Analytical method for total d-phenothrin (sum of cis- and trans- isomers) in surface water

Reports:	ECM: EPA MRID No. 50914202. Smith, R.J. 2017. Validation of the Analytical Method for the Determination of *d*-phenothrin in Aqueous Matrix by LC-MS/MS. Smithers Viscient Study No.: 13048.7070. Report prepared by Smithers Viscient, Wareham, Massachusetts, sponsored and submitted by Sumitomo Chemical Company, Ltd., Tokyo, Japan, and submitted by Sumitomo Chemical America, Inc., New York, New York; 76 pages. Final report issued September 8, 2017. ILV: EPA MRID No. 50914203. Jooß, S., and S. Tussetschläger. 2014. Independent Laboratory Validation (ILV) of the Analytical Method for the Determination of d-phenothrin in Surface Water by LC-MS/MS. EAG Laboratories ID: P 4686 G. Report prepared by EAG Laboratories GmbH, Ulm, Germany, sponsored and submitted by Sumitomo Chemical Company, Ltd., Tokyo, Japan, and submitted by Sumitomo Chemical America, Inc., New York, New York; 42 pages. Final report issued April 24, 2018.
Document No.:	MRIDs 50914202 & 50914203
Guideline:	850.6100
Statements:	ECM: The study was conducted in accordance with USEPA FIFRA (40 CFR Part 160) Good Laboratory Practices (GLP), which are accepted by OECD GLP standards (p. 3). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the Quality Assurance statement. ILV: The study was conducted in accordance with German Good Laboratory Practices (GLP; 2011), which are based on OECD GLP standards which are accepted by Regulatory Authorities throughout the European Community, the United States of America (FDA and EPA) and Japan (MHLW, MAFF and METI; pp. 3, 5; Appendix 2, p. 38). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-5; Appendix 2, p. 38). A statement of the authenticity of the study report was included with the Quality Assurance statement.
Classification:	This analytical method is classified as **supplemental**. The number of trials was not reported in the ILV. The method was specified for surface water. ILV/Sponsor communication details were not provided.

PC Code:	069005		
Reviewer:	Kristy Crews, Ph.D., Chemist	**Signature:** **Date:**	Kristy Crews Digitally signed by KRISTY CREWS Date: 2020.05.13 18:04:45 -04'00'
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist	Signature: Date:	Lisa Muto 04/30/2020
	Mary Samuel, M.S., Environmental Scientist	Signature: Date:	Mary Samuel 04/30/2020

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac Joint Venture personnel. The CDM/CSS-Dynamac JV role does not include establishing Agency policies.

Executive Summary

This analytical method, Smithers Viscient Study No. 13048.7070, is designed for the quantitative determination of the total d-phenothrin (sum of cis- and trans- isomers) at 0.005 µg/L in surface water using LC/MS/MS. The LOQ is **equal to** the lowest toxicological level of concern in water[1]. The ECM and ILV validated the method using different characterized surface water matrices. The number of trials was not reported, but the reviewer assumed that the method was validated by the ILV with the first trial as written with insignificant analytical instrument and equipment modifications. Prior to the beginning of the study, the ILV clarified some parts of the extraction method with the Sponsor. All ILV and ECM data regarding repeatability, accuracy, precision, linearity, and specificity were satisfactory for d-phenothrin; the cis- and trans-isomers of phenothrin were not quantified separately.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
d-Phenothrin[1]	50914202	50914203		Surface Water[2,3]	08/09/2017	Sumitomo Chemical Company	LC/MS/MS	0.005 µg/L

[1] Sum of cis- and trans- isomers; Sumithrin.

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[1] MRID 49657901

[2] In the ECM, the surface water matrix (SMV Lot No. 15 May 17 WAT-A; pH 5.81, hardness 12 mg/L $CaCO_3$, alkalinity 6.0 mg/L $CaCO_3$, conductivity 83.6 µS/cm, total organic carbon 9.535 mg/L) was obtained from the Weweantic River, Wareham, Massachusetts (p. 11 of MRID 50914202). The water was collected on May 15, 2017 from an area of the river with *ca*. 30-60 cm of overlying water at a temperature of 13.2°C.

