Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Endosulfan

MRID #: 414686-01

Matrix: Soil/Water

Analysis: GC/ECD

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414686-01

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Date: 01.09.1989
Report No.: CB040/87
Page: 50 (284)

Hoechst

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Date: 27.01.87
Report No.: AL60/86
Page: 0

Hoe 002670 ([redacted])

Residue determination of the active ingredient and Endosulfan-sulfate in soil, water, urine and plant material as well as of Endosulfan-diol and Endosulfan-lactone in soil, water and urine.

Outline of method

The active ingredients and its metabolites are extracted from the sample material with acetone. After dilution of the extract with sodium chloride solution, reextraction is achieved with dichloromethane. After a clean up on a Bio-Beads S-X3 and a mini silica gel column the gaschromatographic determination is carried out using an electron capture detector.

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Page: 1 (21)

Translation of Doc. No. A34468

Hoe 002671 (endosulfan)

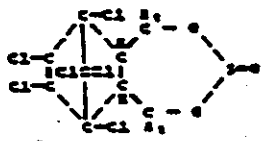
Residue determination of the active ingredient and endosulfan-sulfate in soil, water, urine and plant material as well as of endosulfan-diol and endosulfan-lactone in soil, water and urine.

1. Introduction

1.1 Hoe 002671 (endosulfan)

Chemical name : 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzo-dioxathiepin-3-oxide (IUPAC)

structural formula:



molecular formula : $C_9H_6Cl_6O_3S$

molar mass : 406.9 g/mol

solubility : low solubility in water
readily soluble in dichloromethane, hexane, toluene, acetone and alcohols

other properties : unstable in alkaline media
endosulfan exists in two stereoisomeric forms, the α-(Hoe 052618) and β-endosulfan (Hoe 052619).
The active ingredient has a ratio of 7 : 1 (α : β).

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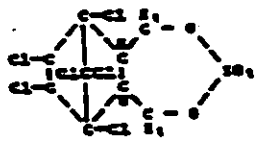
1.2 See 031327 (endosulfan-sulfate)

Chemical name : 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3,3-dioxide (IUPAC)

structural formula:



molecular formula : $C_9H_6Cl_6O_4S$

molar mass : 422.9 g/mol

solubility : low solubility in water
readily soluble in dichloromethane, benzene, toluene, acetone and alcohols

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1.3 Hoe 051328 (endosulfan-lactone)

Chemical name : 4,5,6,7,8,8-hexachloro-1,3,3a,4,7,7a-hexahydro-4,7-ethano-isobenzofuran-1-one (IUPAC)

structural formula:

molecular formula : $C_9H_4Cl_6O_2$

molar mass : 356.8 g/mol

solubility : low solubility in water
readily soluble in toluene, acetone and alcohols

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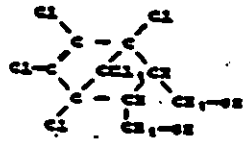
1.4 Hoe 031329 (endosulfan-diol)

Chemical name : 1,4,5,6,7,7-hexachloro-bicyclo-(2,2,1)-hept-5-ene-2,3-dimethanol (IUPAC)

structural formula:



molecular formula : $C_9H_8Cl_6O_2$

molar mass : 360.8 g/mol

solubility : low solubility in water
readily soluble in dichloromethane, toluene, acetone and alcohols

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2. Outline of method

The active ingredient an its metabolites are extracted from the sample material with acetone. After dilution of the extract with sodium chloride solution, reextraction is made by dichloromethane. After clean up on a Bio-Beads S-X3 and a mini silica gel column gaschromatographic determination is carried out using an electron capture detector.

3. Apparatus

beakers 400 mL
Ultraturrax (homogenizer)
measuring cylinder 100 mL
vacuum glass fritted filter funnel
separation funnels 250, 1000 mL
volumetric flasks 2, 25 mL
funnel
round bottom flasks 100, 500 mL
volumetric pipette 5 mL
GPC-Autoprep
GPC-column 400 mm x 25 mm filled with Bio Beads S-X3 (200-400 mesh)
soaked with 50 % ethyl acetate/50 % cyclohexane (v/v)
chromatographic column 25 mm x 7 mm
pasteur pipettes
rotary evaporator (bath temperature 40°C)
gaschromatograph equipped with capillary and ECD
injection bottles
on column syringe
disposable syringe 10 mL

