

**Analytical method for MGK-264 in water**

**Reports:** ECM: EPA MRID No.: 50105601. Acedo, K.P. 2015. Validation of Method GPL-MTH-076: Analytical Procedure for the Determination of MGK-264 in Water (Amended Study Report). GPL Study No.: 110375. Report prepared by Golden Pacific Laboratories, LLC (GPL), Fresno, California, and sponsored and submitted by McLaughlin Gormley King Company, Minneapolis, Minnesota; 78 pages. Final original report issued June 26, 2012; amended report issued May 15, 2015.

ILV: EPA MRID No. 49822501. Claussen, F. 2016. Independent Laboratory Validation of Analytical Method GPL-MTH-076 (Revision 1) for the Determination of MGK-264 in Water. EPL BAS Study No.: 578G1120. Report prepared by EPL Bio Analytical Services (EPL BAS), Niantic, Illinois, sponsored and submitted by McLaughlin Gormley King Company, Minneapolis, Minnesota; 90 pages. Final report issued January 20, 2016.

**Document No.:** MRIDs 50105601 & 49822501

**Guideline:** 850.6100

**Statements:** ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards, with the exception of the surface water characterizations by BSK Laboratories (p. 3 of MRID 50105601). 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 (p. 4). The Amended Final Report Approvals page was also signed and dated.


ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 5 of MRID 49822501). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 4-6). A statement of the authenticity of the study report was included with the quality assurance statement (p. 6).

**Classification:** This analytical method is classified as **acceptable**. Performance data to validate the method at 10×LOQ was not reported for both matrices in the ILV and for one matrix in the ECM. One of the ECM water matrices was not characterized. The LOD for the method was not reported in the ECM or ILV.

**PC Code:** 057001

**Final EPA  
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**Date:** 11/29/18

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Lisa Muto,  
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*This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.*

## Executive Summary

This analytical method, Analytical Method GPL-MTH-076 (Revision 1), is designed for the quantitative determination of MGK-264 in water at the LOQ of 0.05 ng/mL using HPLC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water. The LOQ in the original method was 1.00 ng/mL; the LOQ in the amended method was 0.05 ng/mL. The ILV was based on the amended ECM method. The ECM used uncharacterized drinking (tap) water and characterized surface ground (river) water matrices; the ILV used characterized drinking (tap) water and surface (lake) water matrices. Performance data to validate the method at 10×LOQ was not reported for both matrices in the ILV and for one matrix in the ECM. Although the specific number of trials was not reported, the reviewer assumed that the method was validated for both matrices after one trial with insignificant modifications to the analytical method. All submitted ILV and ECM data pertaining to linearity, repeatability, reproducibility and specificity was acceptable. However, the LOD for the method was not reported the ECM or ILV.

**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						
MGK-264 <sup>1</sup>	50105601 <sup>2</sup>	49822501 <sup>3</sup>		Water	15/05/2015 (Amended) 26/06/2012 (Original)	McLaughlin Gormley King Company	LC/MS/MS	0.05 ng/mL

1 n-Octyl bicycloheptene dicarboximide.

2 In the ECM, the drinking (tap) water matrix was obtained from the Fresno municipal supply at GPL; a Water Quality Report for the tap water was provided, but the water sample was not characterized (p. 14; Appendix 4, pp. 61-78 of MRID 50105601). The surface ground water matrix (pH 7.7, 21 mg/L total hardness as CaCO<sub>3</sub>, 2.2 mg/L total organic carbon and dissolved organic carbon) was obtained from the San Joaquin River in Fresno, California at a point near Gravel Haul Road.

3 In the ILV, the drinking (tap) water matrix (pH 8.0, 123 mg/L total hardness as CaCO<sub>3</sub>, 1.4 ppm total organic carbon, 0.7 ppm dissolved organic carbon) was obtained from a tap at EPL (p. 14; Appendix B, pp. 68-69 of MRID 49822501). The surface water matrix (pH 8.3, 262 mg/L total hardness as CaCO<sub>3</sub>, 3.3 ppm total organic carbon, 2.2 ppm dissolved organic carbon) was obtained from Lake Decatur, Decatur, Illinois.