[3] In the ILV, the surface water matrix (pH 7.90, hardness 2.86 mmol/L, conductivity 583 µS/cm at 25°C, dissolved organic carbon 1.5 mg/L, total organic carbon 1.5 mg/L) was obtained locally from River Brenz, Herbrechtingen, Brenz; it was well characterized by Institut Alpha, Ulm Germany (p. 11; Appendix 3, pp. 39-40 of MRID 50914203). The water was collected on April 12, 2017.

I. Principle of the Method

The ECM test material was d-phenothrin [TGAI, technical grade; Sumithrin®; (1R)-isomer], 96.5% (p. 10; Appendix 2, p. 72 of MRID 50914202). The ILV test material was d-phenothrin [(1R)-isomer], 99.8% purity (p. 11; Appendix 1, p. 37 of MRID 50914203). The ratio of trans/cis- isomer was not reported in the ECM or ILV.

Prior to validation, all non-disposable glassware was rinsed with 20% nitric acid solution (p. 15 of MRID 50914202). Samples (500 mL) in a separatory funnel were fortified with 0.0015 or 0.25 mL of 0.100 mg/L fortification solutions, as necessary, then extracted twice using 200 mL of hexane with manual shaking for 30 seconds (pp. 15-18; Figure 1, p. 38). The separatory funnel was rinsed with 50 mL of hexane. The combined organic extracts were reduced to *ca*. 2 mL by rotary evaporation with minimal heating (<35°C). Acetone (100 mL) was added to the round-bottom flask, then the extracts were reduced to *ca*. 5 mL by rotary evaporation with minimal heating (<35°C). The extract was transferred to glass centrifuge tubes with rinsing the round-bottom flask with hexane then acetone. The volume of the extract was reduced to almost dryness using nitrogen. Acetonitrile (1.60 mL) was added to the residue with vortexing (30 seconds) and sonication (5 minutes). The sample was diluted with 0.40 mL of purified reagent water with vortexing (30 seconds) and sonication (5 minutes) to yield a acetonitrile:purified reagent water (80:20, v:v). High fortification samples were further diluted 10x. Samples were transferred to autosampler vials for HPLC/MS/MS analysis.

Samples were analyzed for d-phenothrin using analyzed using an AB Sciex 5000 Q Trap mass spectrometer coupled with a Waters Acquity HPLC (pp. 11, 19-20 of MRID 50914202). The following LC conditions were used: Agilent Poroshell 120 EC-C8 column (3.0 mm x 50 mm, 2.7 µm; column temperature 40°C), gradient mobile phase of A) 10mM ammonium acetate in purified reagent water and B) methanol [time, percent A:B; 0.00 min. 70.0:30.0, 5.00 min. 10.0:90.0, 7.00-9.00 min. 0.00:100, 9.10-10.0 min 70.0:30.0], injection volume of 100 µL, MS/MS with Electrospray Ionization (ESI) Turbo V source in positive polarity (source temperature 500°C). Ions transitions monitored for d-phenothrin were *m/z* 351.4 → 183.2 (quantitation), *m/z* 351.4 → 249.3 (confirmation 1) and *m/z* 351.4 → 305.4 (confirmation 2). Retention time was *ca*. 5.8 minutes for d-phenothrin.

