Test Material:	Pyrithiobac-Na
MRID:	49155901
Title:	Analytical Method for the Determination of Pyrithiobac Sodium and Metabolites in Water Using LC/MS/MS
MRID:	49332101
Title:	Independent Laboratory Validation of DuPont-36965, "Analytical Method for the Determination of Pyrithiobac Sodium and Metabolites in Water Using LC/MS/MS"
EPA PC Code:	078905
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lynne Binari **Signature:** 

Date: 2/16/15

Secondary Reviewer: Lisa Muto **Signature:** 

Date: 2/16/15

QC/QA Manager: Joan Gaidos **Signature:** 

Date: 2/16/15

Analytical method for pyrithiobac-Na and its transformation products IN-B5363 and IN-JW212 in water

Reports: ECM: EPA MRID No.: 49155901. Henze, R. and J. Stry. 2013. Analytical Method for the Determination of Pyrithiobac Sodium and Metabolites in Water Using LC/MS/MS. Project Identification No.: DuPont-36965. Report prepared by E. I. du Pont de Nemours and Company, DuPont Crop Protection, Newark, Delaware, sponsored and submitted by E. I. du Pont de Nemours and Company, Wilmington, Delaware; 78 pages. Final report issued May 24, 2013.
ILV: EPA MRID No. 49332101. Grant, J. 2014. Independent Laboratory Validation of DuPont-36965, "Analytical Method for the Determination of Pyrithiobac Sodium and Metabolites in Water Using LC/MS/MS". ABC Study No.: 80142. DuPont Study No.: DuPont-37868. Report prepared by ABC Laboratories, Inc., Columbia, Missouri, sponsored and submitted by E. I. du Pont de Nemours and Company, Wilmington, Delaware; 140 pages. Final report issued February 27, 2014.

Document No.: MRIDs 49155901 & 49332101

Guideline: 850.6100

Statements: ECM: The study was not conducted under the restriction of compliance with USEPA Good Laboratory Practice (GLP) standards; however the study was conducted in a GLP compliant facility following Standard Operating Procedures (p. 3 of MRID 49155901). Signed and dated Data Confidentiality, GLP, and Authenticity Certification statements were provided (pp. 2-4). A Quality Assurance statement was not provided.
ILV: The study was conducted in compliance with USEPA GLP standards (p. 3 of MRID 49332101). Signed and dated Data Confidentiality, GLP, Quality Assurance, and Authenticity Certification statements were provided (pp. 2-5).

Classification: This analytical method and its Independent Laboratory Validation are classified as supplemental. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

PC Code: 078905

Reviewer:

Final Reviewer: **Ibrahim Abdel-Saheb** Signature:
EPA Reviewer Date: 01-11-2016

Executive Summary

This analytical method, DuPont-36965, is designed for the quantitative determination of pyrithiobac-Na and its transformation products IN-B5363 and IN-JW212 in water using LC/MS/MS. The method is quantitative for the analytes at the stated LOQ of 0.10 µg/kg (ppb). The LOQ is [less than/equal to/greater than] the lowest toxicological level of concern in water. The independent laboratory validated the method for analysis of pyrithiobac-Na, IN-B5363, and IN-JW212 in surface, ground, and drinking water matrices after one trial. No major modifications were made by the independent laboratory.

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						
Pyrithiobac-Na	49155901	49332101		Surface, ground, and drinking water	24/05/2013	E. I. du Pont de Nemours and Company	LC/MS/MS	0.10 µg/kg (ppb)
IN-B5363								
IN-JW212								

I. Principle of the Method

Water (10.0 g ± 1%) was fortified with a mixed standard of pyrithiobac-Na, IN-B5363, and IN-JW212 in methanol for procedural recoveries (pp. 11-12 of MRID 49155901). Water samples (10.0 g ± 1%) are filtered (0.20-µm Acrodisc PTFE syringe filter) and analyzed directly by LC/MS/MS (pp. 10, 13).