Page numbers cited for MRID 49822501 refer to those listed in the bottom-center of the document pages.

## I. Principle of the Method

Water samples (5 mL) were fortified with MGK-264 and diluted with 5 mL of acetonitrile in a 16-mL vial (pp. 16-17 of MRID 50105601). After shaking gently, the samples were filtered through a PTFE 0.45- $\mu$ m syringe filter. The samples were further diluted with acetonitrile:water (50:50, v:v), if necessary, to a concentration within the range of the standard curve and analyzed by HPLC/MS/MS.

Samples were analyzed for MGK-264 using an AB Sciex API 4000 or API5000 LC/MS/MS equipped with a Phenomenex Luna 3  $\mu$  C18 100A column (2.00 mm x 30 mm, 3  $\mu$ m) using a mobile phase of (A) 0.2% formic acid in acetonitrile and (B) 0.2% formic acid in water [API4000: percent A:B at 0 min. 50:50, 3.0 min. 80:20, 3.1-4.5 min. 50:50; API5000: percent A:B at 0 min. 50:50, 3.4 min. 80:20, 3.5-5.0 min. 50:50] with MS/MS-ESI (electrospray ionization) detection in positive ion mode and multiple reaction monitoring (MRM; pp. 17-18 of MRID 50105601). Injection volume was 20  $\mu$ L [0.05 ng/mL LOQ; 10  $\mu$ L (1.0 ng/mL LOQ)]. MGK-264 was identified using two ion transitions (quantitation and confirmation, respectively):  $m/z$  276.2 $\rightarrow$ 210.1 and  $m/z$  276.2 $\rightarrow$ 98.0. Expected retention times were *ca.* 2.2 and 2.5 minutes for MGK-264 (2 isomers).

In the ILV, the ECM was performed as written with insignificant modifications to the re-issued analytical method (pp. 15-16 of MRID 49822501). An Agilent 1290 Infinity Series LC System was coupled to an API Sciex Triple Quad 6500 MS/MS Detector for analyte identification. The ECM mobile phase for API5000 was used; injection volume was 20  $\mu$ L. MGK-264 was identified using the same two ion transitions; expected retention times were *ca.* 1.8 and 2.2 minutes for MGK-264 (2 isomers). No other modifications of the ECM were reported.

The Limit of Quantification (LOQ) for water was 0.05 ng/mL in the ECM and ILV (pp. 11, 20-21 of MRID 50105601; pp. 2, 13, 19 of MRID 49822501). In the ECM, the LOQ of the original method was 1.0 ng/mL; the amendment to the method added the extra validation set with the lower LOQ. The Limit of Detection (LOD) was not reported in the ECM and ILV.

## II. Recovery Findings

ECM (MRID 50105601): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD  $\leq$ 20%) for analysis of MGK-264 at fortification levels of 0.05 ng/mL (LOQ), 1.00 ng/mL (20 $\times$ LOQ), 20.0 ng/mL (400 $\times$ LOQ) and 500 ng/mL (10,000 $\times$ LOQ) in the tap water matrix and 0.05 ng/mL (LOQ) and 0.5 ng/mL (10 $\times$ LOQ) in the surface ground water matrix (Tables 1-3, pp. 23-25). No tap water samples were prepared at 10 $\times$ LOQ. The LOQ in the original method was 1.00 ng/mL; the LOQ in the amended method was 0.05 ng/mL. Two ion transitions were reported in the method, but data for only the quantitation ion transition was reported. A confirmatory method is not usually required when LC/MS and GC/MS is the primary method. The drinking (tap) water matrix was obtained from the Fresno municipal supply at GPL; a Water Quality Report for the tap water was provided, but the water sample was not characterized (p. 14; Appendix 4, pp. 61-78). The surface ground water matrix (pH 7.7, 21 mg/L total hardness as CaCO<sub>3</sub>, 2.2 mg/L total organic carbon and dissolved

organic carbon) was obtained from the San Joaquin River in Fresno, California at a point near Gravel Haul Road. The reviewer assumed that the same tap water source was used for the original and amended method.

