

Test Material: Tioxazafen/MON 102100

MRID: 49304241

Title: Method Validation Study for the Determination of Residues of MON 102100 and Its Degradates in Water Matrices using Liquid Chromatography with Tandem Mass Spectrometry Detection

MRID: 49304242

Title: Independent Laboratory Validation Study for the Determination of MON 102100 and Environmental Degradates in Ground, Surface and Drinking Water

EPA PC Code: 074752

OCSPP Guideline: 850.6100

For CDM Smith

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Analytical method for MON 102100 (tioxazafen) and its transformation products, MON 102130 and benzamidine, in water

- Reports:** ECM: EPA MRID No.: 49304241. Bendler, S.E. 2014. Method Validation Study for the Determination of Residues of MON 102100 and Its Degradates in Water Matrices using Liquid Chromatography with Tandem Mass Spectrometry Detection. EPL BAS Study No.: 115G761. Monsanto Study No.: EBS-2014-0101 and Report No.: MSL0025809. Report prepared by EPL Bio Analytical Services (EPL BAS), Niantic, Illinois, sponsored and submitted by Monsanto Company, St. Louis, Missouri; 183 pages (including 2 non-paginated pages). Final report issued October 29, 2014.
ILV: EPA MRID No. 49304242. Wang, H. 2014. Independent Laboratory Validation Study for the Determination of MON 102100 and Environmental Degradates in Ground, Surface and Drinking Water. CPS Study No.: 14-CPS-007. Monsanto Study No. CPS-2014-0262 and Report No.: MSL0025810. Report prepared by Critical Path Services, LLC (CPS), Garnet Valley, Pennsylvania, sponsored and submitted by Monsanto Company, St. Louis, Missouri; 140 pages. Final report issued October 31, 2014.
- Document No.:** MRIDs 49304241 & 49304242
- Guideline:** 850.6100
- Statements:** ECM: The study was conducted in accordance with USEPA FIFRA Good Laboratory Practice (GLP) standards (p. 3 of MRID 49304241). Signed and dated No Data Confidentiality, GLP, Quality Assurance and Authenticity statements were provided (pp. 2-5).
ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3 of MRID 49304242). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity was included with the quality assurance statement.
- Classification:** This analytical method is **scientifically sound** and classified as **Supplemental**. The confirmation ion analysis which was included in EPL BAS Method 115G761A was not performed by the ILV. The ILV water matrices were not characterized. The sources of the ECM and ILV water matrices were not reported. The LOD values were not reported in the ILV.

All page numbers of MRID 49304241 refer to those listed in the upper right-hand corner of the pages, with the exception that Page 1 was written at the bottom center of the page. All page numbers of MRID 49304242 refer to those listed in the bottom center of the page.

Executive Summary

The analytical method, EPL BAS Method 115G761A, is designed for the quantitative determination of MON 102100, MON 102130 and benzamidine in drinking, surface and ground water at the LOQ of 0.10 µg/L using LC/MS/MS with MRM. The LOQ is less than the lowest toxicological level of concern in water (saltwater mysid LOEC = 2.4 ug a.i./L; MRID 49304224) for all analytes. EPL BAS Method 115G761A was validated in drinking, surface and ground water by the ILV with only minor modifications after one trial; however, the confirmation ion analysis included in the method was not performed by the ILV. The water matrices of the ILV were not characterized; sources of the water matrices were not reported in the ECM and 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						
MON 102100	49304241	49304242		Water ²	29/10/2014	Monsanto	LC/MS/MS	0.10 µg/L
MON 102130 ¹								
Benzamidine								

¹ 3-thienyl 102100.

² Drinking, ground and surface water matrices were used in the ECM and ILV. Water matrices were fully characterized in the ECM, but not characterized in the ILV (sources not reported in ECM and ILV; p. 14; Appendix B, pp. 97-99 of MRID 49304241; p. 13 of MRID 49304242).

