**Test Material:** Pyrithiobac-Na

**MRID:** 49155902

**Title:** Analytical Method for the Determination of Pyrithiobac Sodium and Metabolites in Soil Using LC/MS/MS

**MRID:** 49324001

**Title:** Independent Laboratory Validation of DuPont-37904, "Analytical Method for the Determination of Pyrithiobac Sodium and Metabolites in Soil Using LC/MS/MS"

**EPA PC Code:** 078905

**OCSPP Guideline:** 850.6100

**For CDM Smith**

**Primary Reviewer:** Lynne Binari **Signature:** Lynne Binari

**Date:** 2/16/15

**Secondary Reviewer:** Lisa Muto **Signature:** Lisa Muto

**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 soil

**Reports:** ECM: EPA MRID No.: 49155902. Henze, R. and J. Stry. 2013. Analytical Method for the Determination of Pyrithiobac Sodium and Metabolites in Soil Using LC/MS/MS. Project Identification No.: DuPont-37904. 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; 75 pages. Final report issued May 21, 2013.

ILV: EPA MRID No. 49324001. Schierhoff, R. 2014. Independent Laboratory Validation of DuPont-37904, "Analytical Method for the Determination of Pyrithiobac Sodium and Metabolites in Soil Using LC/MS/MS". ABC Study No.: 80148. DuPont Study No.: DuPont-36961. Report prepared by ABC Laboratories, Inc., Columbia, Missouri, sponsored and submitted by E. I. du Pont de Nemours and Company, Wilmington, Delaware; 145 pages. Final report issued February 14, 2014.

**Document No.:** MRIDs 49155902 & 49324001

**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 49155902). 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 49324001). Signed and dated Data Confidentiality, GLP, Quality Assurance, and Authenticity Certification statements were provided (pp. 2-5).

**Classification:** This analytical method is classified as supplemental. Modifications to the method recommended by the independent laboratory were not implemented in the ECM report. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures. The soil used in the ILV was not of an equivalent, or more difficult, analytical sample condition as that used in the ECM.

**PC Code:** 078905

**Reviewer:**

Final Reviewer: **Ibrahim Abdel-Saheb** Signature:
EPA Reviewer Date: 01-12-2016

## Executive Summary

This analytical method, DuPont-37904, is designed for the quantitative determination of pyrithiobac-Na and its transformation products IN-B5363 and IN-JW212 in soil using LC/MS/MS. The method is quantitative for the analytes at the stated LOQ of 0.0010 mg/kg (ppm). The LOQ is [less than/equal to/greater than] the lowest toxicological level of concern in soil. The independent laboratory validated the method for analysis of pyrithiobac-Na and IN-JW212 in loamy sand soil after one trial and IN-B5363 after two trials. Modifications to the method recommended by the independent laboratory were not implemented in the ECM report. The loamy sand soil (11% clay, 1.7% organic matter) used in the ILV was not of an equivalent, or more difficult, analytical sample condition as the silty clay soil (42.8% clay, 4.6% organic matter) used for 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 | | | | | | |
| Pyrithiobac-Na | 49155902 | 49324001 | | Soil | 21/05/2013 | E. I. du Pont de Nemours and Company | LC/MS/MS | 0.0010 mg/kg (ppm) |
| IN-B5363 | | | | | | | | |
| IN-JW212 | | | | | | | | |

