Analytical method for ipflufenoquin and its metabolite QP-1-7 in water

Reports: ECM: EPA MRID No.: 50920988. Rastogi, T., and M. Senciuc. 2018. Development and Validation of an Analytical Method for the Determination of NF-180 and its Metabolite QP-1-7 in Surface and in Ground Water. Report prepared by EAG Laboratories GmbH, Ulm, Germany, sponsored by Nippon Soda Co., Ltd., Tokyo, Japan, and submitted by Nippon Soda Co., Ltd., Tokyo, Japan and Nippon Soda Co., Ltd. (c/o Nisso America, Inc.), New York, New York (pp. 1-2); 75 pages. EAG Laboratories ID: P 4906 G. Sponsor Study Code: JAS-574: Nisso-NF-180. Final report issued November 14, 2018.

ILV: EPA MRID No.: 50920987. Lawson, C.F. 2019. Independent Laboratory Validation of "Development and Validation of an Analytical Method for the Determination of NF-180 and its Metabolite QP-1-7 in Surface and in Ground Water". Report prepared by Eurofins EAG Agroscience, LLC, Columbia, Missouri, sponsored by Nippon Soda Co., Ltd., Tokyo, Japan, and submitted by Nippon Soda Co., Ltd., Tokyo, Japan and Nippon Soda Co., Ltd. (c/o Nisso America, Inc.), New York, New York (pp. 1-2); 147 pages. Eurofins Study No.: 87623. Sponsor Study Code: JAS-573b: Nisso-NF-180. Final report issued September 25, 2019.

Document No.: MRIDs 50920988 & 50920987

Guideline: 850.6100

Statements: ECM: The study was conducted in compliance with German (2013) and OECD (1997 and 2007) Good Laboratory Practice (GLP) standards, which are accepted by Regulatory Authorities in Europe (Directive 2004/10/EC), the United States (FDA and EPA) and Japan (MHLW, MAFF, and METI; pp. 3, 5; Appendix 4, p. 64 of MRID 50920988). The characterization of the water matrices was performed at Institut Alpha (non-GLP, DIN/EN methods). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-5; Appendix 4, p. 64). The statement of authenticity was included with the QA statement.
ILV: The study was conducted in compliance with USEPA FIFRA GLP standards, with the exception that a critical phase audit was inadvertently not conducted by the test facility for this study (pp. 3-4 of MRID 50920987). Signed and dated Data Confidentiality, GLP, Quality Assurance, and Authenticity statements were provided (pp. 2-5).

Classification: This analytical method is classified as **Supplemental**. The ILV was not conducted independently from the ECM since ECM personnel communicated directly with the ILV personnel. The ILV could not reproduce the method for QP-1-7 after two trials and eliminated the analyte from the study report. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method.

PC Code: 129120

EFED Final Reviewer:	Jerrett Fowler Physical Scientist OPP-EFED-ERB2	Signature: Date: 3/18/2021
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, M.S., Environmental Scientist	Signature: Date: 02/27/2020
	Mary Samuel, M.S., Environmental Scientist	Signature: Date: 02/27/2020

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac Joint Venture personnel. The CDM/CSS-Dynamac JV role does not include establishing Agency policies.