In the ILV, the ECM was performed as written, with insignificant modifications to the analytical instruments and parameters (pp. 12-15; Appendix 4, pp. 41-42 of MRID 50914203). Samples were analyzed for d-phenothrin using an Applied Biosystems API5500 Q-Trap mass spectrometer coupled with an Agilent 1290 Infinity Series HPLC (pp. 12, 14-15 of MRID 50914203). The following LC conditions were used: Agilent Poroshell C8 column (3.0 mm x 50 mm, 2.7 µm; column temperature 40°C), Phenomenex C18 precolumn (4 mm length, 3 mm i.d.), gradient mobile phase of A) 10mM ammonium acetate in water and B) methanol [time, percent A:B; 0.00 min. 70.0:30.0, 5.00 min. 10.0:90.0, 7.00-9.00 min. 0.00:100, 9.10-10.0 min 70.0:30.0], injection volume of 100 µL, MS/MS with Electrospray Ionization (ESI) TurboIonSpray source in positive polarity (source temperature 550°C). Ions transitions monitored for d-phenothrin were *m/z* 351 → 183 (quantitation), *m/z* 351 → 249 (confirmation 1) and *m/z* 351 → 305 (confirmation 2). The monitored ions were similar to those of the ECM. Retention time was *ca*. 6.3 minutes for d-phenothrin. The ILV noted that matrix-matched standards were used since strong suppression was observed for the surface water matrix and that samples were kept at room temperature, instead of 5°C, while standing the autosampler (p. 19 of MRID 50914203).

The Limit of Quantification (LOQ) for d-phenothrin in water was reported as 0.005 µg/L in the ECM and ILV (pp. 21-23 of MRID 50914202; pp. 17, 20 of MRID 50914203). The Limit of Detection (LOD) for d-phenothrin in water were reported as 0.0002-0.0003 µg/L and 0.001 µg/L in the ECM and ILV, respectively.

I. Recovery Findings

ECM (MRID 50914202): Mean recoveries and relative standard deviations (RSDs) met requirements (mean 70-120%; RSD ≤20%) for analysis of d-phenothrin (sum of cis- and trans-isomers) in surface water at the LOQ (0.005 µg/L) and 10×LOQ (0.05 µg/L; Tables 1-3, pp. 30-32). The cis- and trans-isomers of phenothrin were not quantified separately. Three ion transitions were monitored; recovery results of the quantitative and confirmatory ion transitions were comparable. The surface water matrix (SMV Lot No. 15 May 17 WAT-A; pH 5.81, hardness 12 mg/L $CaCO_3$, alkalinity 6.0 mg/L $CaCO_3$, conductivity 83.6 µS/cm, total organic carbon 9.535 mg/L) was obtained from the Weweantic River, Wareham, Massachusetts (p. 11). The water was collected on May 15, 2017 from an area of the river with *ca*. 30-60 cm of overlying water at a temperature of 13.2°C.

ILV (MRID 50914203): Mean recoveries and RSDs met requirements for analysis of d-phenothrin (sum of cis- and trans-isomers) in surface water at the LOQ (0.005 µg/L) and 10×LOQ (0.05 µg/L; Table 1, p. 21). The cis- and trans-isomers of phenothrin were not quantified separately. Three ion transitions were monitored; recovery results of the quantitative and confirmatory ion transitions were comparable. The recoveries at the LOQ were consistently lower than the recoveries at 10×LOQ. The surface water matrix (pH 7.90, hardness 2.86 mmol/L, conductivity 583 µS/cm at 25°C, dissolved organic carbon 1.5 mg/L, total organic carbon 1.5 mg/L) was obtained locally from River Brenz, Herbrechtingen, Brenz; it was well characterized by Institut Alpha, Ulm Germany (p. 11; Appendix 3, pp. 39-40). The water was collected on April 12, 2017. The number of trials was not reported, but the reviewer assumed that the method was validated by the ILV with the first trial as written with insignificant analytical instrument and equipment modifications (pp. 10, 12-15, 19).

Table 2. Initial Validation Method Recoveries for d-Phenothrin in Surface Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Quantitation ion transition (*m/z* 351.4 → 183.2)						
d-Phenothrin	0.005 (LOQ)	5	104-119	110	5.68	5.19
	0.05	5	94.6-116	108	8.62	7.98
Confirmation ion transition 1 (*m/z* 351.4 → 249.3)						
d-Phenothrin	0.005 (LOQ)	5	100-115	105	6.02	5.76
	0.05	5	98.8-114	108	6.43	5.96
Confirmation ion transition 2 (*m/z* 351.4 → 305.4)						
d-Phenothrin	0.005 (LOQ)	5	103-116	108	6.44	5.96
	0.05	5	100-114	108	5.22	4.84

Data (uncorrected results, pp. 21-22) were obtained from Tables 1-3, pp. 30-32 of MRID 50914202.