ILV (MRID 49822501): Mean recoveries and RSDs were within guideline requirements for analysis of MGK-264 the tap water and surface ground water matrices at fortification levels of 0.05 ng/mL (LOQ) and 1.00 ng/mL (20×LOQ; Tables 5-6, p. 25). No samples were prepared at 10×LOQ for either water matrix. The ILV was based on the amended ECM method. MGK-264 was identified using two ion transitions; performance data (recovery results) from quantitation and confirmation analyses were comparable. Recovery results were corrected for residues found in the controls (pp. 16-17). The drinking (tap) water matrix (pH 8.0, 123 mg/L total hardness as CaCO<sub>3</sub>, 1.4 ppm total organic carbon, 0.7 ppm dissolved organic carbon) was obtained from a tap at EPL (p. 14; Appendix B, pp. 68-69). The surface water matrix (pH 8.3, 262 mg/L total hardness as CaCO<sub>3</sub>, 3.3 ppm total organic carbon, 2.2 ppm dissolved organic carbon) was obtained from Lake Decatur, Decatur, Illinois. Although the specific number of trials was not reported, the reviewer assumed that the method was validated for both matrices after one trial with insignificant modifications to the analytical method (pp. 13, 15-16, 19).

**Table 2. Initial Validation Method Recoveries for MGK-264 in Water<sup>1,2,3</sup>**

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Drinking (Tap) Water (Original Method)</b>						
MGK-264	1.00 <sup>4</sup>	7	91.4-103	97.3	3.50	3.60
	20.0	7	98.5-104	102	1.82	1.79
	500	7	108-113	110	1.80	1.63
<b>Drinking (Tap) Water (Amended Method)</b>						
MGK-264	0.05 (LOQ) <sup>5</sup>	7	107-117	114	3.55	3.11
<b>Surface Ground (River) Water (Amended Method)</b>						
MGK-264	0.05 (LOQ) <sup>5</sup>	7	105-123	117	6.18	5.28
	0.5 <sup>5</sup>	7	101-107	104	1.99	1.91

Data (uncorrected recovery results, pp. 19-20) were obtained from Tables 1-3, pp. 23-25 of MRID 50105601.

1 The drinking (tap) water matrix was obtained from the Fresno municipal supply at GPL; a Water Quality Report for the tap water was provided, but the water sample was not characterized (p. 14; Appendix 4, pp. 61-78). The surface ground water matrix (pH 7.7, 21 mg/L total hardness as CaCO<sub>3</sub>, 2.2 mg/L total organic carbon and dissolved organic carbon) was obtained from the San Joaquin River in Fresno, California at a point near Gravel Haul Road.

2 The reviewer assumed that the same tap water source was used for the original and amended method.

3 Data for only the quantitation ion transition was reported; a confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

4 LOQ in the original method.

5 Nominal fortification levels were reported in the table above; actual fortification levels were 0.0535 ng/mL (LOQ) and 0.520 ng/mL (10×LOQ; Tables 2-3, pp. 24-25).

**Table 3. Independent Validation Method Recoveries for MGK-264 in Water<sup>1,2</sup>**

Analyte	Fortification Level (ng/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
<b>Drinking (Tap) Water</b>						
Quantitation Ion Transition						
MGK-264	0.0501 (LOQ)	7	96.1-117	108	6.25	5.80
	1.00	7	92.0-102	99.3	3.43	3.46
Confirmation Ion Transition						
MGK-264	0.0501 (LOQ)	7	99.3-125	118	8.95	7.57
	1.00	7	91.7-102	97.9	3.50	3.58
<b>Surface (Lake) Water</b>						
Quantitation Ion Transition						
MGK-264	0.0501 (LOQ)	7	105-112	109	3.01	2.78
	1.00	7	98.8-105	102	2.24	2.20
Confirmation Ion Transition						
MGK-264	0.0501 (LOQ)	7	99.0-114	106	5.75	5.43
	1.00	7	97.0-101	99.5	1.45	1.46

Data (recovery results were corrected for residues found in the controls, pp. 16-17) were obtained from Tables 5-6, p. 25 of MRID 49822501.