Executive Summary

The analytical method, EAG Laboratories GmbH ID: P 4906 G and Sponsor Study Code: JAS-574: Nisso-NF-180, is designed for the quantitative determination of ipflufenoquin and its metabolite QP-1-7 in water at the stated LOQ of 0.05 µg/L using HPLC/MS/MS. The LOQ is less than the lowest toxicological level of concern in water. The ECM used characterized surface and ground water matrices for the internal validation. The ILV performed two trials, each with a unique surface water matrix: the surface water matrix for the first trial was not characterized, and the surface water matrix for the second trial was the ECM surface water matrix. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method. The ILV validated the method for ipflufenoquin in the first and second trials with elimination of the LC pre-column and insignificant analytical instrument and equipment modifications; however, no samples in the first trial were fortified at the method LOQ due to a calculation error. The method could not be validated for metabolite QP-1-7 by the ILV after the two trials with two different surface water matrices due to low recoveries, and, at the request of the Sponsor, the ILV report only included the parent, ipflufenoquin, and not its metabolite QP-1-7. The ILV was not conducted independently from the ECM since the ILV personnel communicated directly with the ECM personnel to discuss causes and solutions for the ILV's failure to validate the method for QP-1-7. All ILV data regarding repeatability, accuracy, precision, linearity and specificity were satisfactory for ipflufenoquin; however, the specificity of the method was not well-supported by the representative chromatograms for ipflufenoquin since the quantitation ion transition matrix interferences were greater than the corresponding calculated LOD. No raw data for QP-1-7 was provided in the ILV. All ECM data regarding repeatability, accuracy, precision, linearity and specificity were satisfactory for ipflufenoquin and QP-1-7 in both water matrices.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide[1]	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Ipflufenoquin (NF-180)	50920988[2]	50920987[3]		Water	14/11/2018	Nippon Soda Co., Ltd. (c/o Nisso America Inc.)	LC/MS/MS	0.05 µg/L
QP-1-7		None submitted[4]						

1 Ipflufenoquin = 2-[2-(7,8-Difluoro-2-methyl-3-quinolyloxy)-6-fluorophenyl]propan-2-ol; QP-1-7 = (2-[2-(7,8-Difluoro-2-methyl-3-quinolyloxy)-6-fluorophenyl]-2-hydroxypropanoic acid.

2 In the ECM, surface (river) water (pH 8.20, conductivity 544 µS/cm at 25°C, total water hardness 2.59 mmol/L, total organic carbon 2.1 mg/L, total dissolved carbon 2.0 mg/L), obtained from Danube River, Landeswasserversorgung Langenau, Germany (near Leipheim), and ground water (pH 7.40, conductivity 827 µS/cm at 25°C, total water hardness 3.31 mmol/L, total organic carbon 1.2 mg/L, total dissolved carbon 1.1 mg/L), obtained from Buxheim, Germany, were characterized by Institut Alpha (non-GLP) and used in the study (p. 12; Appendix 2, pp. 58-61 of MRID 50920988).

3 In the ILV, two trials were performed, and each trial had a unique surface water matrix (p. 13 of MRID 50920987). The surface water matrix for the first trial was collected on July 26, 2017, on the site of the ILV test facility and was not characterized (p. 13; Appendix VI, p. 142 of MRID 50920987). The surface water matrix for the second trial was provided by the Sponsor, received on July 12, 2019, and was the surface water matrix used in the primary lab validation (p. 13; Appendix VI, pp. 142-143 of MRID 50920987). Surface water characterization data for the second trial matrix was not provided in the study report. No samples in the first trial were fortified at the method LOQ due to a calculation error (Appendix VI, p. 145 of MRID 50920987).

4 At the request of the Sponsor, the ILV only included the parent, ipflufenoquin, and not its metabolite QP-1-7 (Appendix I, pp. 49-50 of MRID 50920987). Recoveries for ipflufenoquin metabolite QP-1-7 were <70% at both fortifications in the first trial and at LOQ in the second trial; individual recoveries not reported (p. 18; Appendix VI, pp. 142-143 of MRID 50920987).

I. Principle of the Method

Water (10 g, equivalent to 10 mL) in 50-mL centrifuge tube was fortified with 50 µL of 0.10 or 0.010 µg/mL fortification solutions, if necessary (pp. 9, 14, 18 of MRID 50920988). The water samples were mixed with 10 mL acetonitrile with vigorous shaking for 1 minute. Citrate Extraction Tubes (4 g magnesium sulphate, 1 g sodium chloride, and buffering citrate salts) were added, and the mixture was shaken intensively for 1 minute followed by centrifugation (5 minutes at 4000 rpm). An aliquot (500 µL) of the acetonitrile layer was mixed with 500 µL of water to obtain a 1.0 mL extract in acetonitrile:water (1:1, v:v). The final extracts were analyzed using LC-MS/MS

