

**Analytical method for imazamox and its metabolites Reg. No. 4110603 (CL 354825) and Reg. No. 4110542 (CL 312622) in water**

**Reports:** ECM: EPA MRID No.: 50105408. Toledo, F. 2015. Method Development and Validation of an Analytical Method for the Determination of BAS 720 H and its 2 Metabolites Reg. No. 4110603 and Reg. No. 4110542 in Water (Analytical Method L0209) (Including 1st Addendum and 2nd Addendum). BASF Study ID: 391110. BASF Registration Document No.: 2015/7008009. SGS Project No.: IF-13/02484111. Report prepared by SGS Institut Fresenius GmbH, Taunusstein, Germany, and sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 74 pages. Final report issued July 22, 2013; 1<sup>st</sup> Addendum completed September 18, 2013; 2<sup>nd</sup> Addendum completed November 30, 2015.

ILV: EPA MRID No. 50105409. Schmitt, J.L., and D. Patel. 2013. Independent Laboratory Validation of BASF Analytical Method L0209/01: "Method for the Determination of BAS 720 H and its 2 Metabolites Reg. No. 4110603 and Reg. No. 4110542 in Water". BASF Study ID No.: 715771. BASF Registration Document No.: 2013/7002701. ADPEN Study No.: 2K13-903-715771. Report prepared by ADPEN Laboratories, Inc., Jacksonville, Florida, sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina; 105 pages. Final report issued November 14, 2013.

**Document No.:** MRIDs 50105408 & 50105409

**Guideline:** 850.6100

**Statements:** ECM: The study was conducted in accordance with OECD and German Good Laboratory Practice (GLP) standards (pp. 3-5 of MRID 50105408). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-6). Authenticity statements were included with the GLP statements (pp. 3, 5). 1<sup>st</sup> Addendum and 2<sup>nd</sup> Addendum Approvals and Quality Assurance statements were signed and dated (1<sup>st</sup> Addendum, pp. 64, 67; 2<sup>nd</sup> Addendum, pp. 69, 74). A signed and dated German GLP compliance statement was provided for the 2<sup>nd</sup> Addendum (2<sup>nd</sup> Addendum, p. 70).

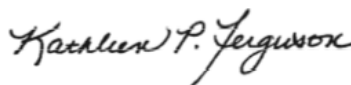
ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (40 CFR 160; p. 3 of MRID 50105409). Signed and dated No Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).

**Classification:** This Environmental Chemistry Method (ECM) (MRID 50105408) is classified as **Acceptable**. The Independent Laboratory Validation (ILV) (MRID 50105409) is classified as **Supplemental**. ILV recommendation to minimize matrix effects by dilution were incorporated into the ECM, but the use of matrix-matched standards was not explored. ILV linearity was not satisfactory for Reg. No. 4110603. 10×LOQ representative chromatograms were not provided in the ECM. The LOD was not reported in the ECM.

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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. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.*

## Executive Summary

The analytical method, BASF Method L0209/01, is designed for the quantitative determination of imazamox (BAS 720 H) and its major metabolites Reg. No. 4110603 (CL 354825) and Reg. No. 4110542 (CL 312622) in water at the LOQ of 0.025 µg/L using LC/MS/MS. The LOQ is **less than** the lowest toxicological level of concern in water for the three analytes. The ECM validated the method using characterized ground and surface water matrices. The ILV validated the method for imazamox, Reg. No. 4110603 and Reg. No. 4110542 in characterized drinking and ground water in the first trial with insignificant modifications to the analytical instruments and parameters; however, the ILV recommended that the ECM be updated to include the directive to dilute samples (1:5) to minimize matrix effects if matrix effects are observed. ECM incorporated the recommendation of the ILV in the 2<sup>nd</sup> Addendum, but the use of matrix-matched standards was not explored. All ECM and ILV data regarding repeatability, accuracy, precision, and specificity were satisfactory for all three analytes, based on the quantitation ion results; however, ECM 10×LOQ fortification representative chromatograms were not provided. ILV performance data for the confirmation ion analysis of Reg. No. 4110542 was insufficient for ground water. A confirmatory method is not typically required where GC/MS and/or LC/MS methods are used as the primary method(s) to generate study data; however, consistently high RSDs were noted for all Reg. No. 4110542 analyses in the ILV. The linearity was unsatisfactory for Reg. No. 4110603 in the ILV. The LOD was not reported in the ECM.