I. Principle of the Method

EPL BAS Method 115G761A

Samples (10-15 mL) of water were transferred to 15-mL culture tubes and centrifuged (4000 rpm for 10 minutes; Appendix C, p. 114 of MRID 49304241). A 0.750 mL aliquot of the supernatant was transferred to a 1.8-mL auto-sampler vial and fortified, as necessary (75 μ L of 1 or 10 ng/mL MON 102100, MON 102130 or benzamidine fortification solution in acetonitrile/water solution; Appendix C, pp. 108-109, 111-112, 114). 0.250 mL of the mixed stable-label internal standards solution [(phenyl- $^{13}\text{C}_6$) MON 102100 and ($^{13}\text{C}_6$) benzamidine; 20 mg/mL] was added to the samples. The solutions were mixed (vortex mixer may be used), and the samples were analyzed by LC/MS/MS. The method noted that the fortified solutions were stable for up to 3 days under refrigeration.

Samples were analyzed for MON 102100, MON 102130 and benzamidine and the internal standards (phenyl- $^{13}\text{C}_6$) MON 102100 and ($^{13}\text{C}_6$) benzamidine by ESI LC/MS/MS using an Agilent 1200 system coupled to an AB Sciex 6500 Q-Trap Mass Spectrometer in positive ion MRM mode (p. 15; Appendix C, p. 113-115 of MRID 49304241). The instrumental conditions consisted of a Waters Xbridge Phenyl column (2.1 x 100 mm, 3.5- μ m; column temperature 20°C), a mobile phase gradient of (A) 0.1% formic acid, 0.1% heptafluorobutyric acid anhydride (HFBA) in water and (B) 0.1% formic acid, 0.1% HFBA in isopropanol:methanol:acetonitrile (50:475:475, v:v:v) [percent A:B (v:v) at 0.0-5.0 min. 98:2, 5.5 min. 70:30, 23.0 min. 30:70, 23.5 min. 10:90, 26.0 min. 5:95, 26.5-30.0 min. 98:2] and MS/MS detection in electrospray ionization mode (temperature, 600°C), and injection volume 20 μ L. The ion source was reported as “turbo V??” (Appendix C, p. 115). Two parent-daughter ion transitions (quantitative and confirmation, respectively) were monitored for the analytes: m/z 228.9 \rightarrow 111.1 and m/z 228.9 \rightarrow 82.9 for MON 102100 and MON 102130, and m/z 121.0 \rightarrow 104.1 and m/z 121.0 \rightarrow 77.0 for benzamidine (Appendix C, p. 115 of MRID 49304241; Appendix 6, p. 126 of MRID 49304242- missing page from ECM Method reproduction). One parent-daughter ion transition (quantitative) was monitored for the internal standards: m/z 235.0 \rightarrow 111.0 for (phenyl- $^{13}\text{C}_6$) MON 102100 and m/z 127.0 \rightarrow 110.0 for ($^{13}\text{C}_6$) benzamidine. (Phenyl- $^{13}\text{C}_6$) MON 102100 was used for MON 102130 quantification, as well as MON 102100 quantification. Retention times were observed at *ca.* 19.5 min. for MON 102130, *ca.* 19.93 min. for MON 102100 and (phenyl- $^{13}\text{C}_6$) MON 102100, and *ca.* 7.7 min. for benzamidine and ($^{13}\text{C}_6$) benzamidine (Figures 4-39, pp. 46-81; Appendix E, Figures E4-E39, pp. 146-181).

ILV

In the ILV, the ECM extraction method was performed as written, except that water samples were centrifuged after fortification instead of before fortification (the entire 10-mL sample was fortified instead of an 0.75 mL aliquot; pp. 15-17; Tables 5-6, pp. 26-27 of MRID 49304242). The analytical method was performed using an Agilent 1200 system coupled to an AB Sciex 4000 Mass Spectrometer in positive ion MRM mode (same LC column as ECM). The mobile phase gradient was adjusted to the following (same solvents): [percent A:B (v:v) at 0.0-5.0 min. 98:2, 5.5 min. 70:30, 23.0 min. 30:70, 26.0 min. 10:90, 26.5 min. 5:95, 30.0-33.0 min. 98:2]. Injection volumes were 10.0 μ L for benzamidine and 75.0 μ L for MON 102100 and MON

