Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Fonofos (Dyfonate)

MRID #: 413087-01

Matrix: Soil

Analysis: GC/MS

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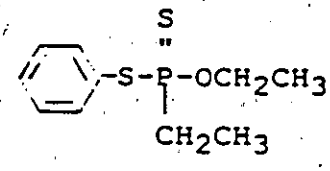
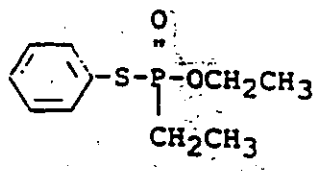
Determination of Fonofos, Fonofos Oxon, and Methylphenylsulfone Residues in Soil by Gas Chromatography

I. SUMMARY/INTRODUCTION

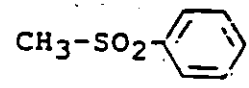
This method is intended for determining residues in soil of fonofos at levels of 0.01 to 10.0 ppm and of fonofos oxon and methylphenylsulfone at levels of 0.01 to 1.0 ppm. Fonofos is the active insecticidal ingredient in the various formulated products marketed by ICI Americas Inc. under the trademark "DYFONATE". The chemical name assigned to fonofos by Chemical Abstracts Service (11th CI) is phosphonodithioic acid, ethyl O-ethyl S-phenyl ester [944-22-9]. Fonofos oxon is a toxic compound derived from the chemical or biochemical oxidation of fonofos. The chemical name for fonofos oxon is phosphonothioic acid, ethyl O-ethyl S-phenyl ester [944-21-8]. Methylphenylsulfone is a metabolite that has been identified in a soil-metabolism study. The chemical name for methylphenylsulfone is methylsulfonylbenzene [3112-85-4]. The chemical structures are given below.



Fonofos



Fonofos oxon



Methylphenylsulfone

Fonofos, fonofos oxon, and methylphenylsulfone are extracted directly from the soil by shaking the soil with water and toluene. The toluene extract is analyzed by capillary gas chromatography with mass-selective detection.

II. MATERIALS/METHODS

The equipment and reagents described below were used to generate the data and chromatograms presented in this report. Equipment with equivalent performance specifications and reagents of comparable purity can be used.

A. Apparatus

1. Gas Chromatograph. Hewlett-Packard model 5880A designed for use with capillary columns and temperature programming of the column oven. The gas chromatograph is equipped with a Hewlett-Packard model 7672A automatic sampler/injector.

2. Mass-Spectrometric Detector. Hewlett-Packard model 5970 mass-selective detector with version 3.1.1 software.

3. Gas-Chromatographic Column. 12 m by 0.2 mm capillary column with a 0.33-µm film thickness of crosslinked methyl silicone and with a minimum of 4800 plates per meter (Hewlett-Packard, Ultra 1, catalog no. 19091A-101).

4. Shaker. Reciprocating movement (Eberbach Corporation, Ann Arbor, MI).

5. Centrifuge. Equipped to accept 4-oz bottles (model K; Damon/International Equipment Company, Needham Heights, MA).

6. Ultrasonic Cleaner. 13.5 x 12 x 11 (height) inches overall; 11.5 x 9.5 x 6 inches for bath (VWR Scientific).

7. Glass Bottles. Four-ounce, widemouth bottles with screwcap lids. Aluminum foil is used to cover the mouth of the bottles prior to capping.

8. Syringes. 10-µL capacity (Hamilton 701N) for autosampler and 500-µL capacity (Hamilton 750N) for fortifications.

B. Reagents

1. Solvents. Methanol, toluene, and water. All solvents must be of high purity and suitable for use in trace organic analyses by gas chromatography.

2. Fonofos, Fonofos Oxon, and Methylphenylsulfone. Analytical reference-standards. Available from ICI Americas Inc., 1200 South 47th Street, Box Number 4023, Richmond, CA 94804-0023; Attention: Environmental Science Department Manager.