[1] The surface water matrix (SMV Lot No. 15 May 17 WAT-A; pH 5.81, hardness 12 mg/L $CaCO_3$, alkalinity 6.0 mg/L $CaCO_3$, conductivity 83.6 µS/cm, total organic carbon 9.535 mg/L) was obtained from the Weweantic River, Wareham, Massachusetts (p. 11). The water was collected on May 15, 2017 from an area of the river with *ca*. 30-60 cm of overlying water at a temperature of 13.2°C.

Table 3. Independent Validation Method Recoveries for d-Phenothrin in Surface Water[1]

Analyte	Fortification Level (µg/L)	Number of Tests[2]	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Quantitation ion transition (*m/z* 351 → 183)						
d-Phenothrin	0.005 (LOQ)	5	74-82	77	3	4
	0.05	5	92-97	95	2	2
Confirmation ion transition 1 (*m/z* 351 → 249)						
d-Phenothrin	0.005 (LOQ)	5	73-82	77	4	5
	0.05	5	85-102	97	7	7
Confirmation ion transition 2 (*m/z* 351 → 305)						
d-Phenothrin	0.005 (LOQ)	5	71-89	78	8	11
	0.05	5	92-98	96	3	3

Data (uncorrected results, pp. 15-16) were obtained from Table 1, p. 21 of MRID 50914203.

[1] The surface water matrix (pH 7.90, hardness 2.86 mmol/L, conductivity 583 µS/cm at 25°C, dissolved organic carbon 1.5 mg/L, total organic carbon 1.5 mg/L) was obtained locally from River Brenz, Herbrechtingen, Brenz; it was well characterized by Institut Alpha, Ulm Germany (p. 11; Appendix 3, pp. 39-40). The water was collected on April 12, 2017.

[2] The recovery value for the first sample of each set of five was the mean of two injections.

III. Method Characteristics

The LOQ for d-phenothrin in water was reported as 0.005 µg/L in the ECM and ILV (pp. 21-23 of MRID 50914202; pp. 17, 20 of MRID 50914203). In the ECM, the LOQ was defined as the lowest fortification level; no justification was reported in the ILV. The LOD for d-phenothrin in water were reported as 0.0002-0.0003 µg/L and 0.001 µg/L in the ECM and ILV, respectively, The LOD was calculated in the ECM using the following equation:

$$LOD = (3x(SN_{ctl})/(Resp_{LS}) \text{ x } Conc_{LS} \text{ x } DF_{CTRL}$$

Where, LOD is the limit of detection of the analysis, SN_{ctl} is the mean signal to noise in height of the control samples (or Blanks), $Resp_{LS}$ is the mean response in height of the two low calibration standards, $Conc_{LS}$ is the concentration of the low calibration standard and DF_{CTRL} is the dilution factor of the control samples (smallest dilution factor used, i.e., 0.0800). No calculations or comparisons to noise level were reported for the LOQ in the ECM or ILV. No calculations or comparisons to noise level were reported for the LOD in the ILV.

Table 4. Method Characteristics

		d-Phenothrin[1]
Limit of Quantitation (LOQ)		0.005 µg/L
Limit of Detection (LOD)	ECM (calc)	0.0003 µg/L (Q) 0.0002 µg/L (C1 & C2)
	ILV	0.001 µg/L
Linearity (calibration curve r and concentration range)	ECM	r = 0.998 (Q & C1) r = 0.997 (C2) (0.00500-0.100 µg/L)
	ILV	r = 0.9994 (Q) r = 0.9988 (C1) r = 0.9996 (C2) (0.00500-0.100 µg/L)[2]
Repeatable	ECM[3]	Yes for LOQ and 10×LOQ (one characterized surface water matrix)
	ILV[4,5]	
Reproducible		Yes for LOQ and 10×LOQ
Specific	ECM	Yes, matrix interferences were <6% of the LOQ (based on peak area) for Q and C1 and *ca*. 11% of the LOQ (based on peak area) for C2. A nearby contaminant was noted in the Q chromatogram (RT 6.12 min.; peak height *ca*. 35% of LOQ peak). Baseline noise noted.
	ILV	Yes, matrix interferences were <1% of the LOQ (based on peak area) for all three monitored ion transitions. Multiple nearby contaminants noted which were only significant in C1 chromatogram. Baseline noise noted.

