RICERCA, INC.
DEPARTMENT OF ENVIRONMENTAL SCIENCES

STUDY TITLE

GENERAL ANALYTICAL PROCEDURE
FOR THE DETERMINATION OF RESIDUES OF
TETRACHLOROISOPTHALONITRILE (CHLOROTHALONIL, SDS-2787),
SDS-3701, SDS-46851, HCB AND PCB ON SELECTED CROPS

DOCUMENT NUMBER: 3136-88-0138-MD-001

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PROJECT IDENTIFICATION
3136-88-0138-MD
REPORT

DISTRIBUTION

DOCUMENT NUMBER: 3136-88-0138-MD-001

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REPORT

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FOR THE DETERMINATION OF RESIDUES OF
TETRACHLOROISOPHTHALONITRILE (CHLOROTHALONIL, SDS-2787),
SDS-3701, SDS-46851, HCB AND PCBN ON SELECTED CROPS

ABSTRACT

The analytical procedure is presented for the determination of chlorothalonil (SDS-2787), SDS-3701, SDS-46851, HCB and PCBN on selected agronomic crops. The crops included in this document are cherries, strawberries, caneberries, cranberries, asparagus, mushrooms, pigeon peas, peaches and dry beans.
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INTRODUCTION

The analytical procedure presented is for the determination of residues of chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile, SDS-2787), its metabolites SDS-3701 (4-hydroxy-2,5,6-trichloroisophthalonitrile) and SDS-46851 (3-carboxy-2,5,6-trichlorobenzamide) and manufacturing impurities hexachlorobenzene (HCB) and pentachlorobenzenitrile (PCBN) on selected agronomic crops. Residues of chlorothalonil, its metabolites and manufacturing impurities HCB and PCBN are extracted from the samples and selectively partitioned into an organic solvent using two separate partitioning procedures. Alternate extraction and partitioning procedures are present for oil containing crops (dry beans).

MATERIALS

Chemicals

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

Acetone, A.C.S. certified, or equivalent

Acetonitrile, A.C.S. certified, or equivalent

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

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

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)

Florisil, 60/100 mesh PR, activated at 120°C ± 5°C for 12 hours before use, available from U. S. Silica Company, Berkeley Springs, West Virginia 25441

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

Hexane, A.C.S. certified, or equivalent

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

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

Methanol, A.C.S. certified, or equivalent

Methylene chloride, (Eluant DCM) A.C.S. certified, or equivalent

Methyllating reagent: A 0.1 g portion of 3-methyl-1-p-tolyltriazene (MTT, Catalog Number 13,933-5, Aldrich Chemical Company) dissolved in 55 ml diethyl ether. The solution is prepared fresh each week and stored at approximately below 20°F.

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

N₂, high purity, or equivalent

Paraffin oil, technical grade, or equivalent

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

Petroleum ether, distilled in glass, nanograde, or equivalent
Sodium bicarbonate ($\text{NaHCO}_3$), A.C.S. certified, or equivalent

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

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

Sodium sulfate ($\text{Na}_2\text{SO}_4$), anhydrous, A.C.S. certified, or equivalent

Sulfuric acid ($\text{H}_2\text{SO}_4$), 10N, A.C.S. reagent grade, or equivalent

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

Toluene, A.C.S. reagent grade, or equivalent

**Equipment**

Blender, stainless steel, explosion proof or explosion resistant

Buchner funnel, Filter paper: Whatman No. 4

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

Culture tubes, 16 mm x 125 mm, with Teflon® lined screw caps, Fisher Scientific Company, Cat. No. 14-930-10E, or equivalent

Gas Chromatograph equipped with a Varian Model 8000 autosampler, or equivalent, and a $^{63}\text{Ni}$ electron capture detector. An acceptable gas chromatograph is the Varian Model 3700 or Model 6000/6500 operating at the following parameters:

**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% OV-7 on 100/120 mesh Supelcoport

Column 3: 6' x 1/4" o.d. x 2 mm i.d. glass column filled with 3-5% Dexsil 300 on 100/120 mesh Supelcoport

Column 4: 6' X 1/4" o.d. x 2 mm i.d. glass column filled with 3% SP-2250-DB on 100/120 mesh Supelcoport

