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

Data Requirement: OECD Data Point: IIA 4.5
PMRA Data Code: 8.2.2.3
EPA Guideline: 835.6200

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: Chuck Peck **Date:** 12-Jul 2010
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ANALYTICAL METHOD: MRID 47824201, Sommer H., November 5, 1999. Enforcement and Confirmatory Method 00608 (MR-385/99) for Determination of RH-2485 in Surface Water by HPLC *Unpublished study prepared by* Bayer and *submitted by* Rohm and Haas, July 21, 2009. 32 *pages.* Rohm and Haas Study ID: 34-99-176

INDEPENDENT LABORATORY VALIDATION: none

EXECUTIVE SUMMARY

This study describes two methods for the quantitative determination of methoxyfenozide in water, specifically surface water. 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.

Method Summary: *Method 1, Enforcement Method:* Water samples are concentrated by on-line or off-line solid phase extraction. Using the on-line method samples are concentrated using a Merck OSP-2A On-line Sample Preparator. In the concentration range of 0.05 to 0.5 µg/L, 50 mL of sample are required. Samples are analyzed by HPLC with UV detection. The limit of detection was not reported. The limit of quantification was 0.05 µg/L. Recoveries were 87% with a relative standard deviation (RSD) of 4.6% at the limit of quantification and 83% with an RSD of 9.9% at 0.5 µg/L.

Method 2, Confirmatory Method: For off-line concentration, a C_{18} cartridge is washed with 10 mL of acetonitrile followed by 10 mL of distilled, deionized water. This conditioning step is then followed by pulling 200 mL of sample water through the column at 5 mL per minute. The cartridge is then dried by pulling ambient air through the cartridge for 1 hr. During drying, an activated carbon cartridge is place before the inlet so the air does not contaminate the cartridge. Following drying, 3 mL of acetonitrile is eluted through the cartridge. The acetonitrile is evaporated to dryness and reconstituted in 1 mL of 2:8 v:v acetonitrile-water solution. Samples are analyzed by HPLC with UV detection. The limit of detection was not reported. The limit of quantification was 0.05 µg/L. Recoveries were 101% with a relative standard deviation (RSD) of 2.1% at 0.05 µg/L and 105% with an RSD of 4.4% at 0.5 µg/L.

METHOD ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS

These methods are **unacceptable** because no independent laboratory validation has been submitted with the method. Additionally, no level of detection was reported.

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 nongrass forage, fodder, hay, and straw, avocados, a variety of green onions and black-eyed and Southern peas.

TABLE A.1. Test Compound Nomenclature

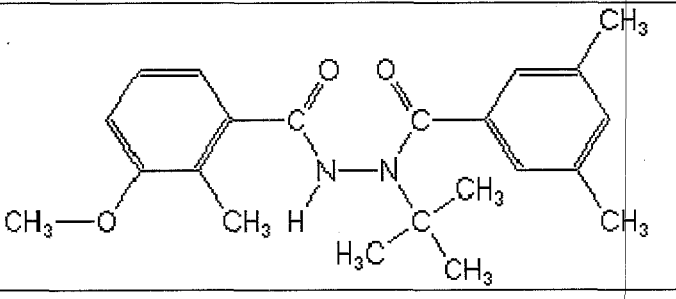
Parameter	Value
Common name	methoxyfenozide
Company experimental name	RH-2485
IUPAC name	N-(1,1-dimethylethyl)-N′-(3-methoxy-2-methylbenzoyl)-3,5-dimethylbenzohydrazide
CAS Name	benzoic acid, 3-methoxy-2-methyl-,2-(3,5-dimethylbenzoyl)-2-(1,1-dimethylethyl)hydrazide
CAS #	161050-58-4
Structure	

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: 3.3×10^{-3}
Solvent solubility (g/L @ 20 °C)	acetone: 100.2 dichloromethane: 45.4 methanol: 152.7 2-propanol: 39.4 xylene: 2.9
Vapor pressure at 25°C	2.46×10^{-8} torr
Dissociation constant (pK_a)	NA

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound	
Parameter	**Value**
Octanol/water partition coefficient	5.25×10^3
UV/visible absorption spectrum	not available