3. Calibration and Fortification Solutions.

To prepare a 1.00 mg/mL (= 1000 µg/mL) stock solution of an analyte, place a known quantity (± 0.1 mg) of approximately 50 mg of primary standard of known purity into a 4-oz narrow-mouth bottle. Calculate the weight of solvent to add, based on the weight of primary standard taken, the purity of the primary standard, and the density of the solvent, as follows:

$$S = \frac{(W \times P \times D)}{L}$$

where S = weight of solvent to add (g),
W = weight of primary standard taken (mg),
P = purity of the standard (100% = 1.00),
D = density of the solvent (g/mL), and
L = required analyte concentration (mg/mL).

Add the calculated weight of the appropriate solvent to the bottle, close the bottle with a Poly-Seal or Teflon-lined cap, and mix thoroughly to dissolve the reference standard. Use toluene (d = 0.867 g/mL) for the calibration solutions and methanol (d = 0.793 g/mL) for fortification solutions.

To prepare working calibration solutions, serially dilute the stock calibration solution by weight with toluene to give 10, 1.0, 0.10, and 0.010 µg/mL solutions or other concentrations as required. Dilute the stock fortification solution by weight with methanol to give a 100, 10, and 1.0 µg/mL solutions, or other concentrations as required.

C. Analytical Procedure

1. Extraction

Place a 25-g subsample of a thoroughly-mixed soil sample into a 4-oz widemouth bottle. Add 25 mL of distilled water. If the soil has been treated with a granular formulation, cap the bottle with an aluminum foil-lined lid and sonicate the sample for 20 min in a ultrasonic cleaning bath. Add 25 mL of toluene. Cap the bottle with an aluminum foil-lined lid. Shake the sample on a reciprocating shaker for 1 hour. Centrifuge the sample for about 10 min at 2000 rpm to separate the phases. Remove the top (toluene) phase for analysis.

2. Fortification

Analyze unfortified and fortified control samples with each sample set to demonstrate method recovery according to the Quality Assurance SOP. For example, for 25-g samples, place 25 g of untreated control soil into a 4-oz widemouth bottle. Add 0.25 mL of the 1.0, 10, 100, or 1000 µg/mL fortification solution to produce a fortification level of 0.01, 0.10, 1.00 or 10.0 ppm. Add 25 mL of water, 25 mL of toluene and extract as detailed in section C.1 above.

D. Instrumentation

1. Operating Conditions

Follow the manufacturer's instructions for operation of the gas chromatograph and mass-selective detector. The specific conditions listed below were used to generate the data and chromatograms presented in this report.

Gas Chromatograph:

Carrier gas:	helium
Column head-pressure:	5 lb/sq. inch
Inlet type:	splitless; 2 mm i.d.
Inlet temperature:	230°C
Interface temperature:	230°C
Initial oven temperature:	100°C
Initial time:	1.0 min
Oven-temperature program-rate:	20°C/min
Final oven temperature:	240°C
Volume injected:	1.0 µL
Valve off:	0.5 min
Total run time:	8.0 min

Mass-Selective Detector:

Software version:	3.1.1
Mode:	low resolution s.i.m.
Dwell time:	100 msec
Tuning:	optics optimized for m/z 219 and 264 with perfluorotributylamine
Quantitation:	Peak height; external standard
Mass monitored:	Parent ion
Fonofos:	m/z 246
Fonofos oxon:	m/z 230
Methylphenylsulfone:	m/z 156

Using the above conditions the elution times of fonofos, fonofos oxon, and methylphenylsulfone were 5.4, 4.9, and 2.9 min, respectively. See Figure 1 for typical chromatograms.

2. Calibration

Calibrate the gas chromatograph by using the analyte calibration solutions specified in section II.B.3 Calibrate the instrument by using the 10.0, 5.00, 1.00, 0.10 and 0.010 µg/mL solutions.

3. Analysis of Extracts

Inject the sample extracts using the same conditions used for calibration. The identity of the analyte peak in the sample chromatogram is assigned based upon the coincidence of retention times (within 0.03 min) with those of the calibration chromatograms. Reinject the calibration solution after injection of every two to four sample extracts and at the end of the chromatographic run. Calculate the concentration of the analyte(s) in the sample extract by comparing it to the closest standard (peak height) or by use of a standard curve.