Samples are analyzed using an Applied Biosystems MDS Sciex API 6500+ triple quadrupole mass spectrometer coupled with an Agilent 1290 HPLC (pp. 13, 16 of MRID 50920988). The following LC conditions were used: Phenomenex C_{18} UPLC pre-column (specifications not reported), Waters Acquity UPLC BEH C18 column (2.1 mm x 100 mm, 1.7 µm; column temperature 40°C), gradient mobile phase of A) water + 0.1% acetic acid and B) acetonitrile + 0.1% acetic acid [time, percent A:B; 0.00 min. 80:20, 5.00-6.00 min. 10:90, 6.10-8.00 min. 80:20], injection volume of 10 µL, MS/MS with TurboIonspray (ESI) source in positive polarity (source temperature 400°C). Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z*

348→330 and *m/z* 348→180 for ipflufenoquin and *m/z* 378→332 and *m/z* 378→314 for QP-1-7. Approximate retention times were 4.9 minutes for ipflufenoquin and 3.8 minutes for QP-1-7.

The ILV performed the ECM method for ipflufenoquin as written, except for the exclusion of the Phenomenex C18 pre-column in the HPLC analysis, as well as insignificant analytical instrument and equipment modifications (pp. 14-16, 20-21; Appendix II, p. 128 of MRID 50920987). The LC/MS/MS instrument was Applied Biosystems/Sciex API 6500 Q-trap mass spectrometer coupled with a Shimadzu UHPLC System. The LC conditions were the same as those of the ECM, except that the MS source temperature was 500°C. The ion pair transitions monitored for ipflufenoquin were the same as those of the ECM. Approximate retention time was 4.75 minutes for ipflufenoquin. The ILV included QP-1-7 when performing the ECM method for ipflufenoquin; however, QP-1-7 recoveries were unacceptable (<70%; p. 18; Appendix VI, pp. 142-143). The Sponsor requested that only ipflufenoquin was included in the ILV (Appendix I, pp. 49-50).

In the ECM, the Limit of Quantification (LOQ) and Limit of Detection (LOD) values were 0.05 µg/L and 0.01 µg/L, respectively, for both analytes in both water matrices (pp. 9, 20, 23 of MRID 50920988). In the ILV, the method LOQ was 0.05 µg/L for ipflufenoquin; the method LOD was not reported (pp. 11, 18; Table 4, p. 25 of MRID 50920987). The LOQ and LOD values for ipflufenoquin were calculated as 0.0257-0.0695 µg/L and 0.00857-0.0232 µg/L, respectively, for surface water matrix in the ILV.

II. Recovery Findings

ECM (MRID 50920988): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of ipflufenoquin and its metabolite QP-1-7 at fortification levels of 0.05 µg/L (LOQ) and 0.5 µg/L (10×LOQ) in two water matrices (Tables 1-2, pp. 24-25; DER Attachment 2). Two ion pair transitions were monitored, one quantitation and one confirmation; quantitation and confirmation; recovery results were comparable. The first sample of each set was injected twice, and the mean of those two injections was used for the recovery value. Surface (river) water (pH 8.20, conductivity 544 µS/cm at 25°C, total water hardness 2.59 mmol/L, total organic carbon 2.1 mg/L, total dissolved carbon 2.0 mg/L), obtained from Danube River, Landeswasserversorgung Langenau, Germany (near Leipheim), and ground water (pH 7.40, conductivity 827 µS/cm at 25°C, total water hardness 3.31 mmol/L, total organic carbon 1.2 mg/L, total dissolved carbon 1.1 mg/L), obtained from Buxheim, Germany, were characterized by Institut Alpha (non-GLP) and used in the study (p. 12; Appendix 2, pp. 58-61).