Temperature: Column 1: 130° to 160°C for HCB
               160° to 200°C for SDS-2787
               160° to 200°C for methyl SDS-3701
               210° to 230°C for methyl SDS-46851
               150° to 200°C for PCBN

Column 2: 130° to 200°C for PCBN
          180° to 220°C for methyl SDS-46851
          170° to 190°C for SDS-2787, methyl SDS-3701, HCB

Column 3: 200° to 250°C for methyl SDS-46851

Column 4: 200° to 250°C for methyl SDS-46851

Injection Port Temperature: 220°C to 340°C

Detector Temperature: 300°C to 350°C

Carrier Gas: High purity N₂, 30 ml/min to 50 ml/min or
          Ultra high purity 10% methane in argon, 30-40 ml/min.

Hobart Food Cutter, Model 8181-D or other similar stainless steel food chopper

MNNG - Diazomethane Kit with O-ring joint (available from Aldrich Chemical Company, Cat. No. Z10,100-1)
Micropipettor, Series H, 10 ul, Labindustries, Inc., Cat. No. MP-10H

Mill, general purpose, Fisher Scientific 08-415 or equivalent

Needle, 22g B-D No. 5156, or equivalent

pH Meter equipped with standard combination electrode

Standard laboratory glassware: beakers, bottles, flasks, separatory funnels, chromatographic columns, etc.

Syringe, 1 cc Tuberculin B-D No. 5602, or equivalent

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

PREPARATION OF STANDARD SOLUTIONS

Chlorothalonil, HCB and PCBN

A standard solution of chlorothalonil is prepared by weighing 0.1 g chlorothalonil to the nearest 0.1 mg into a tared weighing pan. The chlorothalonil is quantitatively transferred to a 100 ml volumetric flask using toluene. The contents of the volumetric flask is diluted to volume with toluene to produce a stock solution of 1000 ug chlorothalonil per ml. This stock solution is serially diluted with toluene to result in a "working standard" of 0.08 to 0.10 ug per ml for the quantitation of chlorothalonil. Separate 1000 ug/ml stock and "working standard" solutions of HCB (0.03 ug per ml) and PCBN (0.05 ug per ml) are prepared in a similar manner as described for chlorothalonil. Additional appropriate dilutions are done as necessary for quantitation.

SDS-3701

A standard solution of SDS-3701 is prepared by weighing 0.1 g SDS-3701 to the nearest 0.1 mg into a tared weighing pan. The SDS-3701 is quantitatively transferred to a 100 ml volumetric flask using acetone.
The contents of the volumetric flask is diluted to volume with acetone to produce a stock solution of 1000 ug SDS-3701 per ml. This stock solution is serially diluted with acetone to final concentrations of 100, 10 and 1 ug per ml. One drop of concentrated hydrochloric acid is added to all solutions. Concentrations of standard solutions between 1000 and 1 ug SDS-3701 per ml are utilized to amend recovery samples.

A one ml aliquot of the 10 ug per ml solution is transferred by pipet to a 125 ml Erlenmeyer flask which has been rinsed with acid water (pH <2), deionized water, acetone and allowed to dry prior to use. The solvent is evaporated and SDS-3701 is derivatized to the methyl ether as described under DERIVATIZATION OF SDS-3701 AND SDS-46851 WITH DIAZOMETHANE. A 100 ml portion of toluene is added to result in a concentration of 0.1 ug SDS-3701 per ml. The methylated standard solution and appropriate dilutions are utilized for quantitation by electron capture gas chromatography.

SDS-46851

A standard solution of SDS-46851 is prepared at a concentration of 1000 ug/ml in methanol as described for SDS-3701. This stock solution is serially diluted with methanol to final concentrations of 100, 10 and 1 ug each per ml. These standard solutions are utilized to amend recovery samples.

A 0.8 ml to one ml aliquot of the 10 ug/ml solution is transferred by pipet to an acid washed 125 ml Erlenmeyer flask. The solvent is carefully evaporated to dryness and the residue is methylated as described under DERIVATIZATION . . . The methylated standard is dissolved in 100 ml of toluene (0.08 to 0.10 ug/ml) and utilized for quantitation by electron capture gas chromatography or diluted to a suitable "working standard" concentration. Additional appropriate dilutions are done as necessary for quantitation.
PREPARATION AND EXTRACTION OF SAMPLES

A. Cherries, Strawberries, Caneberries and Cranberries

From each field sample, duplicate subsamples, each consisting of approximately 100-200 gm of whole fruit are selected and placed into a tared beaker. The weight of the fruit is measured and recorded. If the fruit has been received frozen, the fruit is allowed to come to room temperature before proceeding to the extraction. If fresh fruit are received and are to be frozen, it is recommended that individual 100-200 gm subsamples be made and placed in individual plastic bags prior to placing in frozen storage.