## I. Principle of the Method

Soil (10.0 g ± 1%) was fortified with a mixed standard of pyrithiobac-Na, IN-B5363, and IN-JW212 in methanol for procedural recoveries, with the fortified sample air-dried for 15 minutes to allow evaporation of the vehicle solvent (pp. 11-13 of MRID 49155902). Soil samples (10.0 g ± 1%) are sequentially extracted three times with 20 mL of 90:10 (v:v) acetone:0.1M aqueous ammonium carbonate, followed by 10 mL of 50:50 acetone:0.1M aqueous ammonium carbonate, and finally 10 mL of 20:80 acetone:0.1M aqueous ammonium carbonate (p. 13). For each extraction, soil is homogenized using a bead mill (Genogrinder, two ¼-inch steel balls) for 3 minutes at *ca*. 1,200 strokes/minute. Soil and extract are separated by centrifugation (*ca*. 3,000 rpm, 10 minutes). Extracts are combined and brought to 50 mL with the 20:80 acetone:0.1M ammonium carbonate extraction solvent. A 10-mL aliquot is concentrated under nitrogen (N-Evap, *ca*. 30°C) to *ca*. 4 mL, then partitioned with ethyl acetate (1 mL) plus hexane (1 mL). The sample is centrifuged (*ca*. 3,000 rpm, 5 minutes) and the upper organic phase (ethyl acetate:hexane) discarded. The remaining aqueous phase is further concentrated under nitrogen (N-Evap, 15 minutes) to remove any trace of ethyl acetate:hexane, then diluted to 4 mL with water for LC/MS/MS analysis.

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. 8, 14-15 of MRID 49155902). 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. 14-17 of MRID 49324001). Most specifically, injection volumes were 10 µL for pyrithiobac-Na and IN-JW212 and 3 µL for IN-B5363, and timing of the mobile phase conditions was adjusted. The independent laboratory indicated that the acetone:0.1M aqueous ammonium carbonate solutions are not stable and may need to be prepared the day of extraction. The independent laboratory also recommended that section 5.1.4 *Limit of Quantitation and Limit of Detection* of the ECM (p. 18 of MRID 49155902) be updated to indicate the correct LOQ and LOD of 0.0010 ppm and 0.0003 ppm, respectively. The soil matrix was obtained from Agvise Laboratories, Inc., Northwood, North Dakota (p. 14 of MRID 49324001).

LOQ and LOD: In the ECM and ILV, the LOQ and LOD for all analytes were 0.0010 and 0.0003 mg/kg (ppm), respectively (p. 8 of MRID 49155902; p. 12 of MRID 49324001).

## II. Recovery Findings

ECM (MRID 49155902): 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 a sandy loam soil and a silty clay soil at fortification levels of 0.0010 mg/kg (LOQ) and 0.010 mg/kg (10x LOQ; p. 13; Tables 1-2, pp. 22-27). Analytes were identified and quantified using two ion transitions; quantitation ion and confirmation ion recovery results were comparable. The soil matrices were characterized (p. 12).

ILV (MRID 49324001): 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 loamy sand soil at fortification levels of 0.0010 mg/kg (LOQ) and 0.010 mg/kg (10x LOQ; p. 11). The method was validated for pyrithiobac-Na and IN-JW212 at both fortification levels after one trial and validated for IN-B5363 at both fortification levels after a second trial (pp. 10, 20). Results from the confirmatory method were not reported. The soil matrix was characterized (Appendix 3, p. 127). The loamy sand soil (11% clay, 1.7% organic matter) used in the ILV was not of an equivalent, or more difficult, analytical sample condition as the silty clay soil (42.8% clay, 4.6% organic matter) used for the ECM.

