Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Dicofol

MRID #: 413818-01

Matrix: Soil

Analysis: GC/ECD

This method is provided to you by the Environmental Protection Agency's (EPA) Environmental Chemistry Laboratory (ECL). This method *is not* an EPA method but one which was submitted to EPA by the pesticide manufacturer to support product registration. EPA recognizes that the methods may be of some utility to state, tribal, and local authorities, but makes no claim of validity by posting these methods. Although the Agency reviews *all* Environmental Chemistry Methods submitted in support of pesticide registration, the ECL evaluates only about 30% of the currently available methods. Most methods perform satisfactorily but some, particularly the older methods, have deficiencies. Moreover, the print quality of the methods varies considerably because the methods originate from different sources. Therefore, the methods offered represent the best available copies.

If you have difficulties in downloading the method, or further questions concerning the methods, you may contact Elizabeth Flynt at 228-688-2410 or via e-mail at flynt.elizabeth@epa.gov.

"This is an exact copy of
The original document"

By R.F.Kennedy date 10-16-89

HAAS COMPANY PHILADELPHIA

APPENDIX I

METHOD OF ANALYSIS

"This is an exact copy of
The original document"
By R.E. Kennedy date 10.16.89

34-89-28

Table of Contents

000227

ISK BIOSCIENCES CORPORATION COMPANY PHILADELPHIA

ABC LABS #3b9342

"This is an exact copy of
The original document"
By R.F. Kennedy date 10.16.89

List of Tables

ROHM AND HAAS COMPANY PHILADELPHIA

34-89-28 "This is an exact copy of The original document"

000229

1.0 Scope

By R.F. Kennedy date 10-16-89

This method is used for the extraction, clean-up, derivatization, and final determination of dicofol and dicofol metabolite residues in a California sandy loam soil. The limit of detection is 0.01 ppm or 0.03 ppm, depending upon the component. Chemical structures for o,p'- and p,p'-dicofol, o,p'- and p,p'-FW152, o,p'- and p,p'-DCBP, 3-OH-p,p'-DCBP, o,p'-DCBH, p-CBA, and o-CBA are shown in Figure 1.

2.0 Principle

Dicofol and its metabolites are extracted from soil with acidic methanol. Sample cleanup is performed via liquid-liquid-partition using deionized water and methylene chloride followed by gel-permeation chromatography. Three compounds (p-CBA, o-CBA, and 3-OH-p,p'-DCBP) are derivatized (methylated) and analyzed via GC. The remaining seven compounds are analyzed via GC directly.

3.0 Materials

3.1 Equipment (where brand names are listed, equivalent items may be used)

HDPE bottles and caps, Nalgene, 8-oz.
Shaker, Eberbach
Funnels, Buchner, 12.5 cm
Filter paper, Whatman GF/A, 12.5 cm
Separatory funnels, 1000-ml
Flasks, flat-bottom, 500-ml
Funnels, glass powder
Cotton balls
Rotary evaporator
GPC Autoprep Model 1001, ABC Laboratories, Columbia, Mo. 65202
Pipets, Class A, various volumes
Syringes, Glass luer-lock, 10 cc
Pipets, disposable 9-inch Pasteur
Centrifuge, IEC Clinical
Vortex - Thermolyne®
Thermometer, VWR (-10 to 260°C)
Syringes, Hamilton (various volumes)
pH Meter Corning, Model 140
Diazald® Kit, Aldrich Diazomethane Generator, Catalog Number 210,025-0
Pipets, disposable: 1-, 5-, and 10-ml
Culture tubes and caps, 16 X 125 mm, 15-mm caps
Culture tubes and caps, 13 X 100 mm, 13-mm caps
Muffle furnace, Lab-Heat, Blue M

34-89-28

Dual Magnetic Stirrer/Heater, VWR Dyla-Dual
Cylinder graduated, 500-ml
Tippetor, 50-ml
Tippetor, 100-ml
Flask, Erlenmeyer, 2000-ml
Flask, Erlenmeyer, 4000 ml
Separatory funnel, 2000-ml
Mixing jug, glass, 5 gallon
Buffer, pH 4, VWR
Buffer, pH 7, VWR
Balance, Mettler PM200
Balance, Sartorius 1702
Spatulas, measuring
Cylinder, graduated, 1000-ml
Cylinder, graduated, 50-ml
Volumetrics and caps, Class A, 25, 50, 100 and 500-ml
Ultrasonic Cleaner, Branson, B-220