102130. The ion source was specified as turbo ion spray. Only the quantitation ion transitions were monitored (p. 17). Retention times were observed at *ca.* 23.7 min. for MON 102130, *ca.* 24.1 min. for MON 102100 and (phenyl-¹³C₆) MON 102100, and *ca.* 11.5 min. for benzamidine and (¹³C₆) benzamidine (p. 17; Appendix 1, Figures 2-63, pp. 32-93).

LOQ/LOD

The LOQ for MON 102100, MON 102130 and benzamidine was the same in the ECM and ILV at 0.10 µg/L (0.10 ppb; pp. 20-21; Tables 22-24, p. 38 of MRID 49304241; pp. 10, 20 of MRID 49304242). The LODs for MON 102100 were calculated in the ECM as 0.00636-0.01044 ppb and 0.02232-0.02808 ppb for quantitation and confirmation ions, respectively. The LODs for MON 102130 were calculated in the ECM as 0.00568-0.00798 ppb and 0.00918-0.02024 ppb for quantitation and confirmation ions, respectively. The LODs for benzamidine were calculated in the ECM as 0.00270-0.01380 ppb and 0.00682-0.01546 ppb for quantitation and confirmation ions, respectively. The LOD was not reported in the ILV.

II. Recovery Findings

ECM (MRID 49304241): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of MON 102100, MON 102130 and benzamidine in drinking, ground and surface water matrices at fortification levels of 0.10 µg/L (0.10 ppm; LOQ) and 1.00 µg/L (1.00 ppm; 10×LOQ; uncorrected results; pp. 16-18; Tables 19-21, pp. 36-37 ; Appendix E, Tables 16E-18E, pp. 141-142). Performance data (recovery results) from quantitation and confirmation ion analyses were comparable or generally comparable (some variability was seen for LOQ MON 102100 samples in drinking and ground water). The water matrices were well characterized by Agvise Laboratories, Northwood, ND; however, sources were not reported (p. 14; Appendix B, pp. 97-99).

ILV (MRID 49304242): Mean recoveries and relative standard deviations (RSDs) were within guidelines for analysis of MON 102100, MON 102130 and benzamidine in drinking, ground and surface water matrices at fortification levels of 0.10 µg/L (0.10 ppm; LOQ) and 1.00 µg/L (1.00 ppm; 10×LOQ; uncorrected results; Tables 1-3, pp. 22-24; Appendix 3, p. 103). Only the quantitation ion transitions were monitored; confirmation ion analyses were not included. The water matrices were supplied by the sponsor, but water characterization data and sources were not reported (p. 13). The method was validated after one trial (p. 10).

Table 2. Initial Validation Method Recoveries for MON 102100 and Its Transformation Products in Waters^{1,2}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Drinking Water						
Quantitation ion						
MON 102100	0.10 (LOQ)	5	80-85	82	2	3
	1.00	5	81-86	84	2	3
MON 102130 (3-thienyl 102100)	0.10 (LOQ)	5	78-85	81	2	3
	1.00	5	87-90	88	1	1
Benzamidine	0.10 (LOQ)	5	105-107	106	1	1
	1.00	5	102-106	104	2	1
Confirmation ion						
MON 102100	0.10 (LOQ)	5	85-105	99	8	8
	1.00	5	80-90	86	5	5
MON 102130 (3-thienyl 102100)	0.10 (LOQ)	5	93-108	99	7	7
	1.00	5	88-94	92	2	3
Benzamidine	0.10 (LOQ)	5	101-108	104	3	3
	1.00	5	102-105	103	1	1
Surface Water						
Quantitation ion						
MON 102100	0.10 (LOQ)	5	80-86	83	2	3
	1.00	5	86-88	87	1	1
MON 102130 (3-thienyl 102100)	0.10 (LOQ)	5	94-100	97	3	3
	1.00	5	89-96	93	2	3
Benzamidine	0.10 (LOQ)	5	108-112	111	2	2
	1.00	5	103-107	106	2	2
Confirmation ion						
MON 102100	0.10 (LOQ)	5	75-99	86	9	11
	1.00	5	81-91	84	4	5
MON 102130 (3-thienyl 102100)	0.10 (LOQ)	5	88-95	91	3	3
	1.00	5	89-95	92	2	3
Benzamidine	0.10 (LOQ)	5	107-113	110	2	2
	1.00	5	104-107	106	1	1
Ground Water						
Quantitation ion						
MON 102100	0.10 (LOQ)	5	75-84	81	3	4
	1.00	5	86-90	88	2	2
MON 102130 (3-thienyl 102100)	0.10 (LOQ)	5	93-98	95	2	2
	1.00	5	91-94	93	1	1
Benzamidine	0.10 (LOQ)	5	91-102	97	5	5
	1.00	5	101-106	104	2	2