**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						
Imazamox (BAS 720 H)	50105408	50105409		Water <sup>1,2</sup>	22/07/2013 (Final Report)	BASF Corporation	LC/MS/MS	0.025 µg/L
Reg. No. 4110603 (CL 354825)					18/09/2013 (1 <sup>st</sup> Addendum)			
Reg. No. 4110542 (CL 312622)					30/11/2015 (2 <sup>nd</sup> Addendum)			

1 In the ECM, the ground water (130206571; pH 6.00, 0.0049 mmol/L water hardness, 0.116 mg/L dissolved organic carbon), obtained from a well in Glashuetten, Germany, and surface water (130160769; pH 7.81, 0.0169 mmol/L water hardness, 0.931 mg/L dissolved organic carbon), obtained from a natural water course known as Burbach in Dietzhoelztal, Germany, were used (p. 14; Table 3, p. 32 of MRID 50105408).

2 In the ILV, the ground water (Jordan Lake; pH 6.9, 33 mg equiv. CaCO<sub>3</sub>/L, 98 ppm total dissolved solids, 7.9 ppm total organic carbon) and drinking water (Tap Water; pH 7.2, 25 mg equiv. CaCO<sub>3</sub>/L, 120 ppm total dissolved solids, 3.1 ppm total organic carbon) matrices were provided by the Sponsor (p. 14; Appendix B, pp. 91-92 of MRID 50105409). The sources of the water matrices were not further detailed.

## I. Principle of the Method

Samples (10 g) were transferred to a 15-mL centrifuge tube, acidified with 0.5 mL of 2 N HCl, and fortified, as necessary, with mixed fortification solutions of imazamox, Reg. No. 4110603, and Reg. No. 4110542 in methanol (pp. 16-18, 20-21 of MRID 50105408). A Varian Bond Elut C-18 Solid Phase Extraction (SPE) column (0.2 g, 3 mL) was pre-conditioned by washing the column subsequently with 3 mL of the following solutions: hexane, dimethylene chloride (DCM), methanol, water, and 0.01 N HCl. The entire sample was loaded onto the Varian column. The column was washed with 3 mL of water followed by 3 mL of hexane; column flow was stopped when liquid passed just below frit. 3 mL of DCM was passed through the column, and the eluate was collected into a 15-mL glass centrifuge vial. The DCM eluate was reduced to dryness unto a gentle stream of nitrogen at 40°C. An Isolute/Biotage SCX column was pre-conditioned by washing with 3 mL of methanol, then filling the column with 2 mL of methanol. The Varian column (post-DCM elution) was attached to the top of the SCX column containing 2 mL of methanol. The analytes were transferred from the Varian column to the SCX column using 3 mL of methanol. The Varian column was detached and discarded. The SCX column was washed with 3 mL of methanol. The analytes were eluted with 6 mL of water:methanol (20:80, v:v) into the same 15-mL glass centrifuge vial used for the DCM eluate. The final eluate was reduced to dryness unto a gentle stream of nitrogen at 70°C (water bath). The residue was reconstituted in 0.1% formic acid in water:methanol (80:20, v:v) and analyzed by LC/MS/MS.

Samples were analyzed for analytes using an Agilent 1200 HPLC system coupled to an Applied Biosystems API 4000 mass spectrometer (pp. 18-20 of MRID 50105408). The LC/MS conditions consisted of a Zorbax Eclipse XDB-C18 column (4.6 x 50.0 mm, 1.8-µm; column temperature not reported), a mobile phase of (A) water with 0.1% formic acid and (B) methanol with 0.1% formic acid [percent A:B (v:v) at 0.0-0.5 min. 80:20, 3.0 min. 50:50, 7.0-10.0 min. 5:95, 10.1-15.0 min. 80:20] and MS/MS detection in positive ion mode (ionization temperature 500°C). Injection volume was 20 µL. Two ion transitions were monitored (quantitation and confirmatory, respectively) as

follows:  $m/z$  306.3→261.2 and  $m/z$  306.3→86.3 for imazamox (BAS 720 H),  $m/z$  278.1→233.1 and  $m/z$  278.1→165.0 for Reg. No. 4110603, and  $m/z$  306.3→261.2 and  $m/z$  306.3→264.2 for Reg. No. 4110542. Retention times were 5.2, 7.1, and 4.7 minutes for imazamox (BAS 720 H), Reg. No. 4110603, and Reg. No. 4110542, respectively.