1 The drinking (tap) water matrix (pH 8.0, 123 mg/L total hardness as CaCO<sub>3</sub>, 1.4 ppm total organic carbon, 0.7 ppm dissolved organic carbon) was obtained from a tap at EPL (p. 14; Appendix B, pp. 68-69). The surface water matrix (pH 8.3, 262 mg/L total hardness as CaCO<sub>3</sub>, 3.3 ppm total organic carbon, 2.2 ppm dissolved organic carbon) was obtained from Lake Decatur, Decatur, Illinois.

2 MGK-264 was identified using two ion transitions (quantitation and confirmation, respectively):  $m/z$  276.2→210.1 and  $m/z$  276.2→98.0.

### III. Method Characteristics

The LOQ for water was 0.05 ng/mL in the ECM and ILV (pp. 11, 20-21 of MRID 50105601; pp. 2, 13, 19 of MRID 49822501). In the ECM, the LOQ of the original method was 1.0 ng/mL; the amendment to the method added the extra validation set with the lower LOQ. No justification was provided for the LOQ in the ECM or ILV. No calculations or comparisons to background levels were reported to justify the LOQ for the method. The LOD was not reported in the ECM and ILV.

**Table 4. Method Characteristics<sup>1</sup>**

Analyte		MGK-264	
		Tap Water	Surface Water
Limit of Quantitation (LOQ)		0.05 ng/mL	
Limit of Detection (LOD)		<b>Not reported</b>	
Linearity (calibration curve $r^2$ and concentration range) <sup>2</sup>	ECM <sup>3</sup>	$r^2 = 0.9998$ (Q, 0.05 ng/mL LOQ) (0.0125-1.00 ng/mL) $r^2 = 0.9984$ (Q, 1.0 ng/mL LOQ) (0.250-10.0 ng/mL)	
	ILV	$r^2 = 0.9996$ (Q) $r^2 = 0.9995$ (C) (0.005-1 ng/mL)	
Repeatable	ECM <sup>3,4</sup>	Yes at LOQ, 20×LOQ and 400×LOQ and 10,000×LOQ. <b>No</b> samples were prepared at 10×LOQ.	Yes at LOQ and 10×LOQ.
	ILV <sup>5,6</sup>	Yes at LOQ and 20×LOQ. <b>No</b> samples were prepared at 10×LOQ.	
Reproducible		Yes at LOQ. <b>Could not be determined</b> for 10×LOQ since inadequate performance data was submitted.	
Specific	ECM	Some irregular integration was noted at the LOQ and 20×LOQ.	
		Yes, no matrix interferences were observed.	Yes, matrix interferences for one of the analyte isomers were observed at <i>ca.</i> 20% of the LOQ (based on peak height); no matrix interferences were observed for the other analyte isomer.
	ILV	At the LOQ and in the controls, background baseline noise levels were high, especially in the confirmation ion chromatograms.	
		Yes, matrix interferences were observed at <i>ca.</i> 6% of the LOQ (based on residue recovery; quantitation ion only).	Yes, matrix interferences were observed at <i>ca.</i> 3% (Q) and <i>ca.</i> 5% (C) of the LOQ (based on residue recovery).

Data were obtained from pp. 11, 16, 20-21; Tables 1-3, pp. 23-25 (recovery data); Figures 1-2, pp. 44-45 (calibration curve); Figures 6-13, pp. 49-56 (chromatograms) of MRID 50105601; pp. 2, 13, 19; Tables 5-6, p. 25 (recovery data); Figures 1-2, p. 26 (calibration curve); Figures 5-19, pp. 29-43 (chromatograms) of MRID 49822501; DER Attachment 2. Q = Quantitation ion transition; C = Confirmatory ion transition.

1 Based on the Amended ECM Method since it was the most recent version of the method and the ILV was based on the Amended ECM Method, but all data was included.