The fruit in the beaker is quantitatively transferred to a blender cup using an extraction solvent consisting of 380 ml acetone and 20 ml 10N $\text{H}_2\text{SO}_4$ at a minimum ratio of 3 ml per gram of subsample. The fruit and extraction solvent are blended for two minutes. The sample is then processed as described under FILTRATION OF SAMPLE EXTRACT.

B. Asparagus, Mushrooms and Pigeon Peas

Each received field sample is chopped and thoroughly mixed using a Hobart Food Cutter or similar stainless steel food chopper. If the sample was frozen prior to chopping, it is to be chopped and mixed in a semi-frozen state using a Hobart food chopper. From each field sample, duplicate subsamples, each consisting of approximately 100 gm of chopped material, are selected and placed in a tared beaker or blender cup. If the plant material has been received frozen, the chopped material is allowed to come to room temperature before proceeding to the extraction.

The material in the container is quantitatively transferred to a blender cup using an extraction solvent consisting of 380 ml acetone and 20 ml 10N $\text{H}_2\text{SO}_4$ at a minimum ratio of 3 ml per gram of subsample. The sample and extraction solvent are blended for two minutes. The sample is then processed as described under FILTRATION OF SAMPLE EXTRACT.
C. Peaches

From each field sample, duplicate subsamples, each consisting of 3 to 4 whole peaches are selected and placed into a tared appropriately sized beaker. The weight of the fruit is measured and recorded. If the fruit has been received frozen, the fruit is allowed to come to room temperature before removal of the pits. The pits are removed and the de-pitted fruit weight recorded. The analytical procedure, aliquot sizes and initial assay results are determined on the fruit weight with the pits removed for consistency in the procedure. After the analyses are completed, the analytical results are adjusted to represent the fruit with pits which is the raw agricultural commodity.

The pitted fruit in the beaker is quantitatively transferred to a blender cup using an extraction solvent consisting of 380 ml acetone and 20 ml 10N H₂SO₄ at a minimum ratio of 3 ml per gram of sample. The fruit and extraction solvent are blended for two minutes. The sample is now processed as described under FILTRATION OF SAMPLE EXTRACT.

E. Dry Beans

Each field sample of dry beans is ground with dry ice using a general purpose grinding mill. The dry ice is allowed to sublime off overnight in the freezer.

DRY BEANS - EXTRACTION/PARTITIONING OF RESIDUES OF CHLOROTHALONIL, HCB AND PCB

Duplicate 50 g portions of the resulting powder after the dry ice has sublimed is blended with acetonitrile in a minimum ratio of 5 ml per gram of subsample for approximately two minutes.
The resulting homogenate is quantitatively transferred using acetonitrile to qualitative filter paper (Whatman No. 4) supported on a Buchner funnel and vacuum filtered. The resulting pad is rinsed with acetonitrile. The filtrate is brought to an appropriate known and recorded volume using acetonitrile. The total extract is thoroughly mixed. A portion of the filtrate equivalent to approximately 10 grams of the subsample is transferred to an appropriately sized separatory funnel. The acetonitrile extract is diluted with water (five times the volume of the acetonitrile). To the aqueous acetonitrile are added 5 ml of 10N H₂SO₄ and 50 ml petroleum ether. The separatory funnel is vigorously shaken manually for two minutes, the phases are allowed to separate and the lower aqueous phase is drained into an appropriately sized beaker. The petroleum ether is poured from the top of the separatory funnel to an appropriately sized acetone rinsed Erlenmeyer flask or 4 oz. disposable glass bottle taking care not to transfer any water with the ether. The aqueous phase is quantitatively transferred to the separatory funnel and the partitioning is repeated using an additional 50 ml portion of petroleum ether.