In the ILV, the ECM was performed as written, except for a few minor modifications of analytical instruments or parameters (pp. 16-18 of MRID 50105409). An Agilent 1200 HPLC System coupled to an AB Sciex API4000 TurboIonSpray® mass spectrometer was used. Most of the LC/MS conditions were the same (column temperature was reported as 40°C), except that the ionization temperature was 550°C. Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows:  $m/z$  306.0→261.0 and  $m/z$  306.0→86.0 for imazamox (BAS 720 H),  $m/z$  278.0→233.0 and  $m/z$  278.0→165.0 for Reg. No. 4110603, and  $m/z$  306.0→261.0 and  $m/z$  306.0→264.0 for Reg. No. 4110542. Retention times were *ca.* 4.8, 6.7, and 4.3 minutes for imazamox (BAS 720 H), Reg. No. 4110603, and Reg. No. 4110542, respectively. A flow diagram of the analytical procedure was included (table 13.1, p. 25). No significant modifications were made by the ILV; however, the ILV recommended that the ECM be updated to include the directive to dilute samples (1:5) to minimize matrix effects if matrix effects are observed (pp. 21-22).

The Limit of Quantification (LOQ) was 0.025 µg/L for imazamox (BAS 720 H), Reg. No. 4110603, and Reg. No. 4110542 in water in the ECM and ILV (pp. 7, 24 of MRID 50105408; pp. 11, 19 of MRID 50105409). The Limit of Detection (LOD) was 0.005 µg/L for all analytes in water in the ILV; the LOD was not reported in the ECM.

## II. Recovery Findings

ECM (MRID 50105408): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of imazamox (BAS 720 H; CL 299263) and its metabolites Reg. No. 4110603 and Reg. No. 4110542 in two water matrices at fortification levels of 0.025 µg/L (LOQ) and 0.25 µg/L (10×LOQ; pp. 25-26; Tables 4-9, pp. 33-44). Two ion transitions were monitored for all analytes; performance data (recovery results) from primary and confirmatory analyses were comparable. Ground water (130206571; pH 6.00, 0.0049 mmol/L water hardness, 0.116 mg/L dissolved organic carbon), obtained from a well in Glashuetten, Germany, and surface water (130160769; pH 7.81, 0.0169 mmol/L water hardness, 0.931 mg/L dissolved organic carbon), obtained from a natural water course known as Burbach in Dietzhoelztal, Germany, were used (p. 14; Table 3, p. 32).

ILV (MRID 50105409): Mean recoveries and RSDs were within guideline requirements for analysis of imazamox (BAS 720 H; CL 299263) and its metabolites Reg. No. 4110603 and Reg. No. 4110542 in two water matrices at fortification levels of 0.025 µg/L (LOQ) and 0.25 µg/L (10×LOQ), except for the confirmation ion analysis of Reg. No. 4110542 (CL 312622) in ground water at the LOQ (RSD 24.1%; pp. 11-12). This deviation did not affect the validity of the study since a confirmatory method is not usually required when LC/MS and GC/MS is the primary method. Two ion transitions were monitored for all analytes; performance data (recovery results) from primary and confirmatory analyses were comparable. Ground water (Jordan Lake; pH 6.9, 33 mg equiv. CaCO<sub>3</sub>/L, 98 ppm total dissolved solids, 7.9 ppm total organic carbon) and drinking water (Tap Water; pH 7.2, 25 mg equiv. CaCO<sub>3</sub>/L, 120 ppm total dissolved solids, 3.1 ppm total organic carbon) matrices were provided by the Sponsor (p. 14; Appendix B, pp. 91-92). The sources of the water matrices were not further

detailed. The ECM method, BASF Method L0209/01, for imazamox, Reg. No. 4110603, and Reg. No. 4110542 in drinking and ground water was validated in the first trial with insignificant modifications to the analytical instruments and parameters; however, the ILV recommended that the ECM be updated to include the directive to dilute samples (1:5) to minimize matrix effects if matrix effects are observed (pp. 11, 21-22).