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

| Analyte | Fortification Level (mg/kg) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|---|---|---|---|---|---|---|
| | **Quantitation ion** | | | | | |
| **Pyrithiobac-Na (DPX-PE350)** | **Sandy Loam (Sassafras) Soil** | | | | | |
| | 0.0010 (LOQ) | 5 | 86-102 | 94 | 6.5 | 6.9 |
| | 0.010 | 5 | 74-78 | 76 | 1.5 | 1.9 |
| | **Silty Clay (Tama) Soil** | | | | | |
| | 0.0010 (LOQ) | 5 | 92-97 | 94 | 1.9 | 2.0 |
| | 0.010 | 5 | 76-80 | 78 | 1.7 | 2.1 |
| **IN-B5363** | **Sandy Loam (Sassafras) Soil** | | | | | |
| | 0.0010 (LOQ) | 5 | 83-87 | 85 | 1.8 | 2.1 |
| | 0.010 | 5 | 78-82 | 80 | 2.2 | 2.7 |
| | **Silty Clay (Tama) Soil** | | | | | |
| | 0.0010 (LOQ) | 5 | 75-85 | 80 | 4.4 | 5.6 |
| | 0.010 | 5 | 78-84 | 81 | 2.4 | 2.9 |
| **IN-JW212** | **Sandy Loam (Sassafras) Soil** | | | | | |
| | 0.0010 (LOQ) | 5 | 90-98 | 95 | 3.2 | 3.3 |
| | 0.010 | 5 | 92-96 | 94 | 1.8 | 1.9 |
| | **Silty Clay (Tama) Soil** | | | | | |
| | 0.0010 (LOQ) | 5 | 88-97 | 92 | 3.7 | 4.0 |
| | 0.010 | 5 | 86-94 | 90 | 2.9 | 3.2 |
| | **Confirmation ion** | | | | | |
| **Pyrithiobac-Na (DPX-PE350)** | **Sandy Loam (Sassafras) Soil** | | | | | |
| | 0.0010 (LOQ) | 5 | 82-108 | 97 | 11.7 | 12.0 |
| | 0.010 | 5 | 79-87 | 83 | 3.8 | 4.5 |
| | **Silty Clay (Tama) Soil** | | | | | |
| | 0.0010 (LOQ) | 5 | 83-90 | 86 | 2.9 | 3.4 |
| | 0.010 | 5 | 82-89 | 85 | 3.0 | 3.5 |
| **IN-B5363** | **Sandy Loam (Sassafras) Soil** | | | | | |
| | 0.0010 (LOQ) | 5 | 99-117 | 104 | 7.4 | 7.1 |
| | 0.010 | 5 | 84-87 | 85 | 1.1 | 1.3 |
| | **Silty Clay (Tama) Soil** | | | | | |
| | 0.0010 (LOQ) | 5 | 94-108 | 100 | 5.8 | 5.8 |
| | 0.010 | 5 | 75-91 | 84 | 6.0 | 7.1 |
| **IN-JW212** | **Sandy Loam (Sassafras) Soil** | | | | | |
| | 0.0010 (LOQ) | 5 | 91-94 | 92 | 1.1 | 1.2 |
| | 0.010 | 5 | 89-93 | 91 | 1.8 | 2.0 |
| | **Silty Clay (Tama) Soil** | | | | | |
| | 0.0010 (LOQ) | 5 | 87-96 | 90 | 3.5 | 3.9 |
| | 0.010 | 5 | 85-94 | 89 | 3.4 | 3.8 |

Data (recovery results, corrected as needed for residues detected in matrix control samples) were obtained from Tables 1-2, pp. 22-27 of MRID 49155902.

[1] Soils were characterized (% sand, % silt and % clay, pH, % organic matter; p. 12 of MRID 49155902). The sandy loam (Sassafras) soil was obtained from Chesapeake Farms, Maryland, and the silty clay (Tama) soil from Toulon, Illinois.

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

| Analyte | Fortification Level (mg/kg) | Number of Tests | Recovery Range (%) | Mean Recovery (%) | Standard Deviation (%) | Relative Standard Deviation (%) |
|---|---|---|---|---|---|---|
| **Pyrithiobac-Na (DPX-PE350)** | 0.0010 (LOQ) | 5 | 74-80 | 76 | 2.3 | 3.0 |
| | 0.010 | 5 | 87-91 | 89 | 1.3 | 1.5 |
| **IN-B5363** | 0.0010 (LOQ) | 5 | 83-89 | 86 | 2.2 | 2.6 |
| | 0.010 | 5 | 82-87 | 84 | 1.9 | 2.2 |
| **IN-JW212** | 0.0010 (LOQ) | 5 | 96-110 | 103 | 6.3 | 6.1 |
| | 0.010 | 5 | 80-86 | 83 | 2.2 | 2.6 |

Data (Quantitation ion, uncorrected recovery results) were obtained from p. 21; Tables 1-3, pp. 24-26; Appendix 4, pp. 129, 131, 133 of MRID 49324001.