Samples are analyzed for pyrithiobac-Na and its products IN-B5363 and IN-JW212 by HPLC (Agilent 1200 LC system, MacMod ACE C18-PFP, 3.0 mm x 50 mm column, column temperature 40°C) using a mobile phase of (A) 0.05% aqueous formic acid and (B) methanol [percent A:B (v:v) at 0.0-2.0 min. 90:10, 5.0-7.0 min. 1:99, 8.0-15.0 min. 90:10; flow rate 0.6 mL/minute] with MS/MS-ESI [Applied Biosystems API 5000 MS, electrospray (turbospray) ionization, positive ion mode] detection and multiple reaction monitoring (MRM; pp. 13-15 of MRID 49155901). Injection volume is 25 µL. Analytes are identified using two ion transitions; one for quantitation (Q) and one for confirmation (C). Ion transitions monitored were as follows: *m/z* 327.0→308.9 (Q) and *m/z* 329.0→139.1 (C) or *m/z* 329.0→83.0 (C) for pyrithiobac-Na, *m/z* 157.1→68.0 (Q) and *m/z* 157.1→58.0 (C) for IN-B5363, and *m/z* 313.0→196.0 (Q) and *m/z* 313.0→295.0 (C) for IN-JW212. Expected retention times were 2.1, 6.2, and 6.9 minutes for IN-B5363, IN-JW212, and pyrithiobac-Na (DPX-PE350), respectively.

ILV: The independent laboratory performed the methods as written with equivalent equipment substitutions and minor modifications to optimize LC/MS/MS conditions (pp. 16-18 of MRID 49332101). The surface water and ground water matrices were obtained from Agvise Laboratories, Inc., Northwood, North Dakota (p. 16; Appendix 2, pp. 92-94). The drinking water was Aquafina bottled water (pp. 11, 16; Appendix 4, p. 138).

LOQ and LOD: In the ECM and ILV, the LOQ and LOD for all analytes were 0.10 and 0.03 µg/kg (ppb), respectively (p. 18 of MRID 49155901; p. 14 of MRID 49332101).

II. Recovery Findings

ECM (MRID 49155901): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of pyrithiobac-Na and its transformation products IN-B5363 and IN-JW212 in drinking (tap), ground (well), and surface (pond) water matrices at fortification levels of 0.10 µg/kg (LOQ) and 1.0 µg/kg (10x LOQ; p. 12; Tables 1-2, pp. 21-26). Analytes were identified and quantified using two ion transitions; quantitation ion and confirmation ion recovery results were comparable. The water matrices were characterized (p. 12; Appendix 4, pp. 73-78).

ILV (MRID 49332101): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of pyrithiobac-Na and its products IN-B5363 and IN-JW212 in surface (river), ground (well), and drinking (bottled) water matrices at fortification levels of 0.10 µg/kg (ppb, LOQ) and 1.0 µg/kg (10x LOQ; Tables 3-20, pp. 28-45). The method was validated for all analytes in the three matrices at both fortification levels after one trial (p. 11). Quantitation ion and confirmation ion recovery results were comparable. The surface and ground water matrices were characterized (Appendix 2, p. 92-94). The drinking water was not characterized.

Table 2. Initial Validation Method Recoveries for Pyrithiobac-Na and Its Transformation Products IN-B5363 and IN-JW212 in Water[1]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Quantitation ion					
Pyrithiobac-Na (DPX-PE350)	**Drinking (Tap) Water**					
	0.10 (LOQ)	5	110-115	112	2	2
	1.0	5	97-101	98	2	2
	Ground (Well) Water					
	0.10 (LOQ)	5	105-112	108	3	3
	1.0	5	99-101	100	1	1
	Surface (Pond) Water					
	0.10 (LOQ)	5	106-115	109	4	4
	1.0	5	96-101	99	2	2
IN-B5363	**Drinking (Tap) Water**					
	0.10 (LOQ)	5	97-116	110	8	7
	1.0	5	96-109	102	5	5
	Ground (Well) Water					
	0.10 (LOQ)	5	101-110	105	5	5
	1.0	5	97-109	104	5	5
	Surface (Pond) Water					
	0.10 (LOQ)	5	99-109	105	4	4
	1.0	5	93-105	99	4	4
IN-JW212	**Drinking (Tap) Water**					
	0.10 (LOQ)	5	101-117	110	7	6
	1.0	5	94-104	99	4	4
	Ground (Well) Water					
	0.10 (LOQ)	5	101-107	104	2	2
	1.0	5	96-102	99	3	3
	Surface (Pond) Water					
	0.10 (LOQ)	5	96-110	104	6	6
	1.0	5	94-100	98	2	3
	Confirmation ion					
Pyrithiobac-Na (DPX-PE350)	**Drinking (Tap) Water**					
	0.10 (LOQ)	5	101-113	107	4	4
	1.0	5	86-98	94	5	5
	Ground (Well) Water					
	0.10 (LOQ)	5	91-113	106	9	8
	1.0	5	94-107	99	5	5
	Surface (Pond) Water					
	0.10 (LOQ)	5	100-107	105	3	3
	1.0	5	92-102	97	4	4
IN-B5363	**Drinking (Tap) Water**					
	0.10 (LOQ)	5	94-118	105	10	10
	1.0	5	93-103	98	4	4
	Ground (Well) Water					
	0.10 (LOQ)	5	95-112	105	7	7
	1.0	5	85-95	90	4	4
	Surface (Pond) Water					
	0.10 (LOQ)	5	83-112	98	13	13
	1.0	5	91-103	97	5	5
IN-JW212	**Drinking (Tap) Water**					