E. Interferences

No cleanup was required when this procedure was used as described. However, extractives from soil could potentially contribute peaks with retention times coincident with or near that of the analyte(s). Satisfactory resolution can usually be achieved with appropriate oven temperature manipulations or column selection (length, phase). If resolution cannot be achieved, an alternate ion can be monitored. Figure 1 shows typical chromatograms. Analyze extracts of samples from untreated plots to demonstrate the absence of interferences from sample matrices, solvents, and labware. Fonofos is chemically similar to active ingredients in other organophosphorus insecticides. However, the resolution provided by capillary columns combined with the selectivity afforded by selective-ion-monitoring should eliminate any problems of misidentification.

F. Confirmatory Techniques

Unexpected positive results, as in untreated control or pre-application samples, should be confirmed by other means. Confirmation can be achieved by quantitation using a different m/z ion or by using a different detector type, such as a flame photometric detector with a phosphorus or sulfur bandpass filter or a nitrogen/phosphorus thermionic detector. A list of alternate m/z ions for the three analytes are given below.

Sulfone		Oxon		Fonofos	
m/z	relative % abundance	m/z	relative % abundance	m/z	relative % abundance
51	45	65	47	63	21
77	100	93	100	81	16
94	28	109	20	109	100
141	16	110	21	110	20
156*	16	121	21	137	31
		230*	15	246*	15

* Parent ion

G. Calculations

The concentration of the analyte in the original sample is calculated by using the external standard method, i.e., the response obtained for the analyte in the sample extract is compared to the response obtained from a separate injection of a known amount of analyte (calibration solution). It is assumed for the calculations outlined below that the injection volumes for all calibration solutions and sample extracts are fixed at the same volume.

1. Linear Detector-Response

a. Calibration Factor

Calculate the response factor, F, for injection of a calibration solution as follows:

$$F\ ((ng/\mu L)/\text{response unit}) = \frac{C_{std}}{R_{std} \times S}$$

where C_{std} = concentration of calibration solution, ng/μL

R_{std} = response units (e.g., peak height, peak area, electronic units) from detector for calibration solution

S = ratio of amount (g) of sample extracted to volume (mL) of extraction solvent used.

If the extract has been concentrated or diluted, S can be calculated as follows:

$$S = \frac{W_{sample}}{V_{solvent}} \times \frac{V_{initial}}{V_{final}}$$

where W_{sample} = total weight of sample extracted, g
$V_{solvent}$ = total volume of solvent used in extraction, mL
$V_{initial}$ = volume of initial extract taken for analysis, mL
V_{final} = final volume of extract after concentration or dilution, mL

b. Analyte in Sample

Calculate the analyte concentration, R, in the original sample as follows:

$$R\ (\mu g/g \text{ or } ppm) = F \times R_{sample}$$

where F = response factor, (ng/µL)/response unit
R_{sample} = response units from detector for analyte in the sample extract

Averaged response factors obtained from injections of calibration solution before and after injection of sample extracts may be used for calculation of the analyte concentration in the sample.

2. Nonlinear Detector-Response

a. Analyte in Sample

Generate a standard curve by plotting the concentration of the calibration solution (C_{std}) as the x-axis and the corresponding response units from the detector (R_{std}) as the y-axis for a range of analyte concentrations, as shown in Figure 2. Take the response units from the detector for the analyte in the sample extract (R_{sample}) and determine the concentration of the analyte in the sample extract (C_{sample}) by using the standard curve. Calculate the analyte concentration, R, in the original sample as follows:

$$R\ (\mu g/g \text{ or } ppm) = C_{sample}/S$$

b. Calibration Factor

Calculate the response factor, F, for the theoretical injection of an appropriate calibration solution as follows:

$$F((ng/\mu L)/\text{response unit}) = R/R_{sample}$$

III. DISCUSSION

A. Precision and Accuracy

Fortified soil samples were prepared as described under section II.C.2 and analyzed according to this method to establish accuracy. Table 1 gives the descriptions of the two soils used in this study. As Table 2 shows, recoveries from loamy sand and loam soil samples fortified from 0.01 to 10.0 ppm of fonofos ranged from 66 to 120 % with a mean recovery of 95.4% (n=36 and coefficient of variation (CV) of 13%; CV = 100 x (standard deviation/mean). Recoveries from soil samples fortified from 0.01 to 1.0 ppm of fonofos oxon ranged from 75 to 122% with a mean recovery of 97.1% (n=31) and CV of 12%. Recoveries from soil samples fortified from 0.01 to 1.0 ppm of methylphenylsulfone ranged from 59 to 111% with a mean recovery of 82.3% (n=31) and CV of 13%.