**Table 2. Initial Validation Method Recoveries for Imazamox, Reg. No. 4110603, and Reg. No. 4110542 in Water<sup>1,2</sup>**

Analyte <sup>3</sup>	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%) <sup>2</sup>
<b>Ground Water</b>						
Quantitation ion						
Imazamox (BAS 720 H)	0.025 (LOQ)	5	91-96	93	2.3	2.5
	0.25	5	92-100	96	3.2	3.3
Reg. No. 4110603	0.025 (LOQ)	5	80-87	85	3.3	3.9
	0.25	5	82-92	87	3.5	4.1
Reg. No. 4110542	0.025 (LOQ)	5	80-88	84	3.6	4.3
	0.25	5	76-89	82	4.9	5.9
Confirmation ion						
Imazamox (BAS 720 H)	0.025 (LOQ)	5	90-98	95	2.9	3.0
	0.25	5	87-101	93	5.5	5.9
Reg. No. 4110603	0.025 (LOQ)	5	76-99	84	9.4	11.2
	0.25	5	85-94	89	3.8	4.3
Reg. No. 4110542	0.025 (LOQ)	5	72-81	76	4.7	6.2
	0.25	5	72-89	83	6.8	8.2
<b>Surface Water</b>						
Quantitation ion						
Imazamox (BAS 720 H)	0.025 (LOQ)	5	88-100	94	5.2	5.5
	0.25	5	94-99	96	2.1	2.2
Reg. No. 4110603	0.025 (LOQ)	5	79-85	82	2.6	3.2
	0.25	5	81-88	86	2.6	3.0
Reg. No. 4110542	0.025 (LOQ)	5	84-97	90	5.4	6.0
	0.25	5	80-95	87	6.3	7.2
Confirmation ion						
Imazamox (BAS 720 H)	0.025 (LOQ)	5	91-103	95	5.0	5.2
	0.25	5	92-101	96	3.1	3.2
Reg. No. 4110603	0.025 (LOQ)	5	77-82	80	2.6	3.3
	0.25	5	82-91	86	3.8	4.4
Reg. No. 4110542	0.025 (LOQ)	5	72-83	78	3.9	5.0
	0.25	5	75-83	78	3.2	4.1

Data (uncorrected recovery results, p. 17) were obtained from pp. 25-26; Tables 4-9, pp. 33-44 of MRID 50105408.

1 The ground water (130206571; pH 6.00, 0.0049 mmol/L water hardness, 0.116 mg/L dissolved organic carbon), obtained from a well in Glashuetten, Germany, and surface water (130160769; pH 7.81, 0.0169 mmol/L water hardness, 0.931 mg/L dissolved organic carbon), obtained from a natural water course known as Burbach in Dietzhoelztal, Germany, were used in the study (p. 14; Table 3, p. 32).

2 Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows:  $m/z$  306.3→261.2 and  $m/z$  306.3→86.3 for imazamox (BAS 720 H),  $m/z$  278.1→233.1 and  $m/z$  278.1→165.0 for Reg. No. 4110603, and  $m/z$  306.3→261.2 and  $m/z$  306.3→264.2 for Reg. No. 4110542.

3 Imazamox = BAS 720 H; AC 299263; CL 299263; 2-[(RS)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]-5-methoxymethylnicotinic acid; Reg. No. 4110542 = CL 312622; Diacid; 5-Acid imidazolinone; 2-(4-Isopropyl-4-methyl-5-oxo-1H-imidazol-2-yl)pyridine-3,5-dicarboxylic acid; and Reg. No. 4110603 = CL 354825; Hydroxyl; 5-Hydroxy-6-(4-isopropyl-4-methyl-5-oxo-1H-imidazol-2-yl)pyridine-3-carboxylic acid.