B. MATERIALS AND METHODS

B.1. Principle of Method

Method 1, Enforcement Method: This method concentrates samples on-line prior to injection into the HPLC. Sample concentration is made using a Merck OSP-2A On-line Sample Preparator. In the concentration range of 0.05 to 0.5 µg/L, 50 mL of sample are required. A 50 mL aliquot from the sample preparator is injected into an HPLC using a 250 mm C_{18} column at 40 °C. The eluent is water:acetonitrile at a 55:45 ratio volume to volume at a flow rate of 1 mL/min. Detection of peaks iss made with a UV detector set a 204 nm. Retention time for methoxyfenozide is approximately 15.6 min.

Method 2, Confirmatory Method: For off-line concentration, a C_{18} cartridge is washed 10 mL of acetonitrile followed by 10 mL of distilled, deionized water. This conditioning step is then followed by pulling 200 mL of sample water through the column at 5 mL per minute. The cartridge is then dried by pulling ambient air through the cartridge for 1 hr. During drying, an activated carbon cartridge is place before the inlet so the air does not contaminate the cartridge. Following drying, 3 mL of acetonitrile is eluted through the cartridge. The acetonitrile is evaporated to dryness and reconstituted in 1 mL of 2:8 v:v acetonitrile-water solution.

A 250 µL aliquot of the extract is injected into an HPLC using a 250 mm CN column at 40 °C. The eluent is water:acetonitrile at a 7:3 ratio volume to volume at a flow rate of 1 mL/min. Detection of peaks is made with a UV detector set a 204 nm. Retention time for methoxyfenozide is approximately 14.1 min.

TABLE B.1.1 Summary Parameters for the On-line Concentration Analytical Method Used for the Quantitation of Methoxyfenozide in Water	
Parameter	**Value**
Method ID	"enforcement" or "on-line concentration "
Analyte(s)	methoxyfenozide
Extraction solvent/technique	on-line concentration using OSP-2A method preparatory from Merck
Cleanup strategies	none described
Instrument/Detector	HPLC with C-18 column and UV detection

TABLE B.1.2 Summary Parameters for the Off-line Concentration Analytical Method Used for the Quantitation of Methoxyfenozide in Water	
Parameter	**Value**
Method ID	"confirmatory" or "off-line concentration "
Analyte(s)	methoxyfenozide
Extraction solvent/technique	solid phase extraction on C_{18} cartridge, elution with acetonitrile
Cleanup strategies	none described
Instrument/Detector	HPLC with 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/L)**	**% Recoveries**	**Relative Standard Deviation**
Rhine River water	0.05	87	4.6
Rhine River water	0.5	83	9.9

TABLE C.1.2 Recovery Results from Method Validation of Matrices Studied Using Method 2: Confirmatory Method			
Matrix	**Spiking Level (µg/L)**	**% Recoveries**	**Relative Standard Deviation**
Rhine River water	0.05	101	2.1
Rhine River water	0.5	104	4.4

C.1.1. Method Characteristics

TABLE C.2.1 Enforcement Method Characteristics for Analysis of Methoxyfenozide	
Parameter	**Value**
Analyte	methoxyfenozide
Limit of Quantitation	0.05 µg/L
Limit of Detection (LOD)	not addressed
Accuracy/Precision at LOQ	not addressed
Reliability of the Method/[ILV]	No ILV
Linearity	quadratic term is 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	0.05 µg/L
Limit of Detection (LOD)	not reported
Accuracy/Precision at LOQ	not addressed
Reliability of the Method/[ILV]	No ILV
Linearity	quadratic term is 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 5.4 to 1074 µg/L. The low end of this range is over 100x the quantification limit, so the method is poorly calibrated between the quantification limit and 5.4 µg/L.
2. The authors tested the linearity of the detector in the calibration and cited the correlation coefficient of 0.99996 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 the quadratic term was significant at $p = 0.05$ indicating that there is statistically significant non-linearity in the calibration. However, deviations from the non-linear fit were a trivial component of the residual when compared to the linear fit. As such, extrapolation beyond the calibration range will result in an increase in bias and should not be performed.