The precision of the method depends on variations in extraction and instrumental analysis. The variations in extraction and instrumental analyses can be evaluated from the data obtained during analyses of fortified samples. The coefficient of variations given in Table 2 are a measure of precision.

B. Detection Limit

The detection limit of the method is 0.01 ppm for each of the three analytes as determined by fortifications at the 0.01-ppm level with at least 300 peak-height units.

C. Dry-Weight Basis

This method determines the residues on an as-received basis. If it is desired to express the values on a dry-weight basis, compensation is necessary for water present in the sample. Percent moisture can be determined by drying a subsample at 105°C for 24 hr.

D. Safety Precautions

Personnel untrained in the routine safe-handling of chemicals and good laboratory practices must not attempt to use this procedure. Information on any specific chemical regarding physical properties, hazards, toxicity, and first-aid procedures can be found on the Material Safety Data Sheets accompanying the chemical or available from the chemical supplier. In general, always wear safety glasses with sideshields, work in a well ventilated area, avoid inhaling vapors, and avoid contact of the chemical with skin and clothing. Flammable solvents should be kept away from potential sources of ignition.

1. Flammable Solvents. Methanol and toluene.

2. Fonofos, Fonofos Oxon, Methylphenylsulfone. Remove contaminated clothing and wash affected skin area with soap and water after any accidental contact. Wash eyes with plenty of water after any accidental contact. Fonofos and fonofos oxon are **CHOLINESTERASE INHIBITORS.**

CONCLUSIONS

The method is specific for the analysis of fonofos, fonofos oxon and methylphenylsulfone in soil. Only commercially available laboratory equipment and reagents are required. The analysis can be completed by one person in an 8-hr period if an adequately homogenized sample is available. Untreated and fortified untreated samples should be extracted and analyzed with each set of samples to demonstrate absence of interferences and adequate recovery. If determination of fonofos at a concentration other than 0.01 to 10.0 ppm and of fonofos oxon and methylphenylsulfone each at a concentration other than 0.01 to 1.00 ppm are required, suitably fortified samples must be analyzed to validate the method at that concentration.

V. CERTIFICATION

This is to certify that this is a complete and unaltered report prepared by the Environmental Sciences Department of ICI Americas Inc., Western Research Center.

Y. Iwata, Ph.D., Study Director — Date

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R. J. Bussey, Ph.D.
Environmental Chemistry Supervisor — Date

VI. REFERENCES

WRC Laboratory Notebooks 11706 and 11707.

VII. TABLES AND FIGURES

Table 1. Properties of the Soils used in the Study.

Description	Soil Sample D133	Soil Sample D134
Origin (California):	Visalia	Porterville
Soil depth, inches:	0 - 6	0 - 6
Textural class:	loamy sand	loam
% Organic matter:	0.8	0.7
pH:	7.7	8.2
Cation exchange capacity, meq/g:	9.5	17.5
Composition		
% Sand:	55	48
% Silt:	33	40
% Clay:	12	12
A & L Great Lakes report no.:	F184-173	F260-114
Sample no.:	36/06957	11/14894

Table 2. Recovery of Fonofos, Fonofos Oxon, and Methylphenylsulfone from Loamy Sand and Loam Soils.