**Table 3. Independent Validation Method Recoveries for Imazamox, Reg. No. 4110603, and Reg. No. 4110542 in Water<sup>1,2</sup>**

Analyte <sup>3</sup>	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%) <sup>2</sup>
<b>Ground Water</b>						
Quantitation ion						
Imazamox (BAS 720 H)	0.025 (LOQ)	5	72-102	82	11.9	14.5
	0.25	5	80-104	93	9.3	10.0
Reg. No. 4110603	0.025 (LOQ)	5	70-83	78	5.4	6.9
	0.25	5	75-93	82	6.8	8.3
Reg. No. 4110542	0.025 (LOQ)	5	62-94	77	12.8	16.6
	0.25	5	74-92	81	8.4	10.4
Confirmation ion						
Imazamox (BAS 720 H)	0.025 (LOQ)	5	60-88	70	11.5	16.5
	0.25	5	76-100	86	9.2	10.8
Reg. No. 4110603	0.025 (LOQ)	5	65-82	77	8.6	11.2
	0.25	5	68-94	78	10.8	13.7
Reg. No. 4110542	0.025 (LOQ)	5	67-120	85	20.5	<b>24.1</b>
	0.25	5	69-94	79	12.4	15.7
<b>Drinking Water</b>						
Quantitation ion						
Imazamox (BAS 720 H)	0.025 (LOQ)	5	67-85	73	7.5	10.3
	0.25	5	79-90	85	4.4	5.2
Reg. No. 4110603	0.025 (LOQ)	5	79-85	84	3.0	3.6
	0.25	5	81-93	89	5.3	5.9
Reg. No. 4110542	0.025 (LOQ)	5	65-96	75	11.9	15.8
	0.25	5	59-85	70	11.9	17.0
Confirmation ion						
Imazamox (BAS 720 H)	0.025 (LOQ)	5	66-91	75	10.0	13.4
	0.25	5	84-104	92	7.7	8.4
Reg. No. 4110603	0.025 (LOQ)	5	82-85	83	1.3	1.6
	0.25	5	82-95	88	5.4	6.2
Reg. No. 4110542	0.025 (LOQ)	5	62-99	76	15.0	19.8
	0.25	5	59-92	75	14.2	18.9

Data (uncorrected recovery results, pp. 22-23) were obtained from pp. 11-12 of MRID 50105409.

1 The ground water (Jordan Lake; pH 6.9, 33 mg equiv. CaCO<sub>3</sub>/L, 98 ppm total dissolved solids, 7.9 ppm total organic carbon) and drinking water (Tap Water; pH 7.2, 25 mg equiv. CaCO<sub>3</sub>/L, 120 ppm total dissolved solids, 3.1 ppm total organic carbon) matrices were provided by the Sponsor (p. 14; Appendix B, pp. 91-92). The sources of the water matrices were not further detailed.

2 Two ion transitions were monitored (quantitation and confirmatory, respectively) as follows: *m/z* 306.0→261.0 and *m/z* 306.0→86.0 for imazamox (BAS 720 H), *m/z* 278.0→233.0 and *m/z* 278.0→165.0 for Reg. No. 4110603, and *m/z* 306.0→261.0 and *m/z* 306.0→264.0 for Reg. No. 4110542.

3 Imazamox = BAS 720 H; AC 299263; CL 299263; 2-[(RS)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]-5-methoxymethylnicotinic acid; Reg. No. 4110542 = CL 312622; Diacid; 5-Acid imidazolinone; 2-(4-Isopropyl-4-methyl-5-oxo-1H-imidazol-2-yl)pyridine-3,5-dicarboxylic acid; and Reg. No. 4110603 = CL 354825; Hydroxyl; 5-Hydroxy-6-(4-isopropyl-4-methyl-5-oxo-1H-imidazol-2-yl)pyridine-3-carboxylic acid.

### III. Method Characteristics

The LOQ was 0.025 µg/L for imazamox (BAS 720 H), Reg. No. 4110603, and Reg. No. 4110542 in water in the ECM and ILV (pp. 7, 24 of MRID 50105408; pp. 11, 19 of MRID 50105409). In the ECM, the LOQ was defined as the lowest fortification level giving an acceptable mean recovery (70 to 120%) and a relative standard deviation <20%. In the ILV, the LOQ was defined as the lowest fortification level successfully tested. The LOD was 0.005 µg/L for all analytes in water in the ILV without justification; the LOD was not reported in the ECM. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM and ILV; no calculations were reported to justify the LOD for the method in the ILV.