000230

"This is an exact copy of
The original document"

By R.F. Kennedy date 10/16/89

3.2 Reagents

2,2,4 - Trimethylpentane, reagent grade
Acetone, reagent grade
Ethyl Ether, Burdick and Jackson
Methylene Chloride, Burdick and Jackson
Anhydrous Alcohol, Phototrex, reagent grade
Cyclohexane, Burdick and Jackson
Methanol, reagent grade
Diazald®, Aldrich

3.3 Inorganics

Potassium Hydroxide, pellets
Sodium Sulfate, granular, 12-60 mesh
(Place into a muffle furnace and ignite for 6 to 8 hours at 600°C)
6N HCl
Hydrochloric acid, concentrated reagent, is diluted 1:1 (v/v) with deionized water.

3.4 Solutions

3.4.1 Diazomethane

The method used for diazomethane generation is derived from the Official Methods of Analysis of the AOAC, Section 41.015C, page 779, 14th Ed., 1984. Place 35 ml anhydrous alcohol, 10 ml ethyl ether, 6 g KOH and 10 ml deionized H_2O

ABC LABS#36934Z

"This is an exact copy of
The original document"
By R.F. Kennedy date 10-16-89

into a 250-ml round-bottom flask obtained from the Diazald® kit. Place a magnetic stirring bar into the flask to ensure proper mixing. In the 125-ml separatory funnel from the kit, fitted on one arm of a Claisen adapter, dissolve 21.5 g of Diazald® with a total of 140 ml ethyl ether. Using a thermometer to keep the water bath temperature between 50-60°C, slowly add the Diazald® mixture to the reaction flask (submerged in the water bath) while the generated diazomethane and ethyl ether distill through a distillation apparatus fitted on the other neck of the Claisen adapter. To achieve a closed system, place a flask containing ethyl ether at the gas outlet of the vacuum distilling adapter. Store the generated diazomethane in a non-ground glass container in a freezer.

Note: Diazomethane is very reactive; read all cautionary material before use.

3.4.2 Washed Water, pH=2

A 5-gallon mixing jug is filled with deionized H_2O. A Corning pH probe is calibrated using VWR buffer solutions and is placed into the mixing jug. Acid solution (6N HCl) is added until the pH meter reads 2.0.

Approximately 1600 ml of the pH-adjusted H_2O is placed into a 2000-ml separatory funnel along with 200 ml of methylene chloride. The mixture is shaken and the phases allowed to separate. The lower methylene chloride layer is discarded and the H_2O phase is placed into storage containers.

3.4.3 50% (v/v) Methylene Chloride/Cyclohexane

Mix exactly 2000 ml of methylene chloride and 2000 ml of cyclohexane.

3.4.4 2,2,4-Trimethylpentane with 0.01% OV-101

Add 50 µl of OV-101 (neat) to 500 ml of 2,2,4-trimethylpentane in a 500-ml volumetric flask. This yields a 0.01% OV-101/2,2,4-trimethylpentane solution. The solution is shaken and then sonicated (See 6.0, Note 5).

4.0 Procedure

4.1 Preparation of Sample

Soil is homogenized by processing through a Straub #2 grist mill (or equivalent) in the presence of dry ice. The

232

"This is an exact copy of
The original document"

By R.F.Kennedy date 10.16.89

sample is placed in an upright freezer (at -20°C) until the dry ice has sublimed.

The sample is then placed in a walk-in freezer for storage at -20°C.

4.2 Extraction

Weigh a 50.0-g subsample into an 8 oz. HDPE Nalgene bottle. All fortifications are made at this time. Add 2 ml of 6N HCl and 100 ml of methanol (See 6.0, Note 1). Adjust the 100 ml of methanol to account for the volume of solvent used for fortification. Place the sample in a shaker box and shake for 30 minutes on high speed, then 30 minutes on low speed.