ILV (MRID 50920987): Mean recoveries and RSDs were within guidelines for analysis of ipflufenoquin at fortification levels of 0.05 µg/L (LOQ) and 0.5 µg/L (10×LOQ) in one water matrix (second trial) and at fortification levels of 0.0505 µg/L and 0.505 µg/L in one water matrix (first trial; p. 18; Tables 1-2, p. 24; Appendix VI, pp. 142-143). Two ion pair transitions were monitored, one quantitation and one confirmation; quantitation and confirmation recovery results were comparable. Recoveries for ipflufenoquin metabolite QP-1-7 were <70% at both fortifications in the first trial and at LOQ in the second trial; individual recoveries not reported. The surface water matrix for the first trial was collected on July 26, 2017, on the site of the ILV test facility and was not characterized (p. 13; Appendix VI, p. 142). The surface water matrix for the second trial was provided by the Sponsor, received on July 12, 2019, and was the surface water matrix used in the

primary lab validation (p. 13; Appendix VI, pp. 142-143). Surface water characterization data for the second trial matrix was not provided in the study report. The method was validated for ipflufenoquin in the first and second trials with elimination of the LC pre-column and insignificant analytical instrument and equipment modifications; however, no samples in the first trial were fortified at the method LOQ due to a calculation error (pp. 14-16, 20-21; Appendix VI, pp. 142-143, 145). The method could not be validated for metabolite QP-1-7 by the ILV after two trials with two different surface water matrices, and, at the request of the Sponsor, the ILV report only included the parent, ipflufenoquin, and not its metabolite QP-1-7 (Appendix I, pp. 49-50).

Table 2. Initial Validation Method Recoveries for Ipflufenoquin and QP-1-7 in Water[1,2,3]

Analyte	Fortification Level (µg/L)	Number of Tests[4]	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)[5]	Relative Standard Deviation (%)
	Surface (River) Water					
	Quantitation Ion Transition					
Ipflufenoquin (NF-180)	0.05 (LOQ)	5	85-87	86	1	1
	0.5	5	88-90	88	1	1
QP-1-7	0.05 (LOQ)	5	81-90	87	4	4
	0.5	5	74-77	76	1	8
	Confirmation Ion Transition					
Ipflufenoquin (NF-180)	0.05 (LOQ)	5	87-95	90	3	3
	0.5	5	87-91	89	2	2
QP-1-7	0.05 (LOQ)	5	85-90	88	2	3
	0.5	5	74-77	76	1	2
	Ground Water					
	Quantitation Ion Transition					
Ipflufenoquin (NF-180)	0.05 (LOQ)	5	81-89	85	3	4
	0.5	5	78-85	80	3	3
QP-1-7	0.05 (LOQ)	5	78-85	81	3	4
	0.5	5	76-84	79	3	4
	Confirmation Ion Transition					
Ipflufenoquin (NF-180)	0.05 (LOQ)	5	83-96	89	5	6
	0.5	5	79-86	81	3	3
QP-1-7	0.05 (LOQ)	5	77-88	81	4	5
	0.5	5	76-86	79	4	5

Data (uncorrected recovery results; pp. 17-18) were obtained from Tables 1-2, pp. 24-25 of MRID 50920988 and DER Attachment 2.

1 Ipflufenoquin = 2-[2-(7,8-Difluoro-2-methyl-3-quinolyloxy)-6-fluorophenyl]propan-2-ol; QP-1-7 = (2-[2-(7,8-Difluoro-2-methyl-3-quinolyloxy)-6-fluorophenyl]-2-hydroxypropanoic acid.

2 Surface (river) water (pH 8.20, conductivity 544 µS/cm at 25°C, total water hardness 2.59 mmol/L, total organic carbon 2.1 mg/L, total dissolved carbon 2.0 mg/L), obtained from Danube River, Landeswasserversorgung Langenau, Germany (near Leipheim), and ground water (pH 7.40, conductivity 827 µS/cm at 25°C, total water hardness 3.31 mmol/L, total organic carbon 1.2 mg/L, total dissolved carbon 1.1 mg/L), obtained from Buxheim, Germany, were characterized by Institut Alpha (non-GLP) and used in the study (p. 12; Appendix 2, pp. 58-61 of MRID 50920988).

3 Two ion pair transitions were monitored for each analyte (quantitation and confirmation, respectively): *m/z* 348→330 and *m/z* 348→180 for ipflufenoquin and *m/z* 378→332 and *m/z* 378→314 for QP-1-7.

