Methoxyfenozide/ PC Code: 121027/Rohm and Haas/EPA Company Code: 707/
**ENVIRONMENTAL CHEMISTRY METHOD REVIEW REPORT**

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**Data Requirement:** OECD Data Point: IIA 4.5
PMRA Data Code: 8.2.2.3
EPA Guideline: 835.6100

**Test material:**
Common name: methoxyfenozide
Chemical name: benzoic acid, 3-methoxy-2-methyl-,2-(3,5-dimethylbenzoyl)-2-(1,1-dimethylethyl)hydrazide
IUPAC: N-(1,1-dimethylethyl)-N'-(3-methoxy-2-methylbenzoyl)-3,5-dimethylbenzohydrazide

**Primary Reviewer:** _____ **Date:** 12-JUL-2010
Chuck Peck,
Environmental Engineer,
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**Secondary Reviewer:** _____ **Date:** 7/13/2010
R. David Jones,
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**ANALYTICAL METHOD:** MRID 47809902. Schramel. O. October 15, 1999. Enforcement/Confirmatory Method 005451 (MR-050/99) for Liquid Chromatographic Determination of RH-2485 in Soil *Unpublished study prepared by* Bayer AG and *submitted by* Rohm and Haas. 41 *pages.* Rohm and Haas Study ID: 34-99-175

**INDEPENDENT LABORATORY VALIDATION:** none

## EXECUTIVE SUMMARY

This study describes two methods for the quantitative determination of methoxyfenozide in soil. The first method is identified as an "enforcement method" and the second as the "confirmatory method". The methods were created by Bayer AG in Leverkusen, Germany and submitted by Rohm and Haas in accordance with EPA's Good Laboratory Practice Standards, Title 40 Code of Federal Regulations Part 160. After a thorough review, the Agency found that this method does not meet the criteria for a scientifically valid method and is **not acceptable** for methoxyfenozide because no Independent Laboratory Validation were submitted for the methods. This study can be upgraded to supplemental or acceptable if an acceptable ILV is submitted.

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**Method Summary:** These two methods are essentially identical except that Method 1 uses a Superspher 100 RP-18 column and the second method uses a Zorbax SB-CN column. Soil samples are extracted using boiling methanol/water/1 m HCl in the ratio 900/100/5 v/v/v. The extracts are cleaned up using Envi-carbon SPE-cartridges and the solvent evaporated to dryness. Residues are reconstituted in 2 or 10 mL of 50:50 v:v water/acetonitrile. Identification and quantification is done using high-performance liquid chromatography with UV detection. The limits of detection and quantification were 3 µg/kg and 10 µg/kg, respectively. Mean recoveries in the range 9.8 to 98 µg/kg were 97.8% with a relative standard deviation of 7.0% using the Superspher column and 96.6% with a 11.1% RSD for the Zorbax column

## METHOD ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS

This method is **unacceptable** because no independent laboratory validation study has been submitted with the method.

## COMPLIANCE

Signed and dated statements that this method was conducted in accordance with the requirements for Good Laboratory Practice Standards, 40 CFR 160 were present in the method. Also present was a statement of non-confidentiality on the basis of the method falling within the scope of FIFRA Section 10 (d)(1)(A), (B), or (C).]

## A. BACKGROUND INFORMATION

Methoxyfenozide belongs to the diacyhydrazine class of insecticides that interferes with the binding of the endogenous steroidal molting hormone with its nuclear receptor protein complex. After ingestion of toxic doses, sensitive larvae stop feeding and the molting process initiates prematurely leading to desiccation and ultimately, death. Methoxyfenozide is currently registered for use on corn, cotton, cranberries, ornamentals, cucurbit vegetables, grapes, pome and stone fruits, root vegetables, spearmint and peppermint, berries (including strawberries and cranberries), tree nuts, leafy vegetables, globe artichokes, legume vegetables, a variety of tropical fruits, tuberous and corm vegetables (except potato), dry beans, peanuts, grass and non-grass forage, fodder, hay, and straw, avocados, a variety of green onions and black-eyed and Southern peas.