Vacuum filter the sample through GF/A filter paper into a 1000-ml separatory funnel. Rinse the sample and filter paper with two 50-ml methanol washes and filter into the separatory funnel.

4.3 Liquid-Liquid Partition

Add 500 ml of methylene chloride-washed deionized water (pH=2) to the sample. Partition the sample with three 100-ml portions of methylene chloride. Pass the methylene chloride layer through a powder funnel containing ignited sodium sulfate into a 500-ml flat-bottom flask. Rinse the funnel and sodium sulfate with 50 ml of methylene chloride. Evaporate the sample and bring to 15 ml with cyclohexane: methylene chloride, 50:50 (v/v) GPC solvent.

4.4 Gel-Permeation Chromatography (GPC)

Load a 5-ml aliquot of the sample onto the GPC with the following parameters:

Flow: 5 ml/minute
Mobile Phase: cyclohexane: methylene chloride, 50:50 (v/v)
Dump Time: 25 minutes
Collect Time: 29 minutes
Wash Time: 0 minutes

Collect the sample in a 250-ml flat-bottom flask. Evaporate the sample to dryness and bring to exactly 5 ml with ethyl ether.

"This is an exact copy of The original document"

000233

4.5 Underivatized Sample Analysis By R.F. Kennedy date 10/4/89

Place a 1-ml aliquot of the 5-ml ethyl ether extract into a test tube. Take to dryness under a gentle stream of nitrogen and bring to an appropriate volume with 2,2,4-trimethylpentane/0.01% OV-101; add α-chlordane to yield 20 ng/ml. Submit an aliquot for GC analysis (See 6.0, Note 7).

4.6 Derivatized Sample Analysis

Place a 1-ml aliquot of the 5-ml ethyl ether extract into a test tube. Add 10 µl of 6N HCl and 0.5 ml of diazomethane solution. Cap the sample, vortex, and derivatize undisturbed for 30 minutes.

Add 2,2,4-trimethylpentane (approximately 1 ml) to the sample as a keeper. Evaporate under a gentle stream of nitrogen to remove the diazomethane and the ethyl ether.

Note: Do not take the sample to dryness. The methylated standards are volatile (See 6.0, Note 4).

Bring the sample to an appropriate volume with 2,2,4-trimethylpentane/0.01% OV-101 and add α-chlordane to yield 20 ng/ml. Submit an aliquot for GC analysis.

Note: All ethyl ether must be removed before injection into the GC.

4.7 Capillary GC Determination of Dicofol and Dicofol Metabolites

4.7.1 General

Concentrations of o,p'-and p,p'-dicofol, o,p'- and p,p'-FWIS2, o,p'- and p,p'-DCBP, o,p'-DCBH and methyl derivatives of o- and p-CBA and 3-OH-p,p'-DCBP are determined by Capillary Gas Chromatography using EC detection and a DB-5 column (28.5m X 0.25mm, 0.25µ film thickness). GC conditions for the underivatized and derivatized compound analyses are given in Tables I and II, respectively. The programs may be used interchangeably if interferences permit.

4.7.2 Standardization

Prepare separate stock solutions containing 25 mg of compound in 25 ml of methanol. Prepare a mixed stock

SCOTTS COMPANY, PHILADELPHIA

"This is an exact copy of
The original document"
By R.F. Kennedy date 10-16-87

solution (4 mix) by combining 1 ml of the o,p'- and p,p'-DCBP and o,p'- and p,p'-FW152 1 mg/ml stock solutions and diluting to a final volume of 25 ml with methanol. Prepare a second mixed stock solution (6 mix) by combining 1 ml each of o,p'- and p,p'-dicofol, methylated o-CBA, and methylated 3-OH-p,p'-DCBP and 3 ml each of o,p'-DCBH and methylated p-CBA; dilute to a final volume of 25 ml with methanol. Low EC detector response for o,p'-DCBH and methylated p-CBA dictates the three-fold concentration increase of these compounds over the concentrations of the other compounds in the 6 mix standard solution.

Isomers of FW152 and DCBP cannot be mixed with the dicofol isomers since the latter can degrade to these metabolites upon injection into the GC.

