5-trichlorobenzamide (SDS-47523), batch 0201, 99% pure

Diazomethane solution, methylating reagent, prepared (just prior to use) as follows: The diazomethane solution was prepared using MNNG-Diazomethane Kit with an O-ring joint. A 0.8 g amount of the precursor (N-methyl-N'-nitro-N-nitrosoguanidine) was placed in the inside tube through its screw cap opening along with 1 ml of water. Diethyl ether (approximately 20 ml) was placed in the outside tube and the two parts were assembled with an O-ring and held with a pinch-type clamp. The lower part was immersed in an ice bath and about 2.5 ml of 4N NaOH was injected through the silicone rubber septum via a syringe. The addition was done dropwise to prevent the mixture from getting too hot and to control the column of gas produced. The diazomethane was collected in

the ether ready for use after all evidence of gas production ceased. 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 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

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

Eluant C, consisting of 50% methylene chloride, 48.5% hexane and 1.5% acetonitrile (v/v/v)

Eluant D, consisting of 0.05% acetonitrile in 1:1 hexane:methylene chloride (v/v)

Eluant F, consisting of 50% acetone and 50% methylene chloride (v/v)

Extraction solvent: 5% 10N H_2SO_4 AND 95% Acetone (v/v)

Florisil, 60/100 mesh PR, activated at 120°C $\pm$ 5°C for 12 hours before use, available from Floridin Company, Berkeley Springs, West Virginia 25441.

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

Hexane, pesticide grade

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

2-Hydroxy-5-cyano-3,4,6-trichlorobenzamide (SDS-47525), batch 0201, 98% pure

4-Hydroxy-2,5,6-trichloroisophthalonitrile (SDS-3701), batch 0201, 99.5% pure

"Keeper" solution, consisting of 2% paraffin oil in petroleum ether

Methanol, A.C.S. certified

Methylene chloride, pesticide grade

N-methyl-N'-nitro-N-nitrosoguanidine available from Aldrich Chemical Co., catalog No. 12,944-1

Nitrogen, N_2, high purity

Paraffin oil, technical grade

Pentachlorobenzonitrile (PCBN, SDS-3297), batch 0302, 99.7% pure

Petroleum ether, distilled in glass, nanograde

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

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

Sodium chloride (NaCl), A.C.S. certified

Sodium sulfate (Na_2SO_4), anhydrous, A.C.S. certified

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

2,4,5,6-Tetrachloroisophthalonitrile (chlorothalonil, SDS-2787), batch 1203, 99.7% pure

Toluene, distilled in glass

Equipment

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

Buchner Funnel, Filter paper: Whatman No. 4

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 5840A GC equipped with a Model 7671A 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 3-5% OV-210 on 80/100 mesh Supelcoport (available from Supelco, Inc., Bellefonte, PA 16823)

Column 2: 6' x 1/4" o.d. x 2 mm i.d. glass column filled with 3-5% Dexsil on 100/120 mesh Supelcoport

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

Temperature:

Column 1:	130°C for HCB
	170°C for SDS-2787
	170°C for methyl SDS-3701
	215°C for SDS-19221 and SDS-47523
Column 2:	240°C for methyl SDS-46851 and SDS-47525
Column 3:	180°C for PCBN

Injection Port Temperature: 280°C

Detector Temperature: 350°C

Carrier Gas: High purity Nitrogen, 30-40 ml/min.

pH Meter equipped with standard combination electrode

Chromatographic 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

Chlorothalonil, HCB and PCBN

A standard solution of chlorothalonil was prepared by weighing 0.1 g chlorothalonil to the nearest 0.1 mg into a tared weighing pan. The chlorothalonil 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 chlorothalonil per ml. This stock solution was serially diluted with toluene to result in a "working standard" of 0.05 ug per ml for the quantitation of chlorothalonil. Separate 1000 ug/ml stock and "working standard" solutions of HCB (0.03 ug/ml) and PCBN (0.05 and 0.02 ug/ml) were prepared in a similar manner as described for chlorothalonil. Serial dilutions of all compounds were used to amend check samples for recoveries.

