Analytical method for aldicarb in Temik 15G in soil

Reports:	ECM: EPA MRID No.: 49477402. DeVellis, S. R. 2014. Temik 15G (Aldicarb) – Validation of the Analytical Method for the Determination of a Test Substance in Soil. Smithers Viscient Study No.: 14070.6100. Report prepared by Smithers Viscient, Wareham, Massachusetts, sponsored by Pyxis Regulatory Consulting, Inc., Gig Harbor, Washington, and submitted by AgLogic, LLC, Chapel Hill, North Carolina; 31 pages. Final report issued September 10, 2014. ILV: EPA MRID No. 49477403. Wu, X. 2014. Independent Laboratory Validation (ILV) of the Analytical Method: Temik 15G (Aldicarb) – Validation of the Analytical Method for the Determination of a Test Substance in Soil. Smithers Viscient Study No.: 14070.6102. Report prepared by Smithers Viscient, Wareham, Massachusetts, sponsored by Pyxis Regulatory Consulting, Inc., Gig Harbor, Washington, and submitted by AgLogic, LLC, Chapel Hill, North Carolina; 38 pages. Final report issued September 24, 2014. Addendum: EPA MRID No.: 49513501. Wu, X. 2014. Technical Response Letter prepared by Smithers Viscient, Wareham, Massachusetts, sponsored by Pyxis Regulatory Consulting, Inc., Gig Harbor, Washington, and submitted by AgLogic, LLC, Chapel Hill, North Carolina; 6 pages. Dated November 6, 2014.
Document No.:	MRIDs 49477402, 49477403 & 49513501
Guideline:	850.6100
Statements:	ECM: The study was conducted in accordance with USEPA FIFRA and OECD Good Laboratory Practice (GLP) standards (p. 3 of MRID 49477402). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4). ILV: The study was conducted in accordance with USEPA FIFRA GLP standards (p. 3 of MRID 49477403). Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided (pp. 2-4). A statement of the authenticity of the study report was included with the quality assurance statement (p. 4). Addendum: The Technical Response Letter did not include any No Data Confidentiality, GLP, Authenticity, and Quality Assurance statements; however, it was signed by the author (p. 2 of MRID 49513501).
Classification:	This analytical method is classified as ***supplemental***. However, the study is upgradable when the acceptable performance data of soil fortification at the stated LOQ of 11.0 µg/kg is submitted, along with all the performance data (including chromatograms) of the fortified soil recovery levels in the ECM and ILV, as well as sufficient information to verify that the ECM laboratory was distinct from the ILV. Currently, the ECM cannot be validated with the study's stated LOQ of 11.0 µg/kg because the lowest level of soil

fortification in the study is at 100 µg/kg, which is 10X the LOQ. In the ECM and ILV, no performance data was provided at the LOQ to validate the method. The reviewer reported the LOQ for the method based on the ECM report. Insufficient information was provided to verify that the ECM laboratory was distinct from the ILV laboratory. An insufficient number of samples was prepared for all fortification levels in the ECM. In the ECM, no chromatograms were provided for two of the three fortification levels.

PC Code: 098301

Reviewer: Edmund Wong

Environmental Chemist Date: 07/09/2015

Executive Summary

This analytical method, Smithers Viscient Study No.: 14070.6100, is designed for the quantitative determination of aldicarb in soil at 11 µg/kg using LC/MS/MS. No performance data was provided in the ECM or ILV to validate the method at the LOQ, instead the method was validated at 10×LOQ, 100×LOQ, and 700×LOQ (ECM only). Insufficient information was provided to verify that the ECM laboratory was distinct from the ILV laboratory, although the analytical instruments used were different. The reviewer assumed that the method was validated in the first trial by the ILV 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						
Aldicarb[1]	49477402 & 49513501[2]	49477403 & 49513501[2]		Soil	10/09/2014	AgLogic LLC	LC/MS/MS	11 µg/kg[3]

1 The test substance was Temik 15G, but the LOQ was established for aldicarb.
2 MRID 49513501 was an addendum to the ECM and ILV. It was a technical response letter written by the ILV study author in response to "comments…received from Susan Bartow, Chemical Review Manager at the EPA, OCSPP, OPP, PRD, regarding the aldicarb validation and ILV studies" (p. 1 of MRID 49513501).
3 Method was not validated at LOQ. See also Reviewer Comment #2.