Dilute the mixed stock solutions 1 to 40 with 2,2,4-trimethylpentane to yield 1 µg/ml GC mixed stock solutions. Make serial dilutions of the GC mixed stock solutions using 2,2,4-trimethylpentane containing 0.01% OV-101 to obtain working GC solutions in the range of 5 to 100 ng/ml (o,p-DCBH and methylated p-CBA are 15 to 300 ng/ml). During each final standard dilution, add α-chlordane (internal standard) at the rate of 20 ng/ml (See 6.0, Note 7).

Standardize the gas chromatograph under the conditions stated in Tables I or II by making 1-2 µl injections of the mixed standard solutions. Determine the peak heights or areas of the injected standards using an integrator or computer acquisition system and calculate the ratio of the compound response to the internal standard response. Enter the standardization data into an appropriate electronic calculator or computer (e.g. HP-1000) with a customized program to calculate a standard curve of standard concentration (ng/ml) versus height or area ratio. The curve may be linear or quadratic, depending on the detector response.

4.7.3 Detection of Sample Residues

Inject a 1-2 µl aliquot (equivalent to the standard volumes for that run) of the sample extract into the GC using the same conditions as for the standards. Compare the peak height or area ratio for the unknown sample to the standard curve to determine ng/ml of detected compound in the final GC solution.

Calculate the residue values in ppm using the following equation:

$$ppm = \frac{a \times b \times c \times d}{e \times f \times g}$$

where:

a = concentration in the final solution (ng/ml)
b = final GC volume (ml)
c = sample volume after GPC cleanup (ml)
d = sample volume before GPC cleanup (ml)
e = aliquot taken for derivatization or dilution (ml)
f = aliquot taken for GPC cleanup (ml)
g = sample weight (g)

4.7.4 Correction of Residue Values for On-Column Degradation

Should the breakdown of the dicofol to DCBP or FW152 in the GC be significant (response greater than the lowest injection standard), then sample residues are corrected for this breakdown. Using the calculated standard concentration from the standard curves of dicofol and the metabolite produced, calculate a ratio between the metabolite and its corresponding dicofol parent (e.g. o,p'-DCBP and o,p'-dicofol). Generally, standards from the upper portion of the curve (60 ng/ml and higher) should be used to determine the ratio. Less concentrated standards tend to give variable degradation ratio values. The decision as to which standards will give reliable degradation ratio values is left to the judgement of the analyst. The average ratio of the metabolite to the parent is multiplied by the parent residue value in each sample, and the resulting residue value is then subtracted from the metabolite residue value. An example calculation follows:

Detected residue values: 0.115 ppm p,p'-DCBP and
1.25 ppm p,p'-dicofol

Average metabolite-to-parent standard ratio = 0.075

Corrected residue value for p,p'-DCBP = 0.115 ppm - (1.25 X 0.075) = 0.021 ppm

On-column degradation is deemed unacceptable if the average metabolite-to-parent standard ratio is over 0.20. If this occurs, steps will be taken to reduce the breakdown, and the sample set will be reinjected.

"This is an exact copy of The original document"
By R.F. Kennedy date 10.16.89

ABC LABS COMPANY - PHILADELPHIA, PA

34-89-28

000236

"This is an exact copy of The original document" By R.F. Kennedy date 10/16/89

4.7.5 GC Glass Insert

To minimize the breakdown of the dicofols to their metabolites upon injection into the GC, the glass insert in the injector is treated with phosphoric acid and OV-101 in the following manner:

Boil the insert (Varian #03-94943-7-00) in 4.4N phosphoric acid for 1 hour. Remove the insert from the acid and dry in an oven at ~120°C. Do not rinse; blot dry only. Thoroughly coat the inside and outside of the insert with the liquid OV-101 using a pipe cleaner. Also, coat any metal parts of the injector that may come in contact with the sample. Place the insert into the injector and raise the temperature slowly (5°C/min) to 240°C. Purge the injector with helium (column disconnected) and condition the insert overnight. Reconnect the column and begin injections (See 6.0, Notes 5 and 6).