1 Soil was obtained from and characterized by Agvise Laboratories, Inc., Northwood, Dakota (p. 14; Appendix 3, p. 127 of MRID 49324001).

## III. Method Characteristics

In the ECM and ILV, the LOQ and LOD for all analytes in soil were 0.0010 and 0.0003 mg/kg (ppm), respectively (p. 8 of MRID 49155902; p. 12 of MRID 49324001). 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 49155902). 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 Soil**

| | | **Pyrithiobac-Na** | **IN-B5363** | **IN-JW212** |
|---|---|---|---|---|
| Limit of Quantitation (LOQ) | | 0.0010 mg/kg (ppm) | | |
| Limit of Detection (LOD) | | 0.0003 mg/kg | | |
| Linearity (calibration curve $r^2$ and concentration range)[1] | ECM: | Q ion: $r^2$ = 0.9948-0.996<br>C ion: $r^2$ = 0.9999-1 | Q ion: $r^2$ = 0.9979-0.9995<br>C ion: $r^2$ = 0.9985-0.9999 | Q ion: $r^2$ = 0.9983-0.9999<br>C ion: $r^2$ = 0.9997-0.9999 |
| | ILV: | Q ion: $r^2$ = 0.9988 | Q ion: $r^2$ = 0.9998 | Q ion: $r^2$ = 0.9992 |
| | Range: | 0.05-7.5 ng/mL or 0.1-7.5 ng/mL | | |
| Repeatable | ECM: | Yes at LOQ and 10x LOQ. | | |
| | ILV: | Yes at LOQ and 10x LOQ. | | |
| Reproducible | | Yes; however, the loamy sand soil (11% clay, 1.7% organic matter) used in the ILV was not of an equivalent, or more difficult, analytical sample condition as the silty clay soil (42.8% clay, 4.6% organic matter) used for the ECM. | | |
| Specific | ECM: | Yes; no significant interferences exceeding the LOD (one-third of LOQ). | | |
| | ILV: | Yes, but there was significant baseline noise (*ca*. 30-40% of LOQ) at the retention times of pyrithiobac-Na and IN-JW212.[2] | | |

Data were obtained from pp. 8, 17-18; Figure 2, pp. 31-33; Figure 4, pp. 42-47, 51-56 of MRID 49155902; p. 12; Figures 2-6, pp. 39-45 of MRID 49324001.

Linearity is satisfactory when $r^2 \geq 0.995$.

[1] Linearity of the provided ECM calibration curves could not be verified because individual calibration data were not provided (Figure 2, pp. 31-33 of MRID 49155902). The reviewer used provided calibration standard data to generate additional $r^2$ values and to verify linearity of the standard curves (Figure 3, pp. 34-35; Appendix 2, pp. 62-64 of MRID 49155902; DER Attachment 2). Linearity of the ILV calibration curves was verified by the reviewer (DER Attachment 2). ILV $r^2$ values are reviewer-generated from reported r values of 0.9994-0.9999 (Figures 2-4, pp. 39-41 of MRID 49324001; DER Attachment 2).

[2] Results from confirmatory method were not provided.

## IV. Method Deficiencies and Reviewer's Comments

1. Modifications to the method recommended by the independent laboratory were not implemented in the ECM report. The independent laboratory proposed that the acetone:0.1M aqueous ammonium carbonate solutions are not stable and may need to be prepared the day of extraction (p. 15 of MRID 49324001). The independent laboratory indicated same day of use preparation of the solutions was a critical step and that not using freshly prepared acetone:ammonium carbonate solutions may result in low recovery of IN-B5363.