4 The first sample of each set was injected twice, and the mean of those two injections was used for the recovery value.

5 Standard deviations were reviewer-calculated using the data in the study report since the study author did not report these values (see DER Attachment 2). Rules of significant figures were followed.

Table 3. Independent Validation Method Recoveries for Ipflufenoquin in Water[1,2]

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Surface Water – First Trial[3]					
	Quantitation ion transition					
Ipflufenoquin (NF-180)	0.0505	5	67-89	83	9.3	11
	0.505	5	77-93	86	6.6	7.7
QP-1-7	0.05 (LOQ)	Unacceptable recoveries (<70%); individual recoveries not reported.				
	0.5					
	Confirmation ion transition					
Ipflufenoquin (NF-180)	0.0505	5	65-98	86	12	15
	0.5	5	79-91	84	5.3	6.2
QP-1-7	0.05 (LOQ)	Unacceptable recoveries (<70%); individual recoveries not reported.				
	0.5					
	Surface Water – Second Trial[4]					
	Quantitation ion transition					
Ipflufenoquin (NF-180)	0.0500 (LOQ)	5	79-90	84	4.6	5.5
	0.500	5	83-88	86	2.2	2.5
QP-1-7	0.05 (LOQ)	Unacceptable recoveries (<70%); individual recoveries not reported.				
	0.5	No results reported.				
	Confirmation ion transition					
Ipflufenoquin (NF-180)	0.0500 (LOQ)	5	72-94	85	8.6	10
	0.500	5	79-91	86	4.6	5.3
QP-1-7	0.05 (LOQ)	Unacceptable recoveries (<70%); individual recoveries not reported.				
	0.5	No results reported.				

Data (uncorrected recovery results; pp. 16-17) were obtained from p. 18; Tables 1-2, p. 24; Appendix VI, pp. 142-143 of MRID 50920987.

1 Ipflufenoquin = 2-[2-(7,8-Difluoro-2-methyl-3-quinolyloxy)-6-fluorophenyl]propan-2-ol; QP-1-7 = (2-[2-(7,8-Difluoro-2-methyl-3-quinolyloxy)-6-fluorophenyl]-2-hydroxypropanoic acid.

2 Two ion pair transitions were monitored for ipflufenoquin (quantitation and confirmation, respectively): *m/z* 348→330 and *m/z* 348→180.

3 The surface water matrix was collected on July 26, 2017, on the site of the ILV test facility (p. 13 of MRID 50920987). The surface water matrix was not characterized (Appendix VI, p. 142 of MRID 50920987). No samples in the first trial were fortified at the method LOQ due to a calculation error (Appendix VI, p. 145 of MRID 50920987).

2 The surface water matrix was provided by the Sponsor and received on July 12, 2019 (p. 13 of MRID 50920987). The surface water matrix was the surface water matrix used in the primary lab validation (Appendix VI, pp. 142-143 of MRID 50920987). Surface water characterization data for the second trial matrix was not provided in the study report.

III. Method Characteristics

In the ECM, the LOQ and LOD values were 0.05 µg/L and 0.01 µg/L, respectively, for both analytes in both water matrices (pp. 9, 20, 23 of MRID 50920988). In the ECM, no justification of the LOQ was reported. The LOD was set to 20% of the LOQ and based on the signal obtained for the lowest calibration standard. In the ILV, the method LOQ was 0.05 µg/L for ipflufenoquin; the method LOD was not reported (pp. 11, 18; Table 4, p. 25 of MRID 50920987). The method LOQ was reported from the ECM without further justification. The LOQ and LOD values for ipflufenoquin were calculated as 0.0257-0.0695 µg/L and 0.00857-0.0232 µg/L, respectively, for the surface water matrix in the ILV, based on the following equations:

$LOD_{calc} = (t_{0.99} \times s)$

$LOQ_{calc} = 3 \times LOD_{calc}$

Where, $t_{0.99}$ is the one-tailed t-test value at the 99% confidence interval for n-1 degrees of freedom (where n is the number of replicates) and s is the standard deviation of the analyte recovery measurements at the target LOQ. The LOD and LOQ was calculated for both monitored ions in Trials 1 and 2; the calculated LOQs in the ILV supported the method LOQ. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM or ILV; no calculations or comparisons to background levels were reported to justify the LOD for the method in the ECM.