Data were obtained from pp. 21-23 (LOQ/LOD); Tables 1-3, pp. 30-32 (recovery results); p. 23 (correlation coefficients); Figures 3-6, pp. 41-48 (chromatograms) of MRID 50914202; pp. 17, 20 (LOQ/LOD); Table 1, p. 21 (recovery results); Figure 2, pp. 24-25 (correlation coefficients); Figures 5-8, pp. 29-36 (chromatograms) of MRID 50914203. Q = quantitative ion transition; C1 = confirmatory 1 ion transition; C2 = confirmatory 2 ion transition.

[1] Sum of cis- and trans- isomers. 3-Phenoxybenzyl (1R)-*cis-trans*-chrysanthemate; 3-Phenoxybenzyl (1*RS*)-*cis-trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate. The cis- and trans-isomers of phenothrin were not quantified separately.

[2] The 0.005 µg/L calibration standard in surface water gave no detectable response for both confirmatory ion transitions; the 0.010 µg/L calibration standard was the lowest calibrations standard for those calibration curves (p. 19).

[3] In the ECM, the surface water matrix (SMV Lot No. 15 May 17 WAT-A; pH 5.81, hardness 12 mg/L $CaCO_3$, alkalinity 6.0 mg/L $CaCO_3$, conductivity 83.6 µS/cm, total organic carbon 9.535 mg/L) was obtained from the Weweantic River, Wareham, Massachusetts (p. 11 of MRID 50914202). The water was collected on May 15, 2017 from an area of the river with *ca*. 30-60 cm of overlying water at a temperature of 13.2°C.

[4] In the ILV, the surface water matrix (pH 7.90, hardness 2.86 mmol/L, conductivity 583 µS/cm at 25°C, dissolved organic carbon 1.5 mg/L, total organic carbon 1.5 mg/L) was obtained locally from River Brenz, Herbrechtingen,

Brenz; it was well characterized by Institut Alpha, Ulm Germany (p. 11; Appendix 3, pp. 39-40 of MRID 50914203). The water was collected on April 12, 2017.

[5] The number of trials was not reported, but the reviewer assumed that the method was validated by the ILV with the first trial as written with insignificant analytical instrument and equipment modifications (pp. 10, 12-15, 19 of MRID 50914203).

IV. Method Deficiencies and Reviewer's Comments

1. A method validation for total d-phenothrin (sum of cis- and trans- isomers) in surface water was previously submitted and reviewed by CDM Smith/CSS-Dynamac JV. A DER was prepared for MRIDs 49564001 & 49564002 in 2016 by primary reviewer Lisa Muto and QC/QA manager Joan Gaidos. The ECM, PRTL Europe ID P 3046 G, is designed for the quantitative determination of the total d-phenothrin (sum of cis- and trans- isomers) at 0.1 µg/L in surface water using GC/MS. The reviewer determined that an updated ECM should be submitted to provide precautions and optional steps to prevent loss of analyte during the extraction procedure. In MRIDs 49564001 & 49564002, the recoveries of the cis and trans isomers were independently calculated then summed to determine total d-phenothrin recovery.

2. The number of trials was not reported in the ILV, but the reviewer assumed that the method was validated by the ILV with the first trial as written with insignificant analytical instrument and equipment modifications (pp. 10, 12-15, 19 of MRID 50914203).

3. Only surface water matrices were used in ECM and ILV. It was not reported if the method would be expected to produce acceptable results in other water matrices, such as ground water and drinking water.