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.10 (LOQ)	5	102-110	106	3	3
	1.0	5	96-104	101	3	3
	Ground (Well) Water					
	0.10 (LOQ)	5	102-117	108	6	5
	1.0	5	99-102	100	1	1
	Surface (Pond) Water					
	0.10 (LOQ)	5	100-118	108	7	7
	1.0	5	100-106	102	2	2

Data (recovery results, corrected as needed for residues detected in matrix control samples) were obtained from pp. 16-17; Tables 1-2, pp. 21-26 of MRID 49155901.

1 Water matrices were characterized (Appendix 4, pp. 73-78 of MRID 49155901). The surface water was obtained from Lums Pond, Delaware, the ground water was from Kembelsville Well Water, Pennsylvania, and the drinking water was tap water, Newark, Delaware (p. 12).

Table 3. Independent Validation Method Recoveries for Pyrithiobac-Na and Its Transformation Products IN-B5363 and IN-JW212 in Water[1]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Quantitation ion					
Pyrithiobac-Na (DPX-PE350)	**Surface (River) Water**					
	0.10 (LOQ)	5	86-99	93	4.7	5.1
	1.0	5	78-91	85	4.5	5.2
	Ground (Well) Water					
	0.10 (LOQ)	5	93-102	96	3.5	3.7
	1.0	5	86-88	87	1.2	1.4
	Drinking (Bottled) Water					
	0.10 (LOQ)	5	102-122	111	7.3	6.6
	1.0	5	80-93	85	5.1	6.0
IN-B5363	**Surface (River) Water**					
	0.10 (LOQ)	5	110-127	118	6.5	5.5
	1.0	5	101-122	113	7.9	7.0
	Ground (Well) Water					
	0.10 (LOQ)	5	103-122	113	7.1	6.3
	1.0	5	101-112	108	4.3	4.0
	Drinking (Bottled) Water					
	0.10 (LOQ)	5	108-125	118	7.3	6.2
	1.0	5	75-109	87	12.6	14.4
IN-JW212	**Surface (River) Water**					
	0.10 (LOQ)	5	88-99	92	4.8	5.2
	1.0	5	81-93	86	4.8	5.6
	Ground (Well) Water					
	0.10 (LOQ)	5	90-97	93	3.0	3.2
	1.0	5	88-92	90	1.9	2.1
	Drinking (Bottled) Water					
	0.10 (LOQ)	5	93-105	99	4.1	4.1
	1.0	5	89-94	92	2.2	2.4
	Confirmation ion					
Pyrithiobac-Na (DPX-PE350)	**Surface (River) Water**					
	0.10 (LOQ)	5	63-84	74	7.6	10.3
	1.0	5	77-86	81	3.7	4.6
	Ground (Well) Water					
	0.10 (LOQ)	5	77-82	79	2.1	2.6
	1.0	5	94-99	97	1.8	1.9
	Drinking (Bottled) Water					
	0.10 (LOQ)	5	94-114	104	9.0	8.6
	1.0	5	82-97	87	5.8	6.7
IN-B5363	**Surface (River) Water**					
	0.10 (LOQ)	5	100-131	115	11.4	9.9
	1.0	5	111-129	120	7.3	6.4
	Ground (Well) Water					
	0.10 (LOQ)	5	99-108	105	4.0	3.8
	1.0	5	96-103	100	2.5	2.5
	Drinking (Bottled) Water					
	0.10 (LOQ)	5	107-126	118	8.2	7.0
	1.0	5	76-107	87	11.9	13.6
IN-JW212	**Surface (River) Water**					

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	0.10 (LOQ)	5	78-98	89	8.2	9.2
	1.0	5	83-93	87	4.2	4.8
	Ground (Well) Water					
	0.10 (LOQ)	5	94-113	105	7.2	6.8
	1.0	5	94-98	96	1.6	1.6
	Drinking (Bottled) Water					
	0.10 (LOQ)	5	94-133	109	15.0	13.8
	1.0	5	92-98	95	3.1	3.2

Data (recovery results, corrected as needed for residues detected in matrix control samples) were obtained from pp. 18-19; Tables 3-20, pp. 28-45 of MRID 49332101.