5.0 Method Validation Results

This method was validated by analysis of control soil fortified in triplicate at four levels plus controls and reagent blanks.

Fortification levels were 0.01 ppm, 0.10 ppm, 1.0 ppm and 10.0 ppm for o-CBA, o,p'-DCBP, p,p'-DCBP, 3-OH-p,p'-DCBP, o,p'-FW152, p,p'-FW152, o,p'-dicofol, and p,p'-dicofol. For p-CBA and o,p'-DCBH, the fortification levels were 0.03 ppm, 0.30 ppm, 3.0 ppm and 30 ppm. The results are presented in Table III of this method. Statistical analysis (ANOVA) of these data confirmed significant recovery differences between compounds ($p<0.01$) and levels ($p<0.05$). Compound X level interactions were absent. Thus, limit intervals for acceptable recoveries were calculated from the mean recovery of each compound (ignoring level effects) plus/minus twice the standard deviation. Means, upper and lower limits, and minimum quantifiable limits in soil are noted in Table III of this method.

34-89-28 Table I 000237

GC Operating Conditions for the Determination of o,p'- and p,p'-Dicofol, o,p'- and p,p'-FW152, o,p'- and p,p'-DCBP and o,p'-DCBH

Instrument: Varian Vista 6000 Capillary Gas Chromatograph, or equivalent

Detector: Electron Capture

Column: DB-5 28.5 m X 0.25 mm, 0.25 µ film thickness, or equivalent

Gas Flow Rates: Carrier: 1.7 ml/min Helium
Makeup: 28 ml/min Nitrogen
Split Flow: 50 ml/min

Temperatures: Injector: 240°C
Detector: 300°C
Oven: Initial: 100°C
Initial Hold: 2.1 min.
Ramp: 30°C/min
Final: 220°C
Final Hold: 16.9 min

Injector split times: On = at 2.00 min
Off= at 23.00 min

"This is an exact copy of The original document"
By R.F. Kennedy date 10.16.89

FMC CORPORATION AGRICULTURAL CHEMICAL GROUP PHILADELPHIA

34-89-28

Table II

000238

GC Operating Conditions for the Determination of Methylated o- and p-CBA and 3-OH-p,p'-DCBP.

Instrument: Varian Vista 6000 Capillary Gas Chromatograph, or equivalent

Detector: Electron Capture

Column: DB-5 28.5 m X 0.25 mm, 0.25 µ film thickness, or equivalent

Gas Flow Rates: Carrier: 1.7 ml/min Helium
Makeup: 28 ml/min Nitrogen
Split Flow: 50 ml/min

Temperatures: Injector: 240°C
Detector: 300°C
Oven: Initial: 95°C
Initial Hold: 3.1 min.
Ramp: 10°C/min
1st Final: 135°C
Ramp: 30°C/min
2nd Final: 220°C
Final Hold: 14.50 min

Injector split times: On = at 2.00 min
Off= at 23.00 min

"This is an exact copy of
The original document"
By R.F. Kennedy date 10-16-89

ABC LABS# 36934 2

Table III

Results from the Method Validation for the Analysis of Dicofol and Metabolites in Soil

Compound ID Sample Description (36934-)	A[1] p-CBA Percent Recovery	B o-CBA Percent Recovery	C o,p'-DCBP Percent Recovery	D p,p'-DCBP Percent Recovery	E[1] o,p'-DCBH Percent Recovery	E 3-OH-p,p'-DCBP Percent Recovery	G o,p'-FW152 Percent Recovery	H p,p'-FW152 Percent Recovery	I o,p'-Dicofol Percent Recovery	J p,p'-Dicofol Percent Recovery
89 Reagent Blank	-	-	-	-	-	-	-	-	-	-
90 Reagent Blank	-	-	-	-	-	-	-	-	-	-
91 Reagent Blank	-	-	-	-	-	-	-	-	-	-
92 Control	-	-	-	-	-	-	-	-	-	-
93 Control	-	-	-	-	-	-	-	-	-	-
94 Control	-	-	-	-	-	-	-	-	-	-
95 Control + 0.01 ppm	65	77	84	60	92	81	104	103	100	99
96 Control + 0.01 ppm	56	68	91	59	88	74	106	105	101	101
97 Control + 0.01 ppm	65	79	96	79	91	62	110	106	100	102
98 Control + 0.1 ppm	82	88	94	81	97	82	105	106	102	103
99 Control + 0.1 ppm	87	91	101	88	108	86	115	117	113	114
100 Control + 0.1 ppm	95	100	111	93	121	101	129	130	12[illegible]	126

[1] Fortification levels used for p-CBA and o,p'-DCBH were 0.03 ppm, 0.30 ppm, 3.0 ppm, and 30 ppm.