4. The ILV reported that no communications occurred with the method developing laboratory (p. 18 of MRID 50914203). Prior to the beginning of the study, the ILV clarified some parts of the extraction method with the Sponsor. The details of this communication were not reported. The review believed that this communication should have been detailed to determine what parts of the extraction method required clarification or further explanation.

5. The ratio of trans/cis- isomer of the test material, d-phenothrin, was not reported in the ECM or ILV. The cis- and trans-isomers of phenothrin were not quantified separately in the ECM and ILV.

6. Although the calibration range was 0.00500-0.100 µg/L and the LOQ was 0.005 µg/L, sample preparation caused the analytical response of the LOQ and 10×LOQ samples to be below the two highest calibration standards (Figures 3-6, pp. 41-48 and Figures 10-12, pp. 55-60 of MRID 50914202; Figure 2, pp. 24-25 and Figures 5-8, pp. 29-36 of MRID 50914203).

7. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 21-23 of MRID 50914202; pp. 17, 20 of MRID 50914203). In the ECM, the LOQ was defined as the lowest fortification level; no justification was reported in the ILV. The LOD was calculated in the ECM using the following equation: LOD = $(3x(SN_{ctl})/(Resp_{LS}) \times Conc_{LS} \times DF_{CTRL}$, where LOD is the limit of detection of the analysis, SN_{ctl} is the mean signal to noise in height of the control samples (or Blanks), $Resp_{LS}$ is the mean response in height of the two low calibration standards, $Conc_{LS}$ is the concentration of the low calibration standard and DF_{CTRL} is the dilution factor of the control samples (smallest dilution factor used, i.e., 0.0800). No calculations or comparisons to noise level were reported for the LOQ in the ECM or ILV. No calculations or comparisons to noise level were reported for the LOD in the ILV. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

8. In the ECM, no significant matrix effects were observed (<20%; p. 24; Table 4, p. 33 of MRID 50914202). In the ILV, significant matrix effects (>20%) were observed so matrix-match calibration standards were employed (p. 18; Table 2, p. 22 of MRID 50914203).

9. In the ECM, final fraction storage stability and solvent standard stability were determined to be up to 21 days at *ca*. 1-10°C (p. 24; Tables 5-8, pp. 34-37 of MRID 50914202).

10. It was reported in the ILV that one subset of 13 samples (one reagent blank, two matrix blanks, five samples dosed at the LOQ and 10×LOQ) required *ca*. 9 hours to complete the sample processing (p. 18 of MRID 50914203). Subsequent LC/MS/MS analysis and evaluation required an additional *ca*. 6 hours. The overall time for a sample set was *ca*. 2 calendar days.

V. References

U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

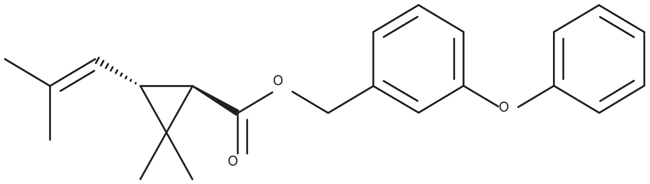
40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

d-Phenothrin (1R trans/cis ratio not reported) [Sumithrin]

IUPAC Name:	3-Phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate. 3-Phenoxybenzyl (1*RS*)-*cis-trans*-2,2-dimethyl-3-(2-methylprop-1-enyl)cyclopropanecarboxylate. 3-Phenoxybenzyl (±)-*cis-trans*-chrysanthemate. 3-Phenoxybenzyl (1R)-*cis-trans*-chrysanthemate.
CAS Name:	(3-Phenoxyphenyl)methyl 2,2-dimethyl-3-(2-methyl-1-propen-1-yl)cyclopropanecarboxylate.
CAS Number:	26002-80-2 51186-88-0 (cis) 26046-85-5 (trans)
SMILES String:	CC(C)=CC3C(C(=O)OCc2cccc(Oc1ccccc1)c2)C3(C)C (EpiSuite version 4.0).

(1R)-*trans*-Phenothrin



(1R)-*cis*-Phenothrin