SDS-3701

A standard solution of SDS-3701 was prepared by weighing 0.1 g SDS-3701 to the nearest 0.1 mg into a tared weighing pan. The SDS-3701 was quantitatively transferred to a 100 ml volumetric flask using acetone. The contents of the volumetric flask were diluted to volume with acetone to produce a stock solution of 1000 ug SDS-3701 per ml. This stock solution was serially diluted with acetone to a final concentration of 1.0 ug per ml. One drop of concentrated hydrochloric acid was added to all solutions. These standards were used to amend check samples for recoveries.

One ml of the 10 ug per ml solution was transferred by pipet to a 4 oz. bottle which had been rinsed with acid water (pH <2), deionized water, acetone and allowed to dry prior to use. The solvent was evaporated and SDS-3701 was derivatized to the methyl ether as described under DERIVATIZATION OF SDS-3701, SDS-47525 and SDS-46851. The residue was dissolved in 100 ml toluene to result in a concentration of 0.1 ug/ml. This solution was diluted to make a 0.05 ug/ml "working standard" for the quantitation of SDS-3701.

SDS-46851, SDS-47525 Mixed Standard

Individual stock solutions of SDS-46851 and SDS-47525 were prepared at a concentration of 1000 ug/ml in methanol as described above for SDS-3701. These stock solutions were serially diluted with methanol to a final concentration of 10 ug each per ml. A 10 ml aliquot of each of the 100 ug per ml solutions was transferred to a single acid washed 100 ml volumetric flask. This 10 ug per ml mixed standard was brought to volume in methanol. Concentrations of these standard solutions were utilized to amend recovery samples.

A one ml aliquot of the individual 10 ug/ml solutions or mixed 10 ug per ml solution was transferred by pipet to an acid washed 4 oz. bottle. The solvent was carefully evaporated to dryness and the residue methylated as described above. The mixed methylated standard was dissolved in 100 ml of toluene (0.10 ug/ml) and utilized for quantitation by electron capture gas chromatography.

SDS-19221, SDS-47523 Mixed Standard

Individual stock solutions of SDS-19221 and SDS-47523 were prepared at a concentration of 1000 ug/ml in methanol as described above for SDS-3701. These stock solutions were serially diluted with methanol to a final concentration of 10 ug each per ml. A 10 ml aliquot of each of the 100 ug per ml solutions were transferred to a single acid washed 100 ml volumetric flask. This 10 ug per ml mixed standard was brought to volume in methanol. Concentrations of these standard solutions were utilized to amend samples.

SDS-47523 was used as the analytical standard. Residue determinations are reported as SDS-47523/47524 (ppm) due to the inability to resolve the two isomers.

A one ml aliquot of each of the individual 10 ug/ml solutions or mixed 10 ug per ml solution was transferred by pipet to a 100 ml volumetric flask. The mixed standard was diluted to 100 ml of toluene (0.10 ug/ml) and utilized for quantitation by electron capture gas chromatography.

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 (10 g if sample size was limited) was transferred to an 8 oz. bottle equipped with Polyseal cap. A 100 ml portion of the extraction solvent was added, and the bottle sealed. The sample was

shaken on a reciprocating shaker for 2 hours. The samples were centrifuged to clear the supernatant. After the solids settled, a 50 ml aliquot of the supernatant, equivalent to 10 g (5 g for limited samples) of soil, was transferred to a 250 ml beaker. A 5 ml portion of "Keeper" solution and 10 ml of water were added to each beaker. The solution was evaporated free of acetone by placing the beaker in the water bath with a stream of dry air impinging on the solvent surface or by allowing the solution to stand overnight in an operating fume hood. This solution was used for selective partitioning of the metabolites. In addition, a 25 ml aliquot of the supernatant, equivalent to 5 g (2.5 g for limited samples) of soil, was transferred to a separate 250 ml beaker for direct partitioning of the parent compounds.