34-89-28

"This is an exact copy of The original document"
By R.F.Kennedy date 10.16.89

000239

ROHM AND HAAS COMPANY PHILADELPHIA

ABC LABS # 36934 2

Table III (continued)

Results from the Method Validation for the Analysis of Dicofol and Metabolites in Soil

Compound ID	A	B	C	D	E[1]	F	G	H	I	J
Sample Description (136934-)	p-CBA Percent Recovery	p-CBA Percent Recovery	o,p'-DCBP Percent Recovery	p,p'-DCBP Percent Recovery	o,p'-DCBH Percent Recovery	3-OH-p,p'-DCBP Percent Recovery	o,p'-FW152 Percent Recovery	p,p'-FW152 Percent Recovery	o,p'-Dicofol Percent Recovery	p,p'-Dicofol Percent Recovery
101 Control + 1.0 ppm	75	77	88	77	103	61	98	98	95	95
102 Control + 1.0 ppm	66	66	93	82	110	74	102	104	100	101
103 Control + 1.0 ppm	81	80	102	87	119	83	110	112	107	108
104 Control + 10 ppm	78	78	100	90	107	78	99	100	95	96
105 Control + 10 ppm	78	80	101	90	108	75	101	101	95	96
106 Control + 10 ppm	92	88	113	99	122	92	114	115	107	108
Mean ($\bar{x}$)	77	81	98	82	106	79	108	108	103	104
Upper Limit	101	100	115	106	130	101	125	126	121	122
Lower Limit	53	62	81	58	82	57	91	90	85	86
Minimum Quantifiable Limits (ppm)	0.03	0.01	0.01	0.01	0.03	0.01	0.01	0.01	0.01	0.01

[1]Fortification levels used for p-CBA and o,p'-DCBH were 0.03 ppm, 0.30 ppm, 3.0 ppm, and 30 ppm.

34-89-28
"This is an exact copy of The original document"
By R.F. Kennedy date 10-6-89
CC0240
WAISCOMPANY PHILADELPHIA
ABC LABS#36342
28011 12 of 3149
2341

"This is an exact copy of The original document"

34-89-28

000241

By R.F.Kennedy date 10.16.89

6.0 Cautionary Notes

There are several critical steps in this method of which the analyst should be aware. They are listed below:

(1) Dicofol is easily degraded to DCBP in neutral or alkaline environments. Sample extraction using an acidic extraction solvent is mandatory (step 4.2).

(2) Substantial chromatographic interferences, especially at the low temperature encountered early in a chromatographic run, can be avoided by pre-extracting the acidified deionized water noted in step 3.4.2 with methylene chloride.

(3) Step 4.3, Liquid-Liquid Partition, uses acidified (pH = 2) water to dilute the methanol extract. Soil (50 g) contains substantial buffer capacity which partially neutralizes the 6N HCl added to the methanol extraction solvent. Failure to use water at pH = 2 results in partial degradation of dicofol to DCBP and substantial losses of both chlorobenzoates ($R-COO^-$ will not partition into the methylene chloride).

(4) Step 4.6 details a solvent exchange from ether to 2,2,4-trimethylpentane. The latter is added to the ether prior to evaporation under nitrogen gas. It acts as a keeper to minimize losses of methylated components, especially the CBA's. The sample must not go dry at any time after derivatization.