**Table 4. Method Characteristics**

Analyte <sup>1</sup>		Imazamox (CL 299263; BAS 720 H)	Reg. No. 4110603 (CL 354825)	Reg. No. 4110542 (CL 312622)
Limit of Quantitation (LOQ)	ECM	0.025 µg/L		
	ILV			
Limit of Detection (LOD)	ECM	<b>Not reported</b>		
	ILV	0.005 µg/L		
Linearity (calibration curve $r^2$ and concentration range) <sup>2,3</sup>	ECM	$r^2 = 0.9996-1.0000$ (Q) $r^2 = 0.9997-0.9998$ (C)	$r^2 = 0.9997-1.0000$ (Q) $r^2 = 0.9993-0.9998$ (C)	$r^2 = 0.9997$ (Q) $r^2 = 0.9998$ (C)
		0.0503-2.515 ng/mL	0.0502-2.515 ng/mL	0.0501-2.505 ng/mL
	ILV	$r^2 = 0.9992$ (Q) $r^2 = 0.9972-0.9984$ (C)	$r^2 = \mathbf{0.9888-0.9916}$ (Q) $r^2 = \mathbf{0.9900-0.9910}$ (C)	$r^2 = 0.9958-0.9972$ (Q) $r^2 = 0.9970-0.9974$ (C)
		0.0002-0.04 ng (ground) 0.001-0.05 ng (drinking)	0.001-0.05 ng	
Repeatable	ECM <sup>4</sup>	Yes at LOQ and 10×LOQ (characterized ground and surface water matrices)		
	ILV <sup>5,6</sup>	Yes at LOQ and 10×LOQ (characterized drinking and ground water matrices)	Yes at LOQ and 10×LOQ (characterized drinking and ground water matrices)	Yes at LOQ and 10×LOQ (characterized drinking and ground water matrices; LOQ C RSD <b>24.1%</b> in ground water) <sup>7</sup>
Reproducible	Yes at LOQ and 10×LOQ			
Specific	ECM	<b>No</b> 10×LOQ representative chromatograms were provided.		
		Yes, no matrix interferences were observed or quantified.	Yes, no matrix interferences were observed or quantified. Minor baseline noise interfered with peak integration at the LOQ.	Yes, no matrix interferences were observed or quantified.
	ILV	Yes, matrix interferences were < 5% of the LOQ (based on peak area). Analyte peak was small and baseline noise was prominent in ground water due to 1:5 dilution.	Yes, no matrix interferences were observed or quantified.	

Data were obtained from pp. 7, 24-26; Tables 4-9, pp. 33-44 (LOQ & recovery data); Tables 4-9, pp. 33-44 (correlation coefficients); Figures 5-24, pp. 48-59 (chromatograms) of MRID 50105408; pp. 11-12, 19 (LOQ/LOD & recovery data); Figures 14.1-14.3, pp. 40-42 (calibration curves); Figures 14.7-14.18, pp. 67-84 (chromatograms) Appendix C, pp. 94-



105 (raw data & correlation coefficients) of MRID 50105409; and DER Attachment 2. Q = Quantitation ion transition; C = Confirmation ion transition.