Sample no.	ppm Added	% Fonofos Recovered	% Oxon Recovered	% Sulfone Recovered
D133-01	0.10	107	116	97
D133-01	0.010	109	83	66
D133-04	0.010	77	86	73
D133-15	10.0	94		
D133-15	1.0	98	92	80
D133-15	0.10	110	102	89
D133-15	0.010	96	100	72
D133-34	10.0	85		
D133-34	1.0	78	82	75
D133-34	0.010	108	85	72
D133-46	5.0	103		
D133-46	0.10	101	110	83
D133-46	0.10	92	101	92
D133-46	0.010	107	96	81
D133-46	0.010	89	97	67
D133-70	1.0	99	85	81
D133-70	1.0	92	89	84
D133-70	0.10	79	75	73
D133-82	0.10	99	87	91
D133-82	0.010	83	102	87
D133-82	0.010	113	94	89
D134-01	0.10	93	86	85
D134-01	0.10	116	122	103
D134-01	0.010	110	102	75
D134-01	0.010	66	89	59
D134-21	10.0	80		
D134-21	1.0	99	95	88
D134-21	0.10	86	111	79
D134-21	0.010	88	95	82
D134-35	10.0	88		
D134-35	1.0	82	109	73
D134-35	0.10	88	88	81
D134-35	0.010	91	113	111
D134-59	1.0	107	112	88
D134-59	0.10	103	100	90
D134-59	0.010	120	106	84
Number of Samples Analyzed:		36	31	31
Range:		66-120	75-122	59-111
Mean:		95.4	97.1	82.3
Coefficient of Variation:		13%	12%	13%

Figure 1A. Sample Chromatograms for the Analysis of Methylphenylsulfone (2.9 min), Fonofos Oxon (4.9 min), and Fonofos (5.4 min) in Soil (D134):

A. Calibration solution containing 0.01 µg of each analyte/mL.
B. Untreated control soil (D134-35).
C. Untreated control soil (D134-35) fortified at 0.01 ppm, each analyte.
D. Treated soil (D134-38); 0-3.5 inch depth; 7-day postapplication after treatment with 4.0 lb a.i./acre with DYFONATE 4-EC. Soil contained 0.01 ppm each of methylphenylsulfone and fonofos oxon and 0.88 ppm of fonofos.

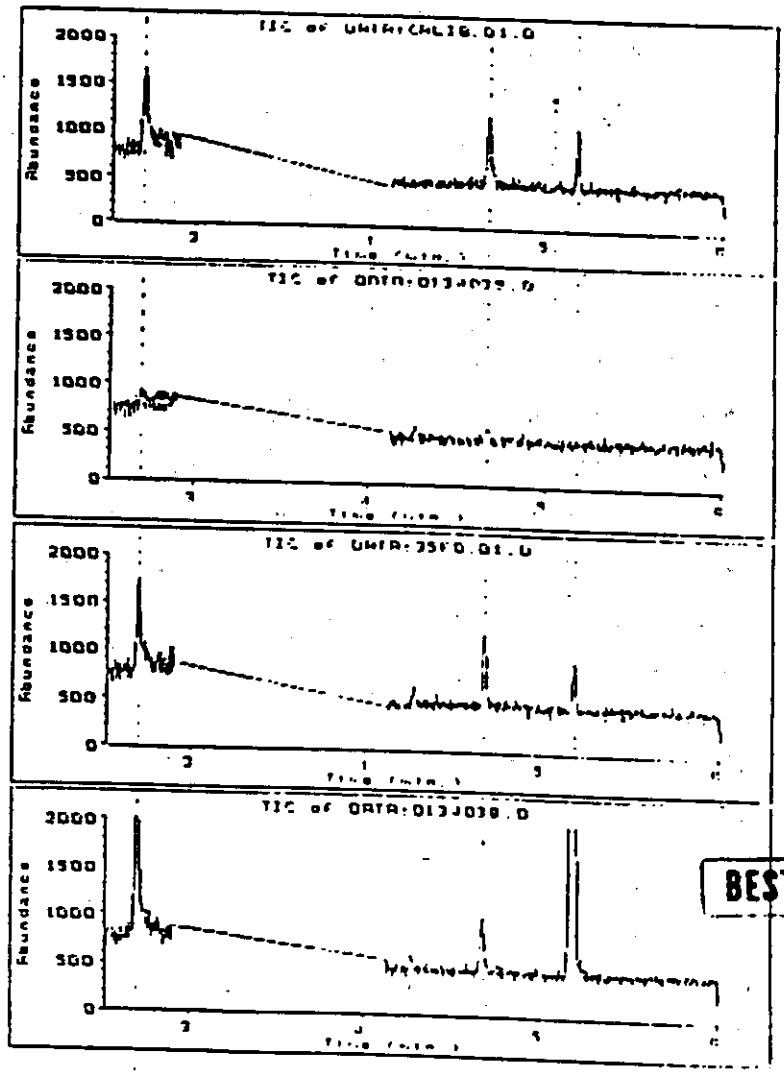

Figure 1B. Sample Chromatograms for the Analysis of Methylphenylsulfone (2.9 min), Fonofos Oxon (4.9 min), and Fonofos (5.4 min) in Soil (D133):