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4. Reagents

acetone Pestanal (Riedel de Haen)
sodium chloride R.G. (Merck)
sodium chloride solution (4 g/100 mL H_2O)
dichloromethane (coffee quality)
Celite R.G.
ethyl acetate, dist.
cyclohexane R.G. (Riedel de Haen)
50 % ethyl acetate/50 % cyclohexane (v/v)
n-hexane for residue analysis (Merck)
quartz wool
silica gel 60 (Merck 7734)
silicia gel desactivated with 1.5 % of water
Bio-Beads S-X3 (200-400 mesh) (Bio-Rad Laboratories)
sodium sulfate anhydrous R.G. (Merck)
toluene, clean up by silica gel and distillation
95 % toluene/5 % acetone (v/v)
MSTFA N-methyl-N-trimethylsilyl-trifluoroacetamide (Machery & Nagel)
α-endosulfan Pestanal (Riedel de Haen)
β-endosulfan Pestanal (Riedel de Haen)
endosulfan-sulfate Pestanal (Riedel de Haen)
endosulfan-lactone Pestanal (Riedel de Haen)
endosulfan-diol Pestanal (Riedel de Haen)

5. Sampling

Aliquots from the laboratory sample reduced to small pieces are taken for analysis. Liquids are mixed well.

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6. Procedure

6.1 Extraction

6.1.1 Plant material

The analysis of plant material was tested only with the active ingredient and the metabolite endosulfan-sulfate.
50 g (W) of sample is weighed into a beaker. 70 mL of acetone are added and the sample is macerated thoroughly with an Ultraturrax. The macerate is filtered through a glass frittad filter funnel. The extract is transferred directly into the separation funnel (1000 mL) containing 600 mL sodium chloride solution. The extraction step is repeated twice with the filter cake.
If problems arise during the filtration process, Celite might be used as filter aid.

6.1.2 Soil samples

50 g (W) of sample are weighed into a separation funnel (250 mL). 70 mL of acetone are added and the sample is shaken for half an hour on a mechanical shaker. The sample is filtered through a glas frittad filter funnel. The extract is transferred directly into a separation funnel (1000 mL, containing 600 mL of sodium chloride solution. The extraction step is repeated twice with the filter cake.

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6.1.3 Samples of surface waters

100 mL (V) of water are measured into a 250 mL separation funnel.

6.1.4 Urine samples

50 mL (V) of urine are measured into a separation funnel (1000 mL) containing 200 mL of acetone and 600 mL of sodium chloride solution.

6.2 Isolation of the active ingredient and metabolites

100 mL of dichloromethane are added to the materials resulting from step 6.1.1, 6.1.2 and 6.1.4. 100 mL of dichloromethane are added to the sample from 6.1.3. The separation funnel is shaken for 1 min. After separation of the phases the lower one is drained off over a funnel containing sodium sulfate and collected in a round bottom flask. The extraction is repeated twice with 50 mL of dichloromethane. The dichloromethane is drained off as well over the sodium sulfate and collected. The sodium sulfate is rinsed with some small portions of dichloromethane. The solvent is evaporated nearly to dryness using a rotary evaporator.

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6.2.1 To extracts from soil-, water- and urine-samples some n-hexane is added. The sample is once more evaporated to near dryness. The reconstitution is repeated until there is no dichloromethane left. The residue is transferred quantitatively with n-hexane into a 25 mL (V_1) volumetric flask, filled up to the mark and shaken.

6.2.2 To extracts from plant materials (6.1.1) a mixture of 50 % ethyl acetate/50 % cyclohexane (v/v) is added. The sample is once more evaporated to near dryness. The reconstitution is repeated until there is no dichloromethane left. The residue is transferred quantitatively with a mixture of 50 % ethyl acetate/50 % cyclohexane (v/v) into a 25 mL (V_1) volumetric flask, filled up to the mark, shaken and the suspended precipitate is allowed to settle.
A 10 mL aliquot of this solution is cleaned by gel permeation as described under 6.3.1.

6.3 Clean up

6.3.1 Gel permeation

The conditions for equipment (dump and collect volumes) are to be determined prior to analysis by testing the column with a standard solution.
Suck 10 mL of the solution into a disposable syringe and inject into the 5 mL loop (T_1) of the GPC. While the sample passes through the column the cleaned extract is collected in a 100 mL round bottom flask. The solvent is evaporated to near dryness using a rotary evaporator. Some n-hexane is added and the sample is once more evaporated to near dryness. The reconstitution is repeated until there is only n-hexane in the round bottom flask.