Table 4. Method Characteristics Ipflufenoquin and QP-1-7 in Water

Test Material[1]			Ipflufenoquin	QP-1-7
Limit of Quantitation (LOQ)	ECM (method)		0.05 µg/L	
	ILV	Method	0.05 µg/L	
		Calculated	0.0519-0.0695 µg/L (First Trial) 0.0257-0.0488 µg/L (Second Trial)	**Not reported**
Limit of Detection (LOD)	ECM (method)		0.01 µg/L (20% of the LOQ)	
	ILV	Method	**Not reported**	
		Calculated	0.0173 µg/L (Q, First Trial) 0.0232 µg/L (C, First Trial) 0.00857 µg/L (Q, Second Trial) 0.0163 µg/L (C, Second Trial)	**Not reported**
Linearity (calibration curve r and concentration range)	ECM		r = 0.9999 (Q & C)	r = 0.9998 (Q) r = 0.9999 (C)
			0.0050-1.0 ng/mL	
	ILV		r = 0.99992 (Q; First Trial) r = 0.99948 (C; First Trial) r = 0.99790894 (Q; Second Trial) r = 0.99743066 (C; Second Trial)	**Not reported**
			0.0050-1.0 ng/mL	
Repeatable	ECM[2]		Yes at LOQ and 10×LOQ in characterized surface and ground water matrices.	
	ILV[3]	First Trial[4]	Yes at 0.0505 µg/L and 0.505 µg/L in uncharacterized surface water matrix.	**No** at 0.0505 µg/L and 0.505 µg/L in uncharacterized surface water matrix (recoveries <70%).
		Second Trial[5]	Yes at LOQ and 10×LOQ in uncharacterized surface water matrix.	**No** at LOQ in uncharacterized surface water matrix (recoveries <70%); no data reported for 10×LOQ.
Reproducible			Yes at LOQ and 10×LOQ.	**No** at LOQ and 10×LOQ.
Specific	ECM		Yes, matrix interferences were <5% of the LOQ (based on peak area). Some minor baseline noise was noted at the LOQ.	Yes, matrix interferences were <11% of the LOQ (based on peak area). Some minor baseline noise interference was noted at the LOQ.
	ILV		Yes, matrix interferences were <18% of the LOQ (based on peak area).[6] Some minor baseline noise interference was noted at the LOQ; LOQ peak was relatively small compared to baseline noise.	**Not provided**

Data were obtained from pp. 9, 20, 23 (LOQ/LOD); Tables 1-2, pp. 24-25 (recovery results); Figures 1-2, pp. 34-37 (calibration curves); Figures 3-18, pp. 38-55 (chromatograms) of MRID 50920988; pp. 11, 18; Table 4, p. 25 (LOQ/LOD); p. 18; Tables 1-2, p. 24; Appendix VI, pp. 142-143 (recovery results); Figure 1, pp. 27-28; Appendix V, pp. 135-139 (calibration curves); Figures 2-6, pp. 29-33 (chromatograms) of MRID 50920987. Q = quantitation ion transition; C = confirmation ion transition.

1 Ipflufenoquin = 2-[2-(7,8-Difluoro-2-methyl-3-quinolyloxy)-6-fluorophenyl]propan-2-ol; QP-1-7 = (2-[2-(7,8-Difluoro-2-methyl-3-quinolyloxy)-6-fluorophenyl]-2-hydroxypropanoic acid.

2 In the ECM, surface (river) water (pH 8.20, conductivity 544 µS/cm at 25°C, total water hardness 2.59 mmol/L, total organic carbon 2.1 mg/L, total dissolved carbon 2.0 mg/L), obtained from Danube River, Landeswasserversorgung Langenau, Germany (near Leipheim), and ground water (pH 7.40, conductivity 827 µS/cm at 25°C, total water hardness 3.31 mmol/L, total organic carbon 1.2 mg/L, total dissolved carbon 1.1 mg/L), obtained from Buxheim, Germany, were characterized by Institut Alpha (non-GLP) and used in the study (p. 12; Appendix 2, pp. 58-61 of MRID 50920988).