(5) Dicofol (o,p'- and p,p'-isomers) degrades to the corresponding DCBP in unprotected GC injectors. At elevated temperatures (>200°C), the conversion is quantitative. At lower temperatures, the conversion is minimal (ex. at 120°C), but the dicofols are not sufficiently volatile at these temperatures and do not load onto the GC column. Two procedures afford a solution, as follows: (1) Step 4.7.5 outlines a procedure for protecting injector and acid-washed injector glass inserts by coating with OV-101. The method is crude, but reproducible; an overnight bake-out at 240°C is, of course, necessary. Repeated dipping of the insert into a 5% OV-101 in CH_2Cl_2 solution affords a satisfactory insert, but the effect is transitory (ca 40 injections) at best. (2) Final sample dilutions are prepared in 2,2,4-trimethylpentane containing 0.01% OV-101. The OV-101 affords additional protection from the dicofol to DCBP conversion.

242

(6) Step 4.7.5 notes that glass inserts are boiled in acid before coating with OV-101. Inserts which are boiled in acid, but not coated with OV-101, completely inhibit the dicofol to DCBP conversion (<2%). However, these same uncoated inserts cause an unusual and unexpected conversion of FW152 isomers to DDE isomers, especially at elevated temperatures (>200°C). Boiling in acid and coating with OV-101 inhibits both processes.

(7) Chlordane (α-isomer) was used as an internal standard for this method; its choice was ideal in that it yielded a sensitive, well-shaped peak at an interference-free retention time. The internal standard was mandatory for reproducible results. The combination of 10 separate components eluting over a wide temperature range (95° - 220°C) yielded irreproducible peak areas and heights between individual injections. However, the ratio between component areas/heights and the internal standard area/height remained remarkably consistent for long periods of time.

"This is an exact copy of
The original document"
By R.F. Kennedy date 10.16.89

34-89-28

000243

7.0 Chemical Structures of Dicofol and Metabolites

The chemical structures of dicofol and dicofol metabolites follow.

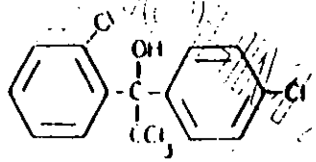
"This is an exact copy of
The original document"
By R.F. Kennedy date 10-4-89

ROHM AND HAAS COMPANY PHILADELPHIA

"This is an exact copy of
The original document"
By R.F. Kennedy date 10-16-89

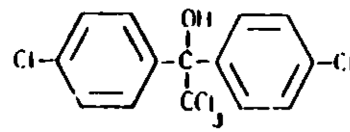
FIGURE 1

Chemical Structures of Dicofol and Metabolites



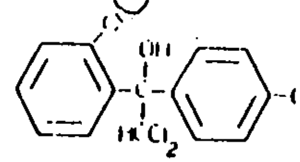
o,p'-dicofol

[1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2,2-trichloroethanol]



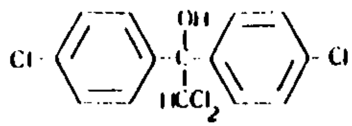
p,p'-dicofol

[1,1-bis(4-chlorophenyl)-2,2,2-trichloroethanol]



o,p'-FW-152

[1-(2-chlorophenyl)-1-(4-chlorophenyl)-2,2-dichloroethanol]

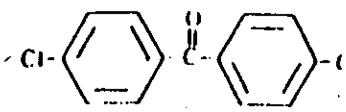


p,p'-FW152

[1,1-bis(4-chlorophenyl)-2,2-dichloroethanol]

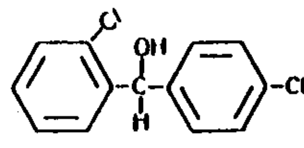
o,p'-DCBP

[2,4'-dichlorobenzophenone]



p,p'-DCBP

[4,4'-dichlorobenzophenone]



o,p'-DCBH

[2,4'-dichlorobenzhydrol]

ROHM AND HAAS COMPANY PHILADELPHIA

"This is an exact copy of The original document"

34-89-28

000245

By R.F. Kennedy date 10.16.82

-19-

FIGURE 1 (Continued)

Chemical Structures of Dicofol and Metabolites

DUPLICATE COMPANY PHILADELPHIA

240

ABC LABS# 36934 2

pg 0177 of 3749

FORM 4685 REV. 12/73