The petroleum ether extracts are combined and 0.4 ml of 2% paraffin oil in petroleum ether is added. The ether is concentrated in the water bath using a stream of clean, dry air impinging on the solvent surface to approximately 2 ml. The remaining solvent is evaporated to dryness using a gentle stream of clean, dry air only. **CAUTION:** The use of the dry air stream after the solvent has evaporated can result in low recovery of chlorothalonil, HCB and PCBN. The residue is dissolved in 10 ml of Eluant A and reserved for cleanup and residue separation by column chromatography.

**DRY BEANS – EXTRACTION OF RESIDUES OF SDS-3701 AND SDS-46851**

Separate duplicate 50 g portions of the ground sample is transferred to a blender cup using an extraction solvent consisting of 380 ml acetone and 20 ml 10N H₂SO₄ at a minimum ratio of 5 ml per gram of subsample. The subsample and solvent is blended for two minutes.
The resulting homogenate is quantitatively transferred using acetone to qualitative filter paper (Whatman No. 4) supported on a Buchner funnel and vacuum filtered. The resulting pad is rinsed with acetone. The filtrate is brought to an appropriate recorded volume using acetone. The total extract is thoroughly mixed. A measured and recorded portion of the filtrate equivalent to approximately 10 g of the subsample is transferred to an appropriately sized beaker, and a 10 ml portion of water is added to the filtrate. The filtrate is 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 filtrate to stand overnight in an operating fume hood. Proceed to PARTITIONING SYSTEM II for only SDS-3701 and SDS-46851. The separation of chlorothalonil, HCB and PCBN have already been accomplished; therefore, Partitioning System II is utilized only for the isolation and separation of SDS-3701 and SDS-46851.

**Filtration of Sample Extract**

The resulting homogenate is quantitatively transferred using acetone to qualitative filter paper (Whatman No. 4) supported on a Buchner funnel and vacuum filtered. The resulting pad is rinsed with acetone. The filtrate is brought to an appropriate recorded volume using acetone. The total extract is thoroughly mixed.

 Appropriately sized aliquots may be processed through PARTITIONING SYSTEM I or PARTITIONING SYSTEM II. The systems are described in the following ANALYSIS FLOW DIAGRAMS:
ANALYSIS FLOW DIAGRAM

PARTITIONING SYSTEM I

Sample
XT acid acetone

Sample Extract
pH 4.5, XT pet ether -------- <pH 2, + 30% NACL XT ethyl ether

SDS-2787, HCB
PCBN Extract

SDS-3701, SDS-46851 Extract
methylate

-------------
Florisil col.
elute Eluant A-Discard
elute Eluant C

SDS-2787 Fraction

-------------
Florisil col.
elute Eluant A
elute Eluant D--

Methyl SDS-3701, SDS-46851 Extract

-------------
HCB Fraction
PCBN Fraction

-------------
Alumina col.
elute DCM

-------------
Methyl SDS-3701
Fraction

-------------
elute Eluant F --------

-------------
Methyl SDS-46851
Fraction
ANALYSIS FLOW DIAGRAM
PARTITIONING SYSTEM II

XT Acid Acetone
↓
Direct Partition
↓
XT Pet Ether
↓
SDS-2787
HCB
PCBN
↓
Selective Partition
↓
pH 4.5
XT Pet Ether (Discard)
pH <2, 30% NaCl
XT Ethyl Ether
↓
Florisil Col
Eluant A-discard
Eluant C
↓
SDS-2787
HCB
PCBN
↓
SDS-3701, SDS-46851
↓
Methylate
Methyl SDS-3701, Methyl SDS-46851
↓
Alumina Col.
Eluant DCM
Eluant F
↓
Methyl SDS-3701
Methyl SDS-46851

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PARTITIONING SYSTEM I

A measured and recorded portion of the filtrate equivalent to approximately 10 gm of the subsample is transferred to an appropriately sized beaker for selective partitioning, 5 ml of "Keeper" solution, and a 10 ml portion of water is added to the filtrate.