**TABLE A.1. Test Compound Nomenclature**

| Parameter | Value |
|---|---|
| Common name | methoxyfenozide |
| Company experimental name | RH-2485 |
| IUPAC name | N-(1,1-dimethylethyl)-N′-(3-methoxy-2-methylbenzoyl)-3,5-dimethylbenzohydrazide |

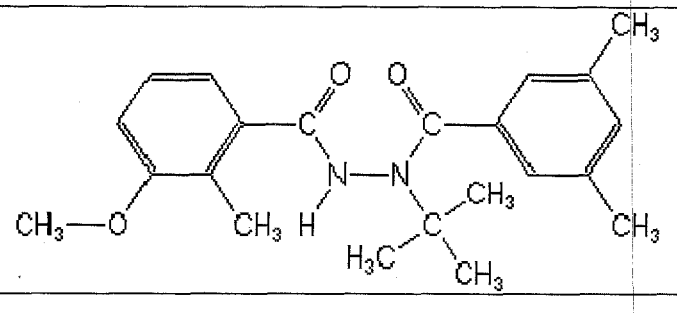
| | |
|---|---|
| CAS Name | benzoic acid, 3-methoxy-2-methyl-,2-(3,5-dimethylbenzoyl)-2-(1,1-dimethylethyl)hydrazide |
| CAS # | 161050-58-4 |
| Structure |  |

**TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound**

| Parameter | Value |
|---|---|
| Melting point/range | 203.8-206.4 |
| pH | not applicable |
| Density | not available |
| Water solubility (g/L 20 °C) | water: $3.3 \times 10^{-3}$ |
| Solvent solubility (g/L at 20 °C) | acetone: 100.2<br>dichloromethane: 45.4<br>methanol: 152.7<br>2-propanol: 39.4<br>xylene: 2.9 |
| Vapor pressure at 25°C | $2.46 \times 10^{-8}$ torr |
| Dissociation constant ($pK_a$) | NA |
| Octanol/water partition coefficient | $5.25 \times 10^{3}$ |
| UV/visible absorption spectrum | not available |

## B. MATERIALS AND METHODS

### B.1. Principle of Method

*Method 1, Enforcement Method*: Soil samples of 25 g are weighed into an extraction thimble and covered with defatted cotton wool plug. 40 mL of methanol/water/1m HCl in the ratio 900/100/5 v/v/v, along with some boiling chips, are placed into aluminum cups and inserted with the thimble into a Soxtec extraction device. The oil bath temperature is set at 200°C. The extraction time is 1 hour. Afterwards, the thimbles are placed in a rinse position for 30 minutes until the extraction is terminated. The residue is flushed quantitatively into a 50 mL centrifuge tube by rinsing the aluminum caps 2 times with 5 mL of ethanol. The extract is evaporated to dryness in a Turbo-Vap evaporator at 50°C and reconstituted in 5mL of methanol/water (40:60, v/v).

The extract is added to a preconditioned SPE cartridge (10 mL of methanol and 10 mL of water using a Varian SPS vacuum manifold or similar apparatus) packed with 0.5g of Envi-carbon and the flask is rinsed with 5 mL of methanol/water (40:60, v/v), with the rinsate also being added to the SPE cartridge. The cartridge is washed with an additional 10 mL of methanol/water (40:60, v/v), with all washings solutions being discarded. Elute the Envi-carbon with 20 mL of methanol and evaporate the methanol solution to dryness. Reconstitute the methanol solution with 2 mL of 50:50 v:v water/acetonitrile. Identification and quantification is done using high-performance liquid chromatography with UV detection.

A 50 µL aliquot from the extract is injected into an HPLC using a 250 mm Superspher column at 40 °C. The eluent was water+1.0 mL phosphoric acid per liter:acetonitrile at a flow rate of 1 mL/min at the following ratios:

| Time (min) | 0 | 7 | 10 | 15 | 18 | 20 |
|---|---|---|---|---|---|---|
| Water+1.0mL phosphoric acid per liter | 35 | 35 | 10 | 10 | 35 | 35 |
| Acetonitrile | 65 | 65 | 90 | 90 | 65 | 65 |

Detection of peaks was made with a UV detector set a 220 nm. Retention time for methoxyfenozide was 5.6 min.

*Method 2, Confirmatory Method:* For this method, the same extraction method used in the enforcement method is used. Identification and quantification is done using high-performance liquid chromatography with UV detection.

A 50 µL aliquot of the extract is injected into an HPLC using a 250 mm Zorbax SB-CN column at 40 °C The eluent was water+1.0 mL phosphoric acid per liter:acetonitrile at a flow rate of 1 mL/min at the following ratios:

| Time (min) | 0 | 10 | 20 | 23 | 26 | 29 | 34 |
|---|---|---|---|---|---|---|---|
| Water+1.0mL phosphoric acid per liter | 80 | 60 | 60 | 10 | 10 | 80 | 80 |
| Acetonitrile | 20 | 40 | 40 | 90 | 90 | 20 | 20 |

Detection of peaks was made with a UV detector set a 220 nm. Retention time for methoxyfenozide was 22.8 min.