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Confirmation ion						
MON 102100	0.10 (LOQ)	5	85-103	93	7	8
	1.00	5	87-95	90	4	4
MON 102130 (3-thienyl 102100)	0.10 (LOQ)	5	83-93	89	4	4
	1.00	5	88-96	92	4	4
Benzamidine	0.10 (LOQ)	5	87-98	94	5	6
	1.00	5	100-104	103	2	2

Data (uncorrected results; pp. 16-18) were obtained from Tables 19-21, pp. 36-37; Appendix E, Tables 16E-18E, pp. 141-142 of MRID 49304241.

1 Water matrices were fully characterized by Agvise Laboratories, Northwood, ND; however, sources were not reported (p. 14; Appendix B, pp. 97-99 of MRID 49304241).

2 Two parent-daughter ion transitions (quantitative and confirmation, respectively) were monitored for the analytes: m/z 228.9 \rightarrow 111.1 and m/z 228.9 \rightarrow 82.9 for MON 102100 and MON 102130, and m/z 121.0 \rightarrow 104.1 and m/z 121.0 \rightarrow 77.0 for benzamidine (Appendix C, p. 115 of MRID 49304241; Appendix 6, p. 126 of MRID 49304242-missing page from ECM Method reproduction).

Table 3. Independent Validation Method Recoveries for MON 102100 and Its Transformation Products in Waters^{1,2}

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Drinking Water						
Quantitation ion						
MON 102100	0.10 (LOQ)	5	72.9-124	102	18.8	18.4
	1.00 (LOQ)	5	80.3-92.7	88.0	5.0	5.7
MON 102130 (3-thienyl 102100)	0.10 (LOQ)	5	98.6-126	108	10.7	9.9
	1.00 (LOQ)	5	88.7-103	94.5	5.2	5.5
Benzamidine	0.10 (LOQ)	5	104-128	116	9.5	8.2
	1.00 (LOQ)	5	98.2-107	102	3.9	3.8
Surface Water						
Quantitation ion						
MON 102100	0.10 (LOQ)	5	81.3-112	97.9	11.5	11.7
	1.00 (LOQ)	5	86.9-92.8	90.1	2.6	2.9
MON 102130 (3-thienyl 102100)	0.10 (LOQ)	5	87.2-104	96.1	7.9	8.2
	1.00 (LOQ)	5	85.2-102	91.8	7.5	8.2
Benzamidine	0.10 (LOQ)	5	103-124	113	8.5	7.5
	1.00 (LOQ)	5	89.7-101	94.9	4.2	4.4
Ground Water						
Quantitation ion						
MON 102100	0.10 (LOQ)	5	80.9-113	98.8	15.9	16.1
	1.00 (LOQ)	5	80.6-89.5	84.1	3.5	4.2
MON 102130 (3-thienyl 102100)	0.10 (LOQ)	5	68.4-97.8	87.1	12.0	13.8
	1.00 (LOQ)	5	81.6-93.5	87.0	4.8	5.5
Benzamidine	0.10 (LOQ)	5	108-127	116	7.3	6.3
	1.00 (LOQ)	5	100-102	101	0.8	0.8

Data (uncorrected recovery results; Appendix 3, p. 103) were obtained from Tables 1-3, pp. 22-24 of MRID 49304242.