DIRECT PARTITIONING OF CHLOROTHALONIL, HCB AND PCBN

The acid/acetone soil extract was quantitatively transferred to a 250 ml separatory funnel using 50 ml petroleum ether. A 25 ml portion of the acid/acetone extraction solvent and 20 ml of water were added. 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 a 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 using a gentle stream of clean, dry nitrogen only. CAUTION: The use of the dry nitrogen stream after the solvent has evaporated can result in low recovery of chlorothalonil, HCB and PCBN. The residue was dissolved in 5 ml of Eluant A and transferred to a culture tube. The culture tube was sealed using a polypropylene screw cap, appropriately labeled with the laboratory sample reference number, refrigerated and reserved for cleanup and residue separation by column chromatography.

SELECTIVE PARTITIONING

SDS-3701, SDS-47525, SDS-46851, SDS-19221 and SDS-47523/47524

After the acetone evaporated, a 100 ml portion of 0.4M $NaHCO_3$ was added to the beaker. The pH of the contents of the beaker was adjusted to 4.5 with the aid of a pH meter using either 0.4M $NaHCO_3$ or 10N H_2SO_4. The aqueous solution at pH 4.5 was quantitatively transferred to a 250 ml separatory funnel using 50 ml petroleum ether. The separatory funnel was vigorously shaken manually for two minutes, the phases were alloved to separate, and the lower aqueous phase was drained into the beaker. The petroleum ether phase was discarded. The aqueous phase was quantitatively transferred to the separatory funnel using an additional 50 ml portion of petroleum ether. The partitioning was repeated as previously described. After the second partitioning, the pH of the aqueous phase containing SDS-3701, SDS-46851, SDS-19221, SDS-47523/47524 and SDS-47525 was adjusted to less than 2 by the addition of 5 ml of 10N H_2SO_4. Sufficient NaCl was added to the aqueous solution to obtain a 30% solution (w/v) and mixed thoroughly using a magnetic stirrer. The resulting solution was quantitatively transferred to the separatory funnel using approximately 10 ml water and 50 ml diethyl ether. The separatory funnel was vigorously shaken manually for two minutes, the phases were alloved to separate and the lower aqueous phase drained into the beaker. The diethyl ether phase was then 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 alloved to dry before use. Care was taken so that no water was transferred with the diethyl ether. The partitioning was repeated once again. The ethyl ether extracts were combined and 0.4 ml of 2% Keeper added. The ether was concentrated in the water bath to approximately 2 ml. The remaining solvent was evaporated to dryness using a gentle stream of clean, dry nitrogen only. Ten ml of acetone was added to the flask and thoroughly mixed. Exactly one-half of the solution was transferred to an acid washed 4 oz. bottle. Both acetone solutions were evaporated as described above. One bottle containing metabolite residues was reserved for derivatization using the procedure described below. The residue in the second bottle was quantitatively transferred to a culture

tube using 5 ml of methylene chloride and reserved for separation and clean up of SDS-47523/SDS-47524 and SDS-19221 by column chromatography prior to quantitation by gas chromatography. Optionally, SDS-19221 and SDS-47523/24 fractions were assayed directly as described in the following procedure. A 2 ml (2 g equivalent) aliquot of the methylene chloride extract containing SDS-19221 and SDS-47523/47524 was put into a 2 oz. bottle, concentrated as described previously, brought up in 6 ml toluene and quantitated by gas chromatography.

DERIVATIZATION OF SDS-3701, SDS-47525 AND SDS-46851

Diazomethane Solution

The SDS-3701, SDS-47525 and SDS-46851 were converted to the methyl ether derivatives and the methyl ester derivative, by the addition of 10 ul of 1:3 concentrated HCL:methanol (v/v) and 4 ml of the diazomethane solution to the dried residue remaining in the bottle after evaporation. The reaction mixture was alloved to set in an operating fume hood at ambient temperature for 60 minutes, after which time the solvent was evaporated using a gentle stream of dry nitrogen. CAUTION: The use of the dry nitrogen stream after the solvent has evaporated can result in lov recovery of methylated SDS-3701, SDS-47525 and SDS-46851. The residue was dissolved in 5 ml of methylene chloride and transferred to a culture tube. The culture tube was sealed with a polypropylene cap, appropriately labeled with the laboratory sample reference number, refrigerated and reserved for separation and cleanup by column chromatography prior to quantitation by gas chromatography.