   The independent laboratory noted that section 5.1.4 *Limit of Quantitation and Limit of Detection* of the ECM (p. 18 of MRID 49155902) should be updated to indicate the correct LOQ and LOD of 0.0010 ppm and 0.0003 ppm, respectively; the ECM currently lists the LOQ and LOD as 0.010 mg/kg (ppm) and 0.003 mg/kg (ppm), respectively (p. 15; Appendix 5, pp. 139-140 of MRID 49324001). The independent laboratory also reported the LOQ incorrectly at 0.010 ppm in section **5.0 Conclusions** of the ILV study report (p. 22 of MRID 49324001).

2. 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 soil were 0.0010 and 0.0003 mg/kg (ppm), respectively (p. 8 of MRID 49155902; p. 12 of MRID 49324001). 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 49155902). 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 soil was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.

3. The loamy sand soil (11% clay, 1.7% organic matter) used in the ILV was not of an equivalent, or more difficult, analytical sample condition as the silty clay soil (42.8% clay, 4.6% organic matter) used for the ECM (p. 12 of MRID 49155902; Appendix 3, p. 127 of MRID 49324001).

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

5. For the ECM, only chromatograms of the 0.05, 0.10, and 1.0 ng/mL standards (calibration standard range 0.05-7.5 ng/mL) were provided (Figure 3, pp. 34-41 of MRID 49155902). While chromatograms of the 0.05 ng/mL calibration standards were provided, the peak area counts were not included in the raw data spreadsheets of the same data sets (Appendix 2, pp. 62-64). Individual calibration standard data were not presented with the provided calibration curves (Figure 2, pp. 31-33).

6. For the ECM, peaks from IN-B5363 confirmation ion analysis at the LOQ were barely measurable above the baseline (Figure 4, pp. 45, 54). The ILV did not provide results from the confirmatory method; however, OSCPP guidelines state that it is not necessary typically to perform another confirmatory procedure where GC/MS and LC/MS methods are used as the primary method(s) to generate study data.

7. The independent lab verified that the soil matrix was "free of any interferences in the area of analyte elution (corresponding to analyte residue levels <30% of the LOQ)" (p. 21 of MRID 49324001). However, for pyrithiobac-Na and IN-JW212, baseline noise appears as *ca*. 30-40% of peak height at the LOQ (Figures 5-6, pp. 42-45).

8. For the ECM, recovery results were corrected for any residues detected in the matrix control samples (pp. 16-17; Appendix 2, pp. 62-64 of MRID 49155902). For the ILV, raw data spreadsheets indicate recoveries were not corrected (Appendix 2, pp. 129, 131, 133 of MRID 49324001).

9. The reviewer noted a typographical error in the ILV report in which the ILV study author reported that “the second method validation trial (Set #2) was successful for pyrithiobac sodium and IN-B5363” (p. 20 of MRID 49324001). Pyrithiobac sodium should not have been included in that statement since the first method validation trial was successful (Tables 1-3, pp. 24-26).

10. It was reported for the ILV that a single analyst completed sample sets of twelve samples during one workday (8 hours) with LC/MS/MS analysis performed overnight (p. 11 of MRID 49324001).

## V. References

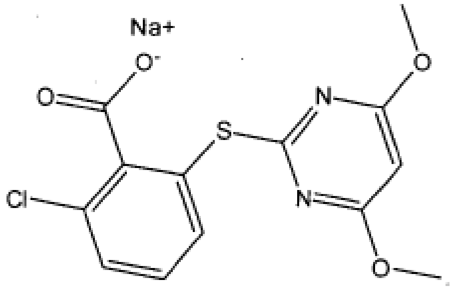
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

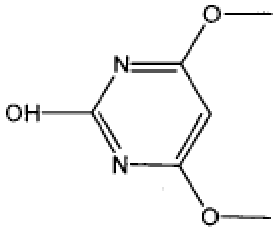
### Pyrithiobac-Na (DPX-PE350, DPX-PE350-4, DPX-PE350-045)

| | |
|---|---|
| **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. |