A. Calibration solution containing 0.01 µg of each analyte/mL.
B. Untreated control soil (D133-34).
C. Untreated control soil (D133-34) fortified at 0.01 ppm, each analyte.
D. Treated soil (D133-38); 0-3.5 inch depth; 7-day postapplication after treatment with 10.0 lb a.i./acre with DYFONATE 20-G. Soil contained 0.02 ppm of methylphenylsulfone, 0.01 ppm of fonofos oxon and 4.4 ppm of fonofos.

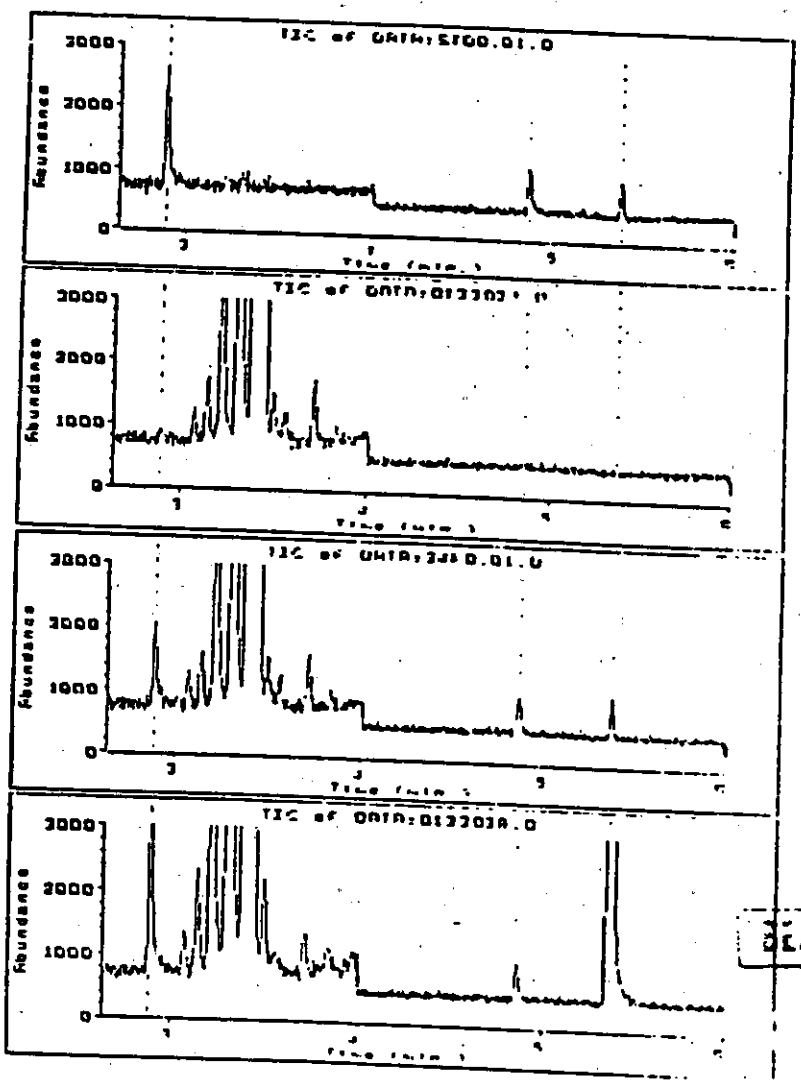

Figure 2. Calibration Curves for Fonofos, Fonofos Oxon, and Methylphenylsulfone Based upon Injection of 0.01, 0.10, 1.0, 5.0, and 10.0 µg of Analyte/mL Solutions.