**TABLE B.1.1 Summary Parameters for the On-line Concentration Analytical Method Used for the Quantitation of Chemical Residues in Matrices Studied**

| Parameter | Value |
|---|---|
| Method ID | "enforcement" |
| Analyte(s) | methoxyfenozide |

| **TABLE B.1.1 Summary Parameters for the On-line Concentration Analytical Method Used for the Quantitation of Chemical Residues in Matrices Studied** | |
|---|---|
| Extraction solvent/technique | methanol/water/1m HCl (900:100:5 v/v/v) |
| Cleanup strategies | SPE cartridge packed with Envi-carbon, rinsed with methanol/water (40:60, v/v) |
| Instrument/Detector | HPLC with Superspher 100 RP-18 column and UV detection |

| **TABLE B.1.2 Summary Parameters for the Off-line Concentration Analytical Method Used for the Quantitation of Chemical Residues in Matrices Studied** | |
|---|---|
| **Parameter** | **Value** |
| Method ID | "confirmatory" |
| Analyte(s) | methoxyfenozide |
| Extraction solvent/technique | methanol/water/1m HCl (900:100:5 v/v/v) |
| Cleanup strategies | SPE cartridge packed with Envi-carbon, rinsed with methanol/water (40:60, v/v) |
| Instrument/Detector | HPLC with Zorbax SB-CN column and UV detection |

## C. RESULTS AND DISCUSSION

### C.1. Recovery Results Summary

**TABLE C.1.1 Recovery Results from Method Validation of Matrices Studied Using Method 1: Enforcement Method**

| Matrix | Spiking Level (µg/kg) | % Recoveries | Relative Standard Deviation | % Recoveries | Relative Standard Deviation |
|---|---|---|---|---|---|
| LUFA 2.2<br>Höfchen soil | 9.8 | 95.7<br>104.8 | 6.8<br>10.9 | 99.8 | 9.7 |
| LUFA 2.2<br>Höfchen soil | 98 | 96.0<br>95.9 | 1.5<br>2.8 | 96.0 | 2.1 |

**TABLE C.1.2 Recovery Results from Method Validation of Matrices Studied Using Method 1: Confirmatory Method**

| Matrix | Spiking Level (µg/kg) | % Recoveries | Relative Standard Deviation | % Recoveries | Relative Standard Deviation |
|---|---|---|---|---|---|
| LUFA 2.2<br>Höfchen soil | 9.8 | 88.3<br>110.0 | 13.1<br>8.6 | 97.9 | 15.5 |
| LUFA 2.2<br>Höfchen soil | 98 | 91.9<br>99.0 | 2.7<br>3.6 | 95.5 | 4.9 |

### C.1.1. Method Characteristics

| **TABLE C.2.1 Enforcement Method Characteristics for Analysis of Methoxyfenozide** | |
|---|---|
| **Parameter** | **Value** |
| Analyte | methoxyfenozide |
| Limit of Quantitation | 10 µg/kg |
| Limit of Detection (LOD) | 3 µg/kg |
| Accuracy/Precision at LOQ | not addressed |
| Reliability of the Method/[ILV] | No ILV |
| Linearity | quadratic term is not statistically significant at $p < 0.05$ |
| Specificity | not addressed |

| **TABLE C.2.1 Confirmatory Method Characteristics for Analysis of Methoxyfenozide** | |
|---|---|
| **Parameter** | **Value** |
| Analyte | methoxyfenozide |
| Limit of Quantitation | 10 µg/kg |
| Limit of Detection (LOD) | 3 µg/kg |
| Accuracy/Precision at LOQ | not addressed |
| Reliability of the Method/[ILV] | No ILV |
| Linearity | quadratic term is not statistically significant at $p < 0.05$ |
| Specificity | not addressed |

### C.2. Independent Laboratory Validation (ILV)

No independent laboratory validation has been submitted to support this study.

## D. CONCLUSION

This study is scientifically sound but unacceptable for use in fulfilling data requirements for the registration of methoxyfenozide as a pesticide as there was no ILV to support the method.

*Comments*

1. The primary method was calibrated using standard ranging from 0.06 to 2.45 µg/L.
2. The authors tested the linearity of the detector in the calibration and cited the correlation coefficient of 0.9999 for the Zorbax SB-CN column and 1.0 for the Superspher 100 RP-18 column as evidence of linearity. However, the correlation coefficient is a measure of the precision of the relation and not the linearity.

Calibration data were fit with a quadratic model ($Y = B_0 + B_1X + B_2X^2$) and there does not appear to be any statistical significance ($p>0.05$) based on non-linearity.