3 The ILV validated the method for ipflufenoquin in the first and second trials with elimination of the LC pre-column and insignificant analytical instrument and equipment modifications; however, no samples in the first trial were fortified at the method LOQ due to a calculation error (pp. 14-16, 20-21; Appendix VI, pp. 142-143, 145 of MRID 50920987). The method could not be validated for metabolite QP-1-7 by the ILV after two trials with two different surface water matrices, and, at the request of the Sponsor, the ILV report only included the parent, ipflufenoquin, and not its metabolite QP-1-7 (Appendix I, pp. 49-50 of MRID 50920987).

4 The surface water matrix for the first ILV trial was collected on July 26, 2017, on the site of the ILV test facility and was not characterized (p. 13; Appendix VI, p. 142 of MRID 50920987).

5 The surface water matrix for the second ILV trial was provided by the Sponsor, received on July 12, 2019, and was the surface water matrix used in the primary lab validation (p. 13; Appendix VI, pp. 142-143 of MRID 50920987). Surface water characterization data for the second trial matrix was not provided in the study report.

6 Based on Figures 4-5, pp. 31-32, of MRID 50920987.

IV. Method Deficiencies and Reviewer's Comments

1. The ILV was not conducted independently from the ECM since ECM personnel Monica Senciuc (Method Developer/Study Director) communicated directly with the ILV personnel (Charles Lawson) after the failure of the second ILV trial for the validation of the method for QP-1-7 (pp. 1, 8 of MRID 50920988; p. 1; Appendix VI, p. 144 of MRID 50920987). Communication between the ECM and ILV personnel involved brainstorming causes and solutions for the ILV's failure to validate the method for QP-1-7, especially since the ILV used the ECM surface water matrix in the second trial so concerns of the water properties were no longer valid. OCSPP guidelines state that the analysts, study director, equipment, instruments, and supplies of the two laboratories must have been distinct and operated separately and without collusion, and the analysts and study director of the ILV must have been unfamiliar with the method both in its development and subsequent use in field studies.

   The reviewer noted that the communication between the ECM and ILV was only initiated due to the failed trials of QP-1-7 (p. 20; Appendix VI, pp. 142-145 of MRID 50920987). All other communication regarding the method was between the ILV personnel (Charles Lawson) and the Study Monitor (Marc Vermeir; pp. 1, 3 of MRID 50920987). No communication between the ECM and ILV was necessary for the validation of the method for ipflufenoquin.

2. The method was not reproducible for QP-1-7 since the ILV performance data was not acceptable at the LOQ (<70%) and 10×LOQ (not reported; p. 18; Appendix VI, pp. 142-143 of MRID 50920987). The individual recovery data, calibration curves, and representative chromatograms for QP-1-7 from the first and second trials were not included in the ILV study report. Only a brief summary of results was provided in the results section and communication details. QP-1-7 was not even included as a test material in the ILV study report due to the fact that the Study Sponsor sent protocol Amendment 2, which removed metabolite QP-1-7 from the study since "QP-1-7 metabolite residues were not part of residue definition"(Appendix VI, p. 145). All raw data should be submitted for review. The ILV study report should have reflected the fact that the validation of the method for QP-1-7 was unsuccessful, instead of not attempted. Additionally, the following ILV statement is inaccurate without further details: "The results of the analyses indicate that the ILV for Method EAG Laboratories GmbH ID: P 4906 G for surface water has passed" (p. 20).

3. The ILV water matrices were not characterized (p. 13; Appendix VI, pp. 142-143 of MRID

50920987). Additionally, the surface water matrix for the second ILV trial was the surface water matrix used in the primary lab validation. It could not be determined if the ILV was provided with the most difficult matrices with which to validate the method. Also, the ILV should validate the method using means which are as or more rigorous than those used by the ECM.