- 1 Imazamox = BAS 720 H; AC 299263; CL 299263; 2-[(RS)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]-5-methoxymethylnicotinic acid; Reg. No. 4110542 = CL 312622; Diacid; 5-Acid imidazolinone; 2-(4-Isopropyl-4-methyl-5-oxo-1H-imidazol-2-yl)pyridine-3,5-dicarboxylic acid; and Reg. No. 4110603 = CL 354825; Hydroxyl; 5-Hydroxy-6-(4-isopropyl-4-methyl-5-oxo-1H-imidazol-2-yl)pyridine-3-carboxylic acid.
- 2 Correlation coefficients ( $r^2$ ) values were reviewer-calculated from  $r$  values provided in the study report (Tables 4-9, pp. 33-44 of MRID 50105408; Appendix C, pp. 94-105 of MRID 50105409; DER Attachment 2). In the ECM and ILV analysis, solvent-based calibration standards were used; however, matrix suppression was noted in the ILV analyses for ground water samples fortified with imazamox (p. 21). The ILV eliminated the matrix effects by diluting the samples 1:5 with 0.1% formic acid in water:methanol (80:20, v:v).
- 3 Correlation coefficients were reported for each sample set in each matrix, but the reviewer combined the correlation coefficients since solvent standards were used (p. 21 of MRID 50105408; of MRID 50105409). The reviewer limited the calculated  $r^2$  to 4 significant figures although 5 significant figures were reported in the ECM for  $r$ .
- 4 In the ECM, the ground water (130206571; pH 6.00, 0.0049 mmol/L water hardness, 0.116 mg/L dissolved organic carbon), obtained from a well in Glashuetten, Germany, and surface water (130160769; pH 7.81, 0.0169 mmol/L water hardness, 0.931 mg/L dissolved organic carbon), obtained from a natural water course known as Burbach in Dietzhoelztal, Germany, were used (p. 14; Table 3, p. 32 of MRID 50105408).
- 5 In the ILV, the ground water (Jordan Lake; pH 6.9, 33 mg equiv.  $\text{CaCO}_3/\text{L}$ , 98 ppm total dissolved solids, 7.9 ppm total organic carbon) and drinking water (Tap Water; pH 7.2, 25 mg equiv.  $\text{CaCO}_3/\text{L}$ , 120 ppm total dissolved solids, 3.1 ppm total organic carbon) matrices were provided by the Sponsor (p. 14; Appendix B, pp. 91-92 of MRID 50105409). The sources of the water matrices were not further detailed.
- 6 The ILV validated ECM method BASF Method L0209/01 for imazamox, Reg. No. 4110603, and Reg. No. 4110542 in drinking and ground water in the first trial with insignificant modifications to the analytical instruments and parameters; however, the ILV recommended that the ECM be updated to include the directive to dilute samples (1:5) to minimize matrix effects if matrix effects are observed (pp. 11, 21-22 of MRID 50105409).
- 7 A confirmatory method is not usually required when LC/MS and GC/MS is the primary method. Linearity is satisfactory when  $r^2 \geq 0.995$ .

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

1. The ILV recommended that ECM method BASF Method L0209/01 be updated to include the directive to dilute samples (1:5) with 0.1% formic acid in water:methanol (80:20, v:v) to minimize matrix effects if matrix effects are observed (pp. 11, 21-22 of MRID 50105409). The ECM incorporated the recommendation of the ILV in the 2<sup>nd</sup> Addendum (2<sup>nd</sup> Addendum, p. 73 of MRID 50105408).

The reviewer noted that usually matrix effects are minimized by the use of matrix-matched standards. The use of a 1:5 dilution for imazamox analyses in ground water caused the analyte peak to be small and baseline noise to be prominent (pp. 21-22; Figure 14.10 and 14.16, pp. 70, 79 of MRID 50105409). The imazamox LOQ quantitation ion analyte peak was 403 cps height and 1706 counts area in the ground water sample, while the imazamox LOQ quantitation ion analyte peak was 3040 cps height and 9132 counts area in the drinking water sample. Also, the maximum height of the baseline noise was *ca.* 38% of the imazamox LOQ quantitation ion analyte peak in ground water versus *ca.* 7% of the imazamox LOQ quantitation ion analyte peak in drinking water. The reviewer acknowledged that acceptable data was obtained from the ILV use of 1:5 dilution; however, the reviewer believed that the use of matrix-matched standards should have been explored for the ground water samples in order to produce more consistent LC/MS/MS raw data. Additionally, the reviewer noted that representative standard chromatograms were not provided for the low concentrations used for the ground water analyses; the lowest concentration standard chromatogram which was provided was 0.05 ng/mL (0.001 ng) while the lowest concentration standard used for ground

water was 0.0002 ng.