Chlorothalonil, HCB and PCBN - System I

After the acetone has evaporated, a 60-100 ml portion of 0.4M NaHCO₃ is added to the beaker. The pH of the contents of the beaker is adjusted to 4.5 with the aid of a pH meter using either 0.4M NaHCO₃ or 10N H₂SO₄. The aqueous solution at pH 4.5 is quantitatively transferred to an appropriately sized separatory funnel using 50 ml petroleum ether. The separatory funnel is vigorously shaken manually for two minutes, the phases are allowed to separate, and the lower aqueous phase is drained into the beaker. The ether phase is poured from the top of the separatory funnel into an appropriately sized acetone rinsed 4 oz. bottle or Erlenmeyer flask. Care must be taken so that no water is transferred with the ether. The aqueous phase is quantitatively transferred to the separatory funnel using an additional 50 ml portion of petroleum ether. The partitioning is repeated as previously described. The petroleum ether extracts are combined and 0.4 ml of 2% paraffin oil in petroleum ether is added. The ether is concentrated in the water bath to approximately 2 ml. The remaining solvent is evaporated to dryness using a gentle stream of clean, dry air only. CAUTION: The use of the dry air stream after the solvent has evaporated can result in low recovery of chlorothalonil, HCB and PCBN. The residue is dissolved and quantitatively transferred to a culture tube using a total of 10 ml Eluant A. The culture tube should be previously rinsed with Eluant A and air dried. The culture tube is sealed using a Teflon® lined screw cap, appropriately labeled with at least the laboratory sample reference number and reserved for cleanup and residue separation by column chromatography.
SDS-3701, SDS-46851 - System I

After the second partitioning, the pH of the aqueous phase containing SDS-3701 and SDS-46851 is adjusted to less than 2 by the addition of 5 ml of 10N H₂SO₄. Sufficient NaCl is added to the aqueous solution to obtain a 30% solution (v/v); this is mixed thoroughly using a magnetic stirrer. The resulting solution is quantitatively transferred to the separatory funnel using approximately 10 ml water and 50 ml diethyl ether. The separatory funnel is vigorously shaken manually for two minutes, the phases allowed to separate and the lower aqueous phase drained into the beaker. The diethyl ether phase is poured from the top of the separatory funnel into an appropriately sized Erlenmeyer flask or 4 oz. disposable bottle which has been rinsed with acid water (pH <2), deionized water, acetone and allowed to dry before use. Care must be taken so that no water is transferred with the diethyl ether. The partitioning is repeated. Both ether extracts are combined, concentrated and evaporated as previously described. The residue containing SDS-3701 and SDS-46851 is reserved for derivatization using either of the procedures described below.

PARTITIONING SYSTEM II

A measured and recorded portion of the filtrate equivalent to approximately 10 g of the subsample is transferred to an appropriately sized beaker for selective partitioning, and a 10 ml portion of water is added to the filtrate. The filtrate is 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 filtrate to stand overnight in an operating fume hood. In addition, an additional aliquot of the filtrate equivalent to approximately 10 g of the subsample is transferred to a second beaker for direct partitioning.
Chlorothalonil, HCB and PCBN - System II

The acid acetone solution is quantitatively transferred to an appropriately sized separatory funnel using 50 ml of petroleum ether and 10 ml of H₂O per 25 ml acetone extract. The separatory funnel is vigorously shaken manually for two minutes, the phases are allowed to separate, and the lower aqueous phase will be drained into the beaker. The ether phase is poured from the top of the separatory funnel into an appropriately sized acetone rinsed Erlenmeyer flask or 4 oz. disposable glass bottle. Care must be taken so that no water is transferred with the ether. The aqueous phase is quantitatively transferred to the separatory funnel using an additional 50 ml portion of petroleum ether. The partitioning is repeated as previously described. The petroleum ether extracts are combined and 0.4 ml of 2% paraffin oil in petroleum ether is added. The ether is concentrated in the water bath to approximately 2 ml. The remaining solvent is evaporated to dryness using a gentle stream of clean, dry air only. **CAUTION:** The use of the dry air stream after the solvent has evaporated can result in low recovery of chlorothalonil, HCB and PCBN. The residue is dissolved in 10 ml of Eluant A and reserved for cleanup and residue separation by column chromatography.

Selective - SDS-3701 and SDS-46851 - System II

After the acetone has evaporated, a 60-100 ml portion of 0.4M NaHCO₃ is added to the beaker. The pH of the contents of the beaker is adjusted to 4.5 with the aid of pH meter using either 0.4M NaHCO₃ or 10N H₂SO₄. The aqueous solution at pH 4.5 is quantitatively transferred to an appropriately sized separatory funnel using 50 ml petroleum ether. The separatory funnel is vigorously shaken manually for two minutes, the phases are allowed to separate, and the lower aqueous phase is drained into the beaker.
The ether phase is poured from the top of the separatory funnel and discarded. The aqueous phase is quantitatively transferred to the separatory funnel using an additional 50 ml portion of petroleum ether. The partitioning is repeated as previously described. The petroleum ether extracts are discarded.