4. In the ILV, the specificity of the method was not well-supported by the representative chromatograms for ipflufenoquin since the quantitation ion transition matrix interferences were *ca*. 17.5% of the LOQ (based on peak area) and the LOQ peak was relatively small compared to baseline noise (Figures 4-5, pp. 31-32, of MRID 50920987). The calculated LOD was 0.00857 µg/L (Q, Second Trial) which was equivalent to *ca*. 17.1% of the LOQ; therefore, the matrix interference was >LOD.

5. The determinations of LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 9, 20, 23 of MRID 50920988; pp. 11, 18; Table 4, p. 25 of MRID 50920987). In the ECM, no justification of the LOQ was reported. The LOD was set to 20% of the LOQ and based on the signal obtained for the lowest calibration standard. In the ILV, the method LOQ was reported from the ECM without further justification. The LOQ and LOD values for ipflufenoquin were calculated in the ILV based on the following equations: $LOD_{calc} = (t_{0.99} \times s)$ and $LOQ_{calc} = 3 \times LOD_{calc}$, where $t_{0.99}$ is the one-tailed t-test value at the 99% confidence interval for n-1 degrees of freedom (where n is the number of replicates) and s is the standard deviation of the analyte recovery measurements at the target LOQ. No calculations or comparisons to background levels were reported to justify the LOQ for the method in the ECM or ILV; no calculations or comparisons to background levels were reported to justify the LOD for the method in the ECM. Detection limits should not be based on arbitrary values.

6. Matrix effects were studied in the ECM and ILV and found to be insignificant (<20%; p. 18; Table 3, p. 26 of MRID 50920988; pp. 19-20; Table 3, p. 25 of MRID 50920987). Solvent-based calibration standards were used for quantification of the residues.

7. The stability of the standard solutions and sample extracts was investigated by the ECM (pp. 18-19; Tables 4-6, pp. 27-29 of MRID 50920988). The stock solutions and calibration solutions were found to be stable under refrigerated storage for 28 and 27 days, respectively. The final sample extracts were found to be stable under refrigerated storage for up to 7 days (recovery within 20% of original for both analytes in both matrices).

8. In the ECM, the total time required to perform the method (extraction and analysis) with one sample set was *ca*. 1.5 working day (p. 19 of MRID 50920988). One set of 13 samples required *ca*. 2 hours (sample processing), *ca*. 3-4 hours (analysis) and *ca*. 3 hours (data processing). In the ILV, the total time required to perform the method (extraction and analysis) with one sample set was *ca*. 11 hours or 1 calendar day (p. 20 of MRID 50920987). One set of 13 samples required *ca*. 8 hours (sample processing) and *ca*. 3 hours (analysis); data processing time not included.

V. References

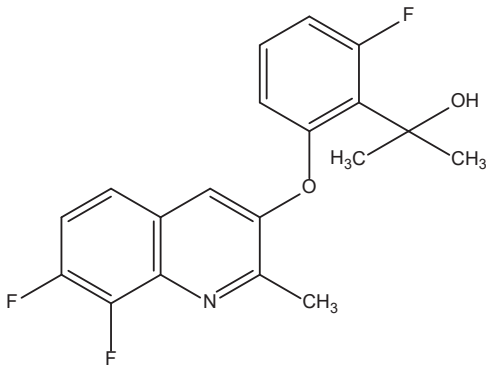
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

Ipflufenoquin (NF-180)

IUPAC Name:	2-[2-(7,8-Difluoro-2-methyl-3-quinolyloxy)-6-fluorophenyl]propan-2-ol
CAS Name:	2-[(7,8-Difluoro-2-methyl-3-quinolinyl)oxy]-6-fluoro-α,α-dimethylbenzenemethanol
CAS Number:	1314008-27-9
SMILES String:	FC1=C(F)C=CC2=C1N=C(C)C(OC3=C(C(C)(C)O)C(F)=CC=C3)=C2



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IUPAC Name:	2-[2-(7,8-Difluoro-2-methyl-3-quinolyloxy)-6-fluorophenyl]-2-hydroxypropanoic acid
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	Not found