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6.3.2 Mini silica gel column

Some glass wool is plugged into the lower end of the chromatographic column. The column is filled with n-hexane. 1 g of desactivated silica gel is added into the column while tapping with a ruler. After sedimentation of the silica gel, a layer of 1 cm of sodium sulfate is added on top. The column is rinsed with 10 mL of n-hexane.
The sample from 6.3.1 is transferred in small portions onto the silica gel column each time draining the solvent until it reaches the upper part of the sodium sulfate layer.
From the n-hexane extracts (6.2.1) 5 mL (T_1) are pipetted onto the silica gel column and the n-hexane is drained off until it reaches the upper part of the sodium sulfate layer.
The column is rinsed with n-hexane until a volume of 10 mL (transfer and liquid for rinsing) is reached.
If only α-endosulfan, β-endosulfan and endosulfan-sulfate are to be determined the column is eluted with 10 mL toluene.
If additionally endosulfan-lactone and endosulfan-diol are to be determined in soil, water and urine all 5 compounds are eluted with 20 mL of 95 % toluene/5 % acetone (v/v). The eluates are evaporated to near dryness using a rotary evaporator and made up to a volume of 2 mL (V_2) with toluene.

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6.4 Gaschromatographic determination

6.4.1 Derivatisation

(This step is only necessary where endosulfan-diol has to be determined in soil, water and urine.)

0.02 ml are taken from the cleaned final extract and are diluted to 0.1 ml in a graduated injection bottle with columns. The silyation is done after adding 0.04 ml MSTFA into the capped injection bottle and heating to 70-80°C for 15 minutes.

After cooling the sample is brought to a final volume of 1 ml. The injection bottle should not be opened anywere during this step. For sampling a short steal cannula funnel shaped by drilling at the luer-extension is pearced through the septum of the capped injection bottle. This guide cannula allowes to sample by GC-syringe. Before a sample is silylated the batch of MSTFA has to be checked for purity to make sure that the determination of endosulfan and its metabolites is not affected by contaminations. This is done by silylation of the solvent and its gaschromatographic analysis.

Experiance has shown not to use the opened bottle with silylation reagent longer than a week.

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6.4.2 GC-conditions

model : C. Erba Model 5160 (Mega)
column: type: capillary (ICT/JSW)
stat. phase: DB-5 (0.25 µm film)
length: 30 m
internal diameter: 0.32 mm
material: fused silica
carrier gas: Helium 4.6 3 ml/min
purge gas : N_2-special 35 ml/min
injector : on column injector
detector : electron capture detector (ECD)
temperature: injector : 45°C
detector : 280°C
column : -
temperature program: oven: standby 85°C 1 min
ramp 1 bal. T_1 200°C 0 min
ramp 2 20 C/min T_2 220°C 15 min
ramp 3 bal. T_3 250°C 5 min

7. Evaluation

7.1 Method of determination

The evaluation is carried out by determining the peak height (F_s) of the sample and comparing it with the peak height (F_c) of a control sample, spiked with standard solution. The volume of the injected sample and the volume of the control sample should be identical.

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7.2 Lower limit of the practical working range and recovery

The lower limit of the practical working range is 0.01 mg/kg. The recoveries (F) from spiking with 0.01, 0.05 and 0.1 mg/kg ranged from 70 to 100 %.

7.3 Calculations

The calculation is carried out in accordance with DFG directives V and XI.

$$R = \frac{V_1 \times V_2 \times V_3 \times V_4 \times M_c \times F_s}{U \times T_1 \times T_2 \times T_3 \times T_4 \times F_c} \times F$$

R = residue in mg/kg or mg/L
U = weight or volume of analytical sample (g or mL)
V_1 = volume of extract (mL)
T_1 = partial volume for clean up (mL)
V_2 = final volume (mL)
T_2 = partial volume for possible dilution (mL)
T_3 = partial volume for possible dilution (mL)
V_3 = volume for possible dilution (mL)
V_4 = volume for possible dilution (mL)
T_4 = volume of injected sample solution (/µl)
M_c = amount of standard injected (ng)
F_s = peak height of sample (cm)
F_c = peak height of standard (cm)
F = recovery factor

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A34558

8. Literature

Deutsche Forschungsgemeinschaft, Rückstandsanalytik von Pflanzenschutzmitteln (1. - 8. Lieferung, 1985)
VCH Verlagsgesellschaft mbH, D-6940 Weinheim, FRG

9. Comments

No comments

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