2 Reported correlation coefficients were reviewer-calculated from  $r$  values reported in the study report (Figures 1-2, pp. 44-45 of MRID 50105601; Figures 1-2, p. 26 of MRID 49822501; DER Attachment 2). Solvent standards were used.

3 Data for only the quantitation ion transition was reported in the ECM; a confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

4 In the ECM, the drinking (tap) water matrix was obtained from the Fresno municipal supply at GPL; a Water Quality Report for the tap water was provided, but the water sample was not characterized (p. 14; Appendix 4, pp. 61-78 of MRID 50105601). The reviewer assumed that the same tap water source was used for the original and amended method. The surface ground water matrix (pH 7.7, 21 mg/L total hardness as CaCO<sub>3</sub>, 2.2 mg/L total organic carbon and dissolved organic carbon) was obtained from the San Joaquin River in Fresno, California at a point near Gravel Haul Road.

5 In the ILV, the drinking (tap) water matrix (pH 8.0, 123 mg/L total hardness as CaCO<sub>3</sub>, 1.4 ppm total organic carbon, 0.7 ppm dissolved organic carbon) was obtained from a tap at EPL (p. 14; Appendix B, pp. 68-69 of

MRID 49822501). The surface water matrix (pH 8.3, 262 mg/L total hardness as CaCO<sub>3</sub>, 3.3 ppm total organic carbon, 2.2 ppm dissolved organic carbon) was obtained from Lake Decatur, Decatur, Illinois.

6 Although the specific number of trials was not reported, the reviewer assumed that the method was validated for both matrices after one trial with insignificant modifications to the analytical method (pp. 13, 15-16, 19 of MRID 49822501).

#### IV. Method Deficiencies and Reviewer's Comments

1. In the ILV, performance data at 10×LOQ was not reported to validate the method for either water matrix (Tables 5-6, p. 25 of MRID 49822501). In the ECM, performance data at 10×LOQ was not reported to validate the method in the drinking (tap) water matrix (Tables 2-3, pp. 19-20 of MRID 50105601). A validation sample set should consist of, at a minimum, a reagent blank, two unspiked matrix control samples, five matrix control samples spike at the LOQ, and five matrix control samples spiked at 10×LOQ for each analyte and matrix.
2. In the ECM, the drinking (tap) water matrix was obtained from the Fresno municipal supply at GPL; a Water Quality Report for the tap water was provided, but the water sample was not characterized (p. 14; Appendix 4, pp. 61-78 of MRID 50105601).
3. The estimation of LOQ in ECM and ILV was not based on scientifically acceptable procedures as defined in 40 CFR Part 136 ILV (pp. 11, 20-21 of MRID 50105601; pp. 2, 13, 19 of MRID 49822501). No justification was provided for the LOQ in the ECM or ILV. No calculations or comparisons to background levels were reported to justify the LOQ for the method. The LOD was not reported in the ECM and ILV.
4. In the ILV, recovery results were corrected for residues found in the controls (pp. 16-17; Tables 5-6, p. 25; Figures 5-19, pp. 29-43 of MRID 49822501). Matrix interferences ranged *ca.* 3-6% in the two water matrices (based on residue recovery).
5. The original ECM method was based on a LOQ of 1.0 ng/mL. Protocol Amendment No. 2 (3/7/2014) set the new LOQ to 0.05 ng/mL (Appendix 1, p. 38 of MRID 50105601).
6. Communications between the ILV and Study Monitor were limited to protocol and test results review and approval (p. 19; Appendix E, p. 90 of MRID 49822501). No communication between the ILV testing facility and the method developer occurred during the conduct of the study.
7. It was reported for the ILV that a set of sixteen samples and a reagent blank required approximately two person-hours for sample processing, with HPLC/MS/MS performed overnight and approximately two hours of data evaluation and transcription (p. 19 of MRID 49822501). Overall, a set can be completed in approximately four person-hours.

## 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****MGK-264****IUPAC Name:** N-(2-Ethylhexyl)-5-norbornene-2,3-dicarboximide**CAS Name:** N-Octyl bicycloheptene dicarboximide**CAS Number:** 113-48-4**SMILES String:** Not found