1 Water matrices were supplied by the sponsor, but water characterization data and sources were not reported (p. 13 (p. 13 of MRID 49304242)).

2 Only the quantitation ion calibration curves and raw data were reported (p. 17 of MRID 49304242). One parent-daughter ion transition (quantitative) was monitored for the analytes: m/z 229 \rightarrow 111 for MON 102100 and MON 102130, and m/z 121 \rightarrow 104 for benzamidine.

III. Method Characteristics

LOQ for MON 102100, MON 102130 and benzamidine was the same in the ECM and ILV at 0.10 µg/L (0.10 ppb; pp. 20-21; Tables 22-24, p. 38 of MRID 49304241; pp. 10, 20 of MRID 49304242). The LOD and LOQ for determination of MON 102100 and its transformation products in water were calculated in the ECM study report using the standard deviation from the 0.10 ppb recovery results. The LOD was calculated as three times the standard deviation ($3s$), and the LOQ was calculated as ten times the standard deviation ($10s$) of the recovery results. The reviewer assumed that the reference for these statistics was Keith, L. H., *et. al.* (1983). The calculated values support the LOQ established for the study and are presented in **Table 4** below. The LOD values were not estimated in the ECM, only calculated. The LOD values were not reported in the ILV.

Table 4. Method Characteristics

		MON 102100	MON 102130 (3-thienyl 102100)	Benzamidine
Limit of Quantitation (LOQ)	Established	0.10 ppb		
	Calculated	0.0212-0.0348 ppb (Q) 0.0744-0.0936 ppb (C)	0.0189-0.0266 ppb (Q) 0.0306-0.0675 ppb (C)	0.0090-0.0460 ppb (Q) 0.0227-0.0515 ppb (C)
Limit of Detection (LOD)	Established	None reported		
	Calculated	0.00636-0.01044 ppb (Q) 0.02232-0.02808 ppb (C)	0.00568-0.00798 ppb (Q) 0.00918-0.02024 ppb (C2)	0.00270-0.01380 ppb (Q) 0.00682-0.01546 ppb (C)
Linearity (Least squares calibration curve r and concentration range)	ECM	$r^2 = 0.9975-0.9994$ (Q) 0.030-15.000 ng/mL $r^2 = 0.9986-0.9995$ (C) 0.060-15.000 ng/mL	$r^2 = 0.9979-0.9996$ (Q) 0.030-15.000 ng/mL $r^2 = 0.9986-0.9997$ (C) 0.060-15.000 ng/mL	$r^2 = 0.9991-0.9998$ (Q) 0.030-15.000 ng/mL $r^2 = 0.9994-0.9998$ (C) 0.060-15.000 ng/mL
	ILV ¹	$r^2 = 0.9974-0.9982$ (Q) 0.06-5.0 ng/mL	$r^2 = 0.9982-0.9992$ (Q) 0.06-5.0 ng/mL	$r^2 = 0.9958-0.9996$ (Q) 0.06-5.0 ng/mL
Repeatable	ECM	Yes at LOQ and 10×LOQ (n = 5). [drinking, ground and surface water matrices (source not specified)] ²		
	ILV	Yes at LOQ and 10×LOQ (n = 5). [drinking, ground and surface water matrices (source not specified)] ²		
Reproducible		Yes		
Specific	ECM	No interferences were observed in the matrix control at the analyte retention time; however, LOQ peak was barely discernable above the baseline in the confirmation ion. ³		Matrix interferences were <i>ca.</i> 0-5% of the LOQ based on residue recovery. ⁴
		Interferences were reported as <20% of the LOQ in reagent blanks/controls. Matrix effects were insignificant (2.8-11.1%; quantitation ion only). Two MRM transitions were monitored in LC/MS/MS analysis.		
	ILV	No interferences were observed; however, some integrations of MON 102100 and benzamidine included small neighboring peaks, as well as the main analyte peak. ⁵		One MRM transition was monitored in LC/MS/MS analysis.