2. In the ILV, the linearity was not satisfactory for Reg. No. 4110603 (CL 354825),  $r^2 = 0.9888-0.9916$  and  $0.9900-0.9910$  for the quantitation and confirmation ion transition, respectively (matrix analyses combined; Appendix C, pp. 94-105 of MRID 50105409). Linearity is satisfactory when  $r^2 \geq 0.995$ . However, in the case of the ILV confirmation ion analysis, the reviewer noted that a confirmatory method is not typically required where GC/MS and/or LC/MS methods are used as the primary method(s) to generate study data.
3. No  $10 \times \text{LOQ}$  representative chromatograms were provided in the ECM. Representative chromatograms should be provided for all fortifications, quantified ions, and matrices to allow evaluation of the specificity of the method.
4. Performance data was not satisfactory for the ILV confirmation ion analysis of Reg. No. 4110542 (CL 312622) in ground water (RSD 24.1%; pp. 11-12, 19 of MRID 50105409). OCSPP guideline requirements state that the mean recovery is 70-120% and the RSD is  $\leq 20\%$ . The reviewer did not consider this guideline deviation to be significant since a confirmatory method is not typically required where GC/MS and/or LC/MS methods are used as the primary method(s) to generate study data. However, the reviewer also noted that RSDs for all Reg. No. 4110542 analyses were high, 10.4-24.1%, indicating that the ILV found the precision and repeatability of the method for Reg. No. 4110542 to be less than optimal, but acceptable. The reviewer noted that RSDs for all imazamox LOQ analyses were high, 10.3-16.5%.
5. The estimations of LOQ and LOD in ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 7, 24 of MRID 50105408; pp. 11, 19 of MRID 50105409). In the ECM, the LOQ was defined as the lowest fortification level giving an acceptable mean recovery (70 to 120%) and a relative standard deviation  $< 20\%$ . In the ILV, the LOQ was defined as the lowest fortification level successfully tested. The LOD was not reported in the ECM. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM and ILV; no calculations were reported to justify the LOD for the method in the ILV. Detection limits should not be based on arbitrary values.
6. The reviewer noted a significant typographical error in the ILV: CL-No. 31622 was reported, instead of CL No. 312622 throughout the Abstract Recovery and Repeatability table (pp. 11-12 of MRID 50105409).
7. The ECM did not find matrix effects to be significant, but the ILV found matrix effects (matrix suppression) to be significant for imazamox in ground water (p. 21 of MRID 50105408; p. 21 of MRID 50105409).
8. The communications between the ILV and BASF Study Monitor (Robert F. Gooding) were discussed (pp. 21-22 of MRID 50105409). The communication involved the communication of the successful trial to the BASF Study Monitor.
9. It was reported for the ILV that one sample set of 13 samples required *ca.* 12 hours of work (*ca.* 1.5 working days) not including LC/MS/MS analysis time (p. 21 of MRID 50105409).

## **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.

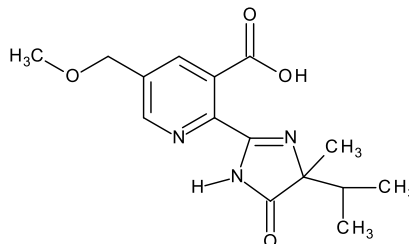
**DER Attachment 1: Chemical Names and Structures****Imazamox (BAS 720 H; AC 299263; CL 299263)**

**IUPAC Name:** 2-[(RS)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]-5-methoxymethylnicotinic acid

**CAS Name:** 2-[4,5-Dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-(methoxymethyl)-3-pyridinecarboxylic acid

**CAS Number:** 114311-32-9

**SMILES String:** OC(=O)c1cc(COC)cnc1C2=NC(C)(C(C)C)C(=O)N2

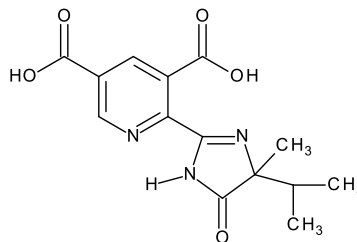
**Reg. No. 4110542 (CL 312622; Diacid; 5-Acid imidazolinone)**

**IUPAC Name:** 2-(4-Isopropyl-4-methyl-5-oxo-1H-imidazol-2-yl)pyridine-3,5-dicarboxylic acid

**CAS Name:** Not reported

**CAS Number:** 146953-32-4

**SMILES String:** CC(C)C1(C)N=C(NC1=O)c2ncc(cc2C(=O)O)C(=O)O

**Reg. No. 4110603 (CL 354825; Hydroxyl)**

**IUPAC Name:** 5-Hydroxy-6-(4-isopropyl-4-methyl-5-oxo-1H-imidazol-2-yl)pyridine-3-carboxylic acid

**CAS Name:** Not reported

**CAS Number:** Not reported

**SMILES String:** CC(C)C1(C)N=C(NC1=O)c2ncc(cc2O)C(=O)O