The pH of the aqueous phase containing SDS-3701 and SDS-46851 is adjusted to less than 2 by the addition of 5 ml of 10N H₂SO₄. Sufficient NaCl is added to the aqueous solution to obtain a 30% solution (w/v); this is mixed thoroughly using a magnetic stirrer. The resulting solution is quantitatively transferred to the separatory funnel using approximately 10 ml water and 50 ml diethyl ether. The separatory funnel is vigorously shaken manually for two minutes, the phases is allowed to separate and the lower aqueous phase is drained into the beaker. The diethyl ether phase is poured from the top of the separatory funnel into an appropriately sized Erlenmeyer flask or 4 oz. disposable glass bottle, which has been rinsed with acid water (pH <2), deionized water, acetone and allowed to dry before use. Care must be taken so that no water is transferred with the diethyl ether. The partitioning is repeated. Both ether extracts are combined, concentrated and evaporated as previously described.

The flask or bottle containing metabolite residues is reserved for derivatization using either of the procedures described below:

DERIVATIZATION OF SDS-3701 AND SDS-46851 WITH DIAZOMETHANE

CAUTION: EXTREME CARE IS 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) IS WORN BY LAB PERSONNEL HANDLING THE REAGENTS AND NO SCRATCHED, CHIPPED OR GLASS-STOPPERED GLASSWARE IS USED. ALL WORK IS PERFORMED IN A FUME HOOD BEHIND A SAFETY SHIELD.
Preparation of Diazomethane Solution

The diazomethane solution is prepared just prior to use by means of the MNNG-Diazomethane Kit with O-ring joint. A minimum 0.8 g portion of the precursor (N-methyl-N'-nitro-N-nitrosoguanidine) is placed in the inside tube through its screw cap opening along with 0.5 ml of water. Diethyl ether (approximately 20 ml) is placed in the outside tube and the two parts are assembled with an O-ring and held with a pinch-type clamp. The lower part is immersed in an ice bath and approximately 2.5 ml of 4N NaOH is injected through the silicone rubber system via a syringe. The addition is done dropwise to prevent the mixture from getting too hot and to control the volume of gas produced. The diazomethane is collected in the ether ready for use after all evidence of gas production has ceased. Any unused reagent is neutralized/destroyed by adding excess glacial acetic acid. Alternatively, diazomethane may be generated by other standard techniques. If so, the method of preparation should be detailed in the final report.

Derivatization

The SDS-3701 is converted to its methyl ether derivative and SDS-46851 to its 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 flask after evaporation. The reaction mixture is allowed to set in an operating fume hood in an ice bath or at ambient temperature for 1 hour, after which time the solvent is evaporated using a gentle stream of dry air. CAUTION: The use of the dry air stream after the solvent has evaporated can result in low recovery of methylated SDS-3701 and SDS-46851. The residue is dissolved using 10 ml of methylene chloride and reserved for separation and cleanup by column chromatography prior to quantitation by gas chromatography. Any unused reagent is destroyed by adding excess glacial acetic acid.
The SDS-3701 is converted to its methyl ether derivative and SDS-46851 to its methyl ester derivative by the addition of 10 µl of 1:3 concentrated HCl:methanol (v/v) and 5 ml of the methylating reagent to the dried residue remaining in the flask after evaporation. The reaction mixture is allowed to set in an operating fume hood at ambient temperature for 30 minutes after which time the solvent is evaporated using a gentle stream of dry air. CAUTION: The use of the dry air stream after the solvent has evaporated can result in low recovery of methylated SDS-3701 and SDS-46851. To insure complete derivatization, an additional 3 ml portion of the methylating reagent is added and the mixture allowed to set for 10 minutes. The solvent is evaporated using a gentle stream of clean, dry air. The residue is dissolved using 10 ml of methylene chloride and reserved for separation and cleanup by column chromatography prior to quantitation by gas chromatography.