Data were obtained from pp. 20-22; Tables 4-9, pp. 28-31; Tables 19-24, pp. 36-38; Tables 29-31, p. 42; Figures 1-39, pp. 43-81; Appendix E, Tables 1E-18E, pp. 133-142; Appendix E, Figures 1E-39E, pp. 143-181 of MRID 49304241; pp. 10, 17, 20; Tables 1-3, pp. 22-24; Appendix 1, Figures 1-63, pp. 31-93 of MRID 49304242. Q = quantitation ion; C = confirmation ion.

¹ Only the quantitation ion calibration curves and raw data were reported (p. 17 of MRID 49304242).

² Water matrices were fully characterized in the ECM, but not characterized in the ILV (p. 14; Appendix B, pp. 97-99 of MRID 49304241; p. 13 of MRID 49304242).

³ Some minor interference in quantitation ion at RT 19.94 min. which was not labelled as MON 102100 since no response was found in the confirmation ion (Figure 31, p. 73; Appendix E, Figure 31E, p. 173 of MRID 49304241).

⁴ Residues in the controls in quantitation and confirmation ions were 0.0052 ng/mL in the control drinking water sample and 0.1048-0.1011 ng/mL at the LOQ (Figure 15, p. 57; Figure 18, p. 60; Appendix E, Figure 15E, p. 157; Appendix E, Figure 18E, p. 160 of MRID 49304241). Some minor interference in quantitation ion was observed at RT 7.70 min. and 7.85 min. in surface and ground water, respectively, which was not labelled as benzamidine

since no response was found in the confirmation ion (Figure 24, p. 66; Figure 33, p. 75; Appendix E, Figure 24E, p. 166; Appendix E, Figure 33E, p. 175).

5 Several notable examples of multi-peak integration in Appendix 1 include Figures 20-21, pp. 50-51; Figures 48-49, pp. 78-79; Figures 62-63, pp. 92-93 of MRID 49304242.

IV. Method Deficiencies and Reviewer's Comments

1. Confirmation ion analysis was included in EPL BAS Method 115G761A and validated by the ECM; however, it was not performed by the ILV (Tables 19-21, pp. 36-37; Appendix E, Tables 16E-18E, pp. 141-142 of MRID 49304241; p. 17; Tables 1-3, pp. 22-24 of MRID 49302042). Confirmatory methods are typically not necessary where GC/MS and LC/MS methods are used as the primary method(s) to generate study data, but the ILV should have included analysis and quantification of both ions in order to validate EPL BAS Method 115G761A. Additionally, the ECM specified that "confirmation of the presence of the analyte requires the observation of the precursor ion as well as an additional structurally significant product ion observed at the same retention time" (p. 19 of MRID 49304241).
2. The ILV water matrices were not characterized, and the sources were not reported (p. 13 of MRID 49304242). The sources of the ECM water matrices were not reported either (p. 14; Appendix B, pp. 97-99 of MRID 49304241).
3. The LOD values were not reported in the ILV.

The lowest toxicological level of concern in water was not reported. A LOQ above toxicological levels of concern results in an unacceptable method classification

4. The EPL BAS Method 115G761A lacked some analytical details: 1) the ion source of the MS was reported as "turbo V??" in the ECM and ILV copy of the method (Appendix C, p. 115 of MRID 49304241; Appendix 6, p. 125 of MRID 49304242); and 2) the monitored ions for MON 102100 confirmatory and MON 102100 IS were reported on a page which was missing in the ECM copy of the method, but present in the ILV copy of the method (Appendix C, p. 115 of MRID 49304241; Appendix 6, p. 126 of MRID 49304242). A complete ECM should have been submitted by the sponsor.

The ion source was specified as turbo ion spray in the ILV (Table 6, p. 27 of MRID 49304242).