1 The surface water and ground water matrices were obtained from and characterized by Agvise Laboratories, Inc., Northwood, Dakota (p. 16; Appendix 2, p. 92-94 of MRID 49332101). The surface water was identified as Goose River. The ground water was identified as well water, source not specified. The drinking water was Aquafina bottled water; uncharacterized (pp. 11, 16; Appendix 4, p. 138).

III. Method Characteristics

In the ECM and ILV, the LOQ and LOD for all analytes in water were 0.10 and 0.03 µg/kg (ppb), respectively (pp. 8, 18 of MRID 49155901; p. 14 of MRID 49332101). The ECM defined the LOQ as the lowest fortification level at which acceptable average recoveries (70-120%, RSD<20%) were achieved, and also reflects the fortification level at which analyte peaks were consistently generated at approximately 10-20 times the signal at the retention time of pyrithiobac-Na in an untreated control sample for the lowest responding analyte (p. 18 of MRID 49155901). The ECM estimated the LOD as the concentration of pyrithiobac-Na at which analyte peaks are approximately three times the chromatographic baseline noise near the expected retention time, or approximately one-third the concentration of the LOQ.

Table 4. Method Characteristics for Pyrithiobac-Na and Its Transformation Products IN-B5363 and IN-JW212 in Water

		Pyrithiobac-Na	IN-B5363	IN-JW212
Limit of Quantitation (LOQ)		0.10 µg/kg (ppb)		
Limit of Detection (LOD)		0.03 µg/kg		
Linearity (calibration curve r^2 and concentration range)[1]	ECM:[2]	Q ion: r^2 = 0.9985 C ion: r^2 = 1	Q ion: r^2 = 0.9983 C ion: r^2 = 0.9996	Q ion: r^2 = 0.9999 C ion: r^2 = 1
	ILV:[3]	Q ion: r^2 = 0.9966 C ion: r^2 = **0.9904**	Q ion: r^2 = 0.9988 C ion: r^2 = 0.9994	Q ion: r^2 = 0.9994 C ion: r^2 = 0.9996
	Range:	0.05-5.0 ng/mL		
Repeatable	ECM:	Yes at LOQ and 10x LOQ.		
	ILV:	Yes at LOQ and 10x LOQ.		
Reproducible		Yes in surface, ground, and drinking water matrices.		
Specific	ECM:	Yes; no significant interferences exceeding the LOD (one-third of LOQ).		
	ILV:	Yes, for quantitation ion analyses for all analytes/matrices. For confirmation ion analyses in all matrices, there was a near co-eluting interference with pyrithiobac-Na that complicated peak interpolation at lower concentrations (0.05 and 0.1 ng/mL standards, LOQ fortifications), and baseline noise was significant (>30% of LOQ) for IN-JW212 at lower concentrations (0.05 ng/mL standard, LOQ fortifications).		

Data were obtained from pp. 8, 18; Tables 1-2, pp. 21-26; Figure 2, pp. 30-32; Figure 4, pp. 39-42, 45-48, 51-54 of MRID 49155901; p. 14; Tables 1-20, pp. 26-45; Figures 1-5, pp. 46-87 of MRID 49332101.
Linearity is satisfactory when $r^2 \geq 0.995$.
1 Linearity of the ECM and ILV calibration curves were verified by the reviewer (Figure 2, pp. 30-32; Appendix 2, pp. 59-61 of MRID 49155901; Figure 1, pp. 46-51 of MRID 49332101; DER Attachment 2). ILV r^2 values are reviewer-generated from reported r values of 0.9952-0.9998 (Figure1, pp. 46-51 of MRID 49332101; DER Attachment 2).
2 No weighting.
3 1/x weighting.

IV. Method Deficiencies and Reviewer's Comments

1. The determination of the LOQ and LOD were not based on scientifically acceptable procedures as defined in 40 CFR Part 136, Appendix B. In the ECM and ILV, the LOQ and LOD for all analytes in water were 0.10 and 0.03 µg/kg (ppb), respectively (pp. 8, 18 of MRID 49155901; p. 14 of MRID 49332101). The ECM defined the LOQ as the lowest fortification level at which acceptable average recoveries (70-120%, RSD<20%) were achieved, and also reflects the fortification level at which analyte peaks were consistently generated at approximately 10-20 times the signal at the retention time of pyrithiobac-Na in an untreated control sample for the lowest responding analyte (p. 18 of MRID 49155901). The ECM estimated the LOD as the concentration of pyrithiobac-Na at which analyte peaks are approximately three times the chromatographic baseline noise near the expected retention time, or approximately one-third the concentration of the LOQ. Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples. Additionally, the lowest toxicological level of concern in water was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.