SAMPLE CLEANUP OF SDS-3701 AND SDS-46851

A glass chromatographic column, 200 mm x 9 mm, is packed 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 is washed with 10 ml of methylene chloride. After the methylene chloride has entered the column, a 50 ml Erlenmeyer flask (acetone rinsed) or 2 oz. disposable glass bottle is placed under the column. The sample (2 gram equivalent) in methylene chloride is quantitatively transferred to the column. After the sample has moved into the column bed, the derivatized SDS-3701 is eluted from the column with 40 ml methylene chloride. After the methylene chloride eluant has moved into the column, a 50 ml Erlenmeyer flask or 2 oz. disposable glass bottle is placed under the column and derivatized SDS-46851 is eluted from the column with 40 ml Eluant F.
SAMPLE CLEANUP AND SEPARATION OF CHLOROTHALONIL, HCB AND PCBN

Column Preparation

A column is packed bottom to top with a 0.5 cm bed of glass wool, 2 g of Florisil and 1 cm of anhydrous sodium sulfate. The column is packed using Florisil directly from the oven and vibrated to settle and compact the Florisil. The Florisil in the column is immediately covered with the anhydrous sodium sulfate and washed with 10 ml hexane. After the hexane enters the sodium sulfate layer, a 50 ml Erlenmeyer flask is placed under the column.

Elution of Chlorothalonil

A 2 g equivalent portion of the sample extract, dissolved in Eluant A, is transferred to the prepared column. After the sample extract migrates into the sodium sulfate layer, a 23 ml portion of Eluant A is added to the column. A total of 25 ml of Eluant A is collected and discarded. A 50 ml Erlenmeyer flask or 2 oz. disposable glass bottle is placed under the column. Chlorothalonil is 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, is transferred to a separately prepared column. After the sample extract migrates into the sodium sulfate layer, HCB is eluted from the column with 23 ml of Eluant A into a 50 ml Erlenmeyer flask or 2 oz. disposable glass bottle. A total of 25 ml of Eluant A is collected. A 125 ml Erlenmeyer flask or 4 oz. disposable glass bottle is placed under the column. The PCBN is eluted from the column with 75 ml Eluant D.
PREPARATION FOR QUANTITATION

A 0.4 ml portion of 2% paraffin oil in petroleum ether is added to each respective eluant. The flask or bottle is placed in the water bath and the solvent is concentrated to approximately 0.5 ml using a gentle stream of dry air directed on the surface of the solvent to aid evaporation. The flask or bottle is removed from the water bath and the contents are evaporated to dryness using a stream of dry air. The residue is dissolved in an appropriate volume of toluene for quantification of chlorothalonil, methyl SDS-3701, methyl SDS-46851, HCB or PCBN by gas chromatography.

QUANTITATION

Portions of the sample extract (approximately 1 ml) are sealed in separate vials designed for use with the autosampler. This includes all samples, the "working standard", and other standards in the concentration range of interest.

The sample weight, sample volume, concentration of the "working standard" and retention time of the compound of interest are entered into the system. Using these parameters, the instrument reduces the data to ppm values (ug of chlorothalonil, SDS-3701, SDS-46851, HCB or PCBN per gram of sample) by external standard calibration using the following calculations:

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

\[
\text{Assay Weight} = \frac{\text{Sample Weight x Aliquot x Dilution to Cleanup}}{\text{Extraction Volume}}
\]

\[
\text{ppm residue} = \frac{\text{Calibration Factor x Vol. for G.C. x Sample Peak Area (or Peak Height)}}{\text{Assay Weight x 10,000}}
\]
A series of varying concentration standards is prepared from the "working standard" to serve as linearity checks. The non-detect level is established by the lowest concentration standard analyzed.

If necessary, quantitation may be done by linear regression of the series of standards (concentration vs. peak area or height).

\[ \text{ppm residue} = \frac{[(\text{Sample Peak Area or Peak Height} \times \text{Slope}) + \text{Intercept}] \times \text{Vol for G.C.}}{\text{Assay Weight}} \]

The normally used range of standard concentrations are from 0.01 μg/ml to 0.10 μg/ml for chlorothalonil, SDS-3701 and SDS-46851. The normally used range of standard concentrations for HCB is from 0.003 μg/ml to 0.03 μg/ml and for PCBN 0.005 μg/ml to 0.05 μg/ml. Instrumental conditions are usually adjusted so that the lowest standard provides a minimum of 4% full scale deflection.