5. The representative chromatograms of the ECM showed minor matrix interferences at or near the retention times of MON 102100 and benzamidine in several samples. Also, confirmation ion analyte peaks of MON 102100 and 102130 were barely visible above the baseline at the LOQ (Figures 1-39, pp. 43-81; Appendix E, Figures 1E-39E, pp. 143-181 of MRID 49304241). The representative chromatograms of the ILV showed that some integrations of MON 102100 and benzamidine included small neighboring peaks, as well as the main analyte peak (Appendix 1, Figures 1-63, pp. 31-93 of MRID 49304242).

6. The ECM extraction method was performed by the ILV as written, except that water samples were centrifuged after fortification instead of before fortification (the entire 10-mL sample was fortified instead of an 0.75 mL aliquot; pp. 15-17; Tables 5-6, pp. 26-27 of MRID 49304242). The analytical method was performed using an Agilent 1200 system coupled to an AB Sciex 4000 Mass Spectrometer in positive ion MRM mode (same LC column as ECM). The mobile phase gradient and injection volumes were optimized for the system. None of these modifications were considered substantial. However, only the quantitation ion transitions were monitored in the ILV; this was considered a substantial modification of the ECM (see Reviewer's Comment #1; p. 17).
7. A set of example chromatograms (two calibration standards, a reagent blank, and a control and LOQ for each water matrix) were included with EPL BAS Method 115G761A (Appendix C, pp. 116-124 of MRID 49304241; Appendix 6, pp. 127-135 of MRID 49304242). 10×LOQ chromatograms were not included.
8. In the ILV, the study author reported that communications with the sponsor were limited to the changes to the HPLC parameters and mobile phase gradient and updates regarding trial results (Appendix 4, pp. 104-105 of MRID 49304242).
9. In the ECM, the stability of the QC fortification solutions and working standard solutions were investigated (p. 21; Table 25, p. 39 of MRID 49304241). The solutions were shown to be stable for 33 days under refrigerated storage (temperature not specified) in amber bottles diluted with DI water. Final sample extracts were also evaluated for stability under refrigeration storage (autosampler vials; p. 21; Tables 26-28, pp. 40-41). Drinking and ground water sample extracts were stable for up to 11 days, and surface water sample extracts were stable for up to 3 days.
10. It was reported for the ILV that the analytical procedure for one set of samples [a reagent blank, two controls and fortified samples (LOQ and 10×LOQ)] required approximately three person hours (p. 20 of MRID 49304242). The preparation and analysis of one set required approximately 2 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.

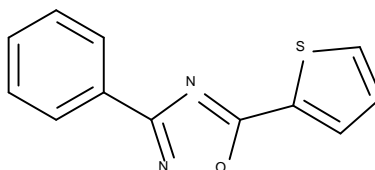
Attachment 1: Chemical Names and Structures**MON 102100 (Tioxazafen)**

IUPAC Name: 3-Phenyl-5-thiophen-2-yl-[1,2,4]oxadiazole
3-Phenyl-5-thiophen-2-yl-1,2,4-oxadiazole

CAS Name: 3-Phenyl-5-(2-thienyl)-1,2,4-oxadiazole

CAS Number: 330459-31-9

SMILES String: c1ccc(cc1)c2nc(on2)c3cccs3

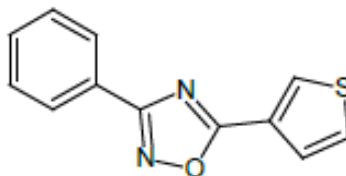
**MON 102130 (3-thienyl 102100)**

IUPAC Name: 3-Phenyl-5-thiophen-3-yl-1,2,4-oxadiazole

CAS Name: Not reported

CAS Number: 255866-91-2

SMILES String: Not found

**Benzamidine**

IUPAC Name: Benzamidine
Benzenecarboximidamide

CAS Name: Not reported

CAS Number: 618-39-3

SMILES String: c1ccccc1C(=N)N