2. For the ILV, it took multiple attempts to obtain a drinking water matrix suitable for the trials (Appendix 4, pp. 131-140 of MRID 49332101). Characterized drinking water from Agvise and Columbia, Missouri, City tap water did not pass control suitability evaluation tests; however the reason for poor analyte recoveries was unclear from the provided

communications. Aquafina bottled water was determined to be suitable for the ILV trials, but characterization of the matrix was not provided (Appendix 4, pp. 133, 138 of MRID 49332101).

3. For both the ECM and ILV, chromatograms were not provided for reagent blanks.

4. For the calibration standards of the ECM, only chromatograms of the 0.05, 0.10, and 1.0 ng/mL standards (calibration standard range 0.05-5.0 ng/mL) were provided (Figure 3, pp. 33-38 of MRID 49155901).

5. Prior to analysis of actual validation samples, the independent lab conducted quantitative control suitability evaluations and verified that the water matrices were "free of any interferences in the area of analyte elution (corresponding to analyte residue levels <30% of the LOQ)" (pp. 20-21; Tables 1-2, pp. 26-27 of MRID 49332101). The independent laboratory reported that "No interferences were detected for any analyte in water, with the exception of one control purified drinking water sample for the confirmatory transition of pyrithiobac sodium at 0.04 ppb (slightly above the LOD of 0.03 ppb)." (p. 24).

   However, the reviewer noted for confirmation ion analyses in all matrices, there was a near co-eluting interference with pyrithiobac-Na that complicated peak interpolation at lower concentrations (0.05 and 0.1 ng/mL standards, LOQ fortifications; Figure 2, pp. 52-53; Figure 3, p. 71; Figure 4, p. 77; Figure 5, p. 83), and baseline noise was significant (>30% of LOQ) for IN-JW212 at lower concentrations (0.05 ng/mL standard, LOQ fortifications; Figure 2, p. 64; Figure 3, p. 75; Figure 4, p. 81; Figure 5, p. 87).

6. For the ECM and ILV, example calculations indicate recovery results were corrected for any residues detected in the matrix control samples (pp. 16-17 of MRID 49155901; pp. 18-19 of MRID 49332101). For the ECM, raw data spreadsheets for pond water samples indicate no residues were detected in the matrix controls; however, raw data for ground water and drinking water samples were not provided (Appendix 2, pp. 59-61 of MRID 49155901). For the ILV, it could not be determined from the raw data spreadsheets whether or not recoveries were corrected (Appendix 3, pp. 95-106, 120-125).

7. In the ILV summary tables (pp. 12, 22 of MRID 49332101), the RSD values of 9.2% and 4.8% for 0.10 and 1.0 ppb pyrithiobac-Na in surface water, respectively, are typographical errors and should be 5.1% and 5.2%, respectively (Table 3, p. 28).

   In Section 7.0 Conclusions of the ECM, the following typographical error is noted: “This method for the determination of residues of pyrithiobac sodium in cotton samples…”; this statement should refer to water samples (p. 19 of MRID 49155901).

8. It was reported for the ILV that a single analyst completed sample sets of thirteen samples during one workday (8 hours) with LC/MS/MS analysis performed overnight (p. 14 of MRID 49332101).

V. References

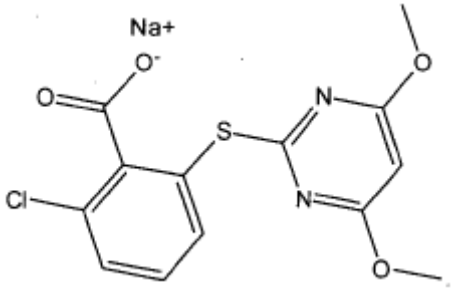
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

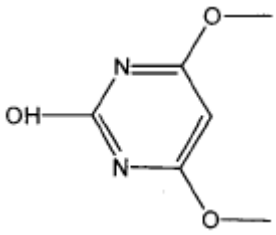
Pyrithiobac-Na (DPX-PE350, DPX-PE350-4, DPX-PE350-045)

IUPAC Name: Sodium 2-chloro-6-(4,6-dimethoxypyrimidin-2-ylthio)benzoate
CAS Name: Sodium 2-chloro-6-[(4,6-dimethoxy-2-pyrimidinyl)thio]benzoate
CAS Number: 123343-16-8
SMILES String: Not found.



IN-B5363 (IN-B5363-000, IN-B5363-002)

IUPAC Name: Not reported.
CAS Name: Not reported.
CAS Number: Not reported.
SMILES String: Not found.



IN-JW212 (IN-JW212-002)

IUPAC Name: Not reported.
CAS Name: Not reported.
CAS Number: Not reported.
SMILES String: Not found.