VALIDATION OF THE ANALYTICAL PROCEDURE

Untreated crops are amended by the addition of separate standard solutions of chlorothalonil, SDS-3701, SDS-46851, HCB and PCBN to the crop in the extraction vessel prior to the addition of the extraction solvent at the start of the analytical procedure. The samples are amended at a minimum of three concentrations covering the range of anticipated residue and assayed prior to the initiation of the study to validate the analytical procedure on that particular crop. A method is considered valid if the analyst has demonstrated adequate recovery of chlorothalonil from the matrix to be analyzed. Adequate recovery is defined as an average of 70% recovery (with a minimum of 60% for any single recovery) of chlorothalonil, SDS-3701, SDS-46851, HCB and PCBN from untreated (control) samples amended at least 3 concentration levels. The control samples are amended at levels which might be reasonable expected to occur in the treated test sample. The lowest level of amendment is at a level two to three times the limit of detection for
each crop. The limit of detection required is determined for each study/crop by the responsible analyst and adhered to during that study. Using this methodology the levels normally utilized are itemized:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Non-Detection Limit</th>
<th>Lowest Recovery Fortification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorothalonil</td>
<td>&lt;0.01 ppm</td>
<td>0.02 to 0.03 ppm</td>
</tr>
<tr>
<td>SDS-3701</td>
<td>&lt;0.01 ppm</td>
<td>0.02 to 0.03 ppm</td>
</tr>
<tr>
<td>SDS-46851</td>
<td>&lt;0.03 ppm</td>
<td>0.06 to 0.10 ppm</td>
</tr>
<tr>
<td>HCB</td>
<td>&lt;0.003 ppm</td>
<td>0.006 to 0.10 ppm</td>
</tr>
<tr>
<td>PCBN</td>
<td>&lt;0.005 ppm</td>
<td>0.01 to 0.015 ppm</td>
</tr>
</tbody>
</table>

The analytical procedure is validated prior to initiation of sample analysis. The recoveries of chlorothalonil, SDS-3701, SDS-46851, HCB and PCBN are determined for each set of samples analyzed.
July 17, 1995

To: Directors of studies with chlorothalonil test substances
RE: Analytical interference potential

The technical staff at Ricerca have conducted preliminary assessments of the potential for analytical interferences between chlorothalonil and several other pesticides which may possibly be used in tank mix with chlorothalonil products which are intended to be treated with the test substance spray preparations. The results of this preliminary assessment are on the attached spreadsheet.

Note the potential interferences of chlorothalonil with Thiodan (endosulfan) and methyl parathion in typical gas chromatographic column packings. Therefore, please do not use either endosulfan or methyl parathion products in studies involving chlorothalonil products as test substances. Please advise each individual involved with the study of this restriction. Please call me if you have any question about this request, at phone number 216-357-4146.

Sincerely,

[Signature]
Dr. John R. French
Manager, Technical Development

Post-it™ Brand Fax Transmittal Memo 767

To: Dave Thompson
From: J.R. French
Co: IR-4 HQ
Co: ISK Biosciences
Dept: Phone #
Fax #: 908-932-8481
ANALYSIS OF PESTICIDES TO DETERMINE POSSIBLE INTERFERENCE WITH CHLOROTHALONIL ANALYSIS

<table>
<thead>
<tr>
<th>PESTICIDE</th>
<th>COLUMN PACKINGS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OV-7-3%</td>
</tr>
<tr>
<td>Guthion</td>
<td></td>
</tr>
<tr>
<td>Sevin</td>
<td></td>
</tr>
<tr>
<td>Thiodan</td>
<td>x</td>
</tr>
<tr>
<td>Benlate</td>
<td></td>
</tr>
<tr>
<td>Ridomil</td>
<td></td>
</tr>
<tr>
<td>Furdan</td>
<td></td>
</tr>
<tr>
<td>Methyl Parathion</td>
<td>x</td>
</tr>
<tr>
<td>Asana XL</td>
<td></td>
</tr>
<tr>
<td>Diazinon</td>
<td></td>
</tr>
<tr>
<td>Poast</td>
<td></td>
</tr>
<tr>
<td>Penncozeb</td>
<td></td>
</tr>
<tr>
<td>Super Tin</td>
<td></td>
</tr>
</tbody>
</table>

x = would cause problems with chlorothalonil analysis.