SAMPLE CLEANUP OF SDS-3701, SDS-47525 AND SDS-46851

A glass chromatographic column, 200 mm x 9 mm, was packed from bottom to top with 0.5 cm bed of glass vool, 3 g of the activated alumina and 1 cm anhydrous sodium sulfate. The column was vashed with 20 ml acetone and 20 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 was placed under the column to collect the eluant. After the sample has moved into the

column bed, the derivatized SDS-3701 was eluted from the column with 40 ml methylene chloride. After the methylene chloride eluant had moved into the column, a 2 oz. bottle was placed under the column and derivatized SDS-47525 and SDS-46851 were eluted from the column with 40 ml Eluant F.

SAMPLE CLEANUP OF SDS-19221 AND SDS-47523/47524

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 vashed with 20 ml acetone and 20 ml methylene chloride. The sample (2 gram equivalent) in methylene chloride was quantitatively transferred to the column. After the sample had moved into the column bed, a 40 ml portion of methylene chloride was added to the column and discarded. A 2 oz. bottle was placed under the column and SDS-19221 and SDS-47523/47524 were eluted from the column with 40 ml Eluant F.

SAMPLE CLEANUP AND SEPARATION OF CHLOROTHALONIL, HCB AND PCBN

Column Preparation

A glass chromatographic column, 200 mm x 9 mm, was packed bottom to top with a 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 vashed with 10 ml hexane. After the hexane entered the sodium sulfate layer, a 2 oz. bottle was placed under the column.

Elution of Chlorothalonil

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

Elution of HCB and PCBN

A separate 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 a 2 oz. bottle. A 4 oz. bottle was placed under the column. The PCBN was eluted from the column with 75 ml Eluant D and collected.

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 chlorothalonil, methyl SDS-3701, methyl SDS-47525, methyl SDS-46851, SDS-19221, SDS-47523/47524, HCB or PCBN by gas chromatography.

QUANTITATION

Sample Assay by Autosampler Injection

Residues of chlorothalonil, methyl SDS-3701, methyl SDS-47525, methyl SDS-46851, SDS-19221, SDS-47523/47524, HCB and PCBN were determined by electron capture gas chromatography (^{63}Ni) using equipment which allowed automated injection and automatic data reduction.

The following automated units were employed:

1. Varian Model 6000/6500 gas chromatograph equipped with a Model 8000 autosampler and a Vista 401 data system.

2. Varian Model 3700 gas chromatograph equipped with a Model 8000 autosampler and a Vista 402 data system.

3. Hewlett Packard 5840A gas chromatograph equipped with Model 7671A autosampler and 5840A terminal.

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.

The instrument was calibrated with the "working standard" (0.10 ug/ml chlorothalonil, 0.10 ug/ml methylated SDS-3701, 0.10 ug/ml methylated SDS-47525, 0.10 ug/ml methylated SDS-46851, 0.10 ug/ml SDS-19221, 0.10 ug/ml SDS-47523, 0.03 ug/ml HCB, or 0.05 ug/ml PCBN). 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 chlorothalonil, SDS-3701, SDS-47525, SDS-46851, SDS-19221, SDS-47523, HCB or PCBN per gram of sample) by external standard calibration.

$$\text{Calibration Factor} = \frac{\text{Standard Concentration (ug/ml)} \times 10{,}000}{\text{Standard Area (or Peak Height)}}$$

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

$$\text{ppm residue} = \frac{\text{Calibration Factor} \times \text{Vol. for G.C.} \times \text{Sample Area (or Peak Height)}}{\text{Assay Weight} \times 10{,}000}$$

A series of varying concentration standards was prepared from the "working standard" to serve as linearity checks. The non-detect level was established by the lowest concentration standard analyzed.