I. Principle of the Method

The test substance was Temik 15G (14.81% aldicarb; pp. 8-11 of MRID 49477402). The fortification levels were based on the aldicarb active ingredient.

Samples (5.00 g dry weight) of soil were fortified, as necessary, then extracted twice with 0.1% formic acid in acetonitrile (2 x 20.0 mL) by shaking via orbital shaker table for 30 minutes at 150 rpm (pp. 12-13 of MRID 49477402). Soil and extract were separated by centrifugation (10 minutes at 3000 rpm) and decanting. The volume of the combined extracts was adjusted to 50 mL using 0.1% formic acid in acetonitrile. Samples were further diluted (200-5000 dilution

factor) into calibration range with acetonitrile:purified reagent water (20:80, v:v) prior to analysis.

Samples were analyzed for aldicarb by HPLC/MS/MS (AB Sciex API 5000 mass spectrometer, equipment number: EQP5368) employing a XBridge C18, 2.1 mm x 50 mm, 2.5 µm column and mobile phase of (A) purified reagent water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid [percent A:B at 0.10-0.50 min. 85.0:15.0, 2.50-5.00 min. 5.0:95.0, 5:10-6.50 min. 85.0:15.0) with an AB Sciex Turbo V ESI Ion Spray source (positive ionization mode and multiple reaction monitoring, MRM; p. 14 of MRID 49477402). Injection volume was 100 µL. Aldicarb was identified using the following ion transition: 213.10→89.00 amu. The retention time was *ca*. 2.9 minutes. The ECM study author noted that similar analysis columns could be used and that the injection volume could be changed (decreased only, 100 µL = maximum).

In the ILV, the method was performed exactly as written in the ECM (pp. 10-15 of MRID 49477403). The extraction procedure and HPLC/MS/MS method were almost identical. However, the analytical instrument used is different (AB Sciex API 5000 mass spectrometer, equipment number: EQP5297). Two noted differences in the analytical conditions were that the retention time for aldicarb was *ca*. 1.8 minutes and that the injection volume was decreased to 50 µL (p. 14).

In the ECM, the Limit of Quantification (LOQ) and Limit of Detection (LOD) were reported as 11.0 µg/kg and 1 µg/kg, respectively (p. 17 of MRID 49477402). In the ILV, the LOQ and LOD were reported as 9.39 µg/kg and 2 µg/kg, respectively (See Reviewer Comment #2; p. 18 of MRID 49477403).

II. Recovery Findings

ECM (MRID 49477402): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of aldicarb in sandy loam soil at fortification levels of 100 µg/kg (10×LOQ), 1000 µg/kg (100×LOQ) and 7000 µg/kg (700×LOQ; Table 1, p. 21). The number of samples was three for all fortification levels. No samples were fortified at the LOQ. Aldicarb was dosed as Temik 15G (14.81% as aldicarb); the fortification rate was based on aldicarb active ingredient (pp. 9, 11). The soil was not characterized (Rochester Sandy Loam soil, SMV Lot No. 021814, Sample ID 2014 100 ROCH LOAM); soil moisture was determined prior to testing (11.44%; p. 10).

ILV (MRID 49477403): Mean recoveries and relative standard deviations (RSDs) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of aldicarb in sandy loam soil at fortification levels of 100 mg/kg (10×LOQ) and 1000 mg/kg (100×LOQ; Table 1, p. 21). No samples were fortified at the LOQ. Aldicarb was dosed as Temik 15G (14.81% as aldicarb); the fortification rate was based on aldicarb active ingredient (pp. 9, 11). The soil was not characterized (Rochester Sandy Loam soil, SMV Lot No. 021814, Sample ID 2014 100 ROCH LOAM); soil moisture was determined prior to testing (27.35%; p. 10). The number of trials was

not specifically reported; however, the reviewer assumed that the method was validated in the first trial (pp. 8, 18).

Table 2. Initial Validation Method Recoveries for Aldicarb in Sandy Loam Soil[1]

Analyte	Fortification Level (µg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy Loam Soil						
Aldicarb[2]	100	3	75.8-82.4	79.4	3.3	4.2
	1000	3	85.3-93.8	88.3	4.8	5.4
	7000	3	83.3-91.0	88.0	4.1	4.7

Data (corrected recovery results) were obtained from Table 1, p. 21 of MRID 49477402 and DER Attachment 2 (means, s. d. and RSD).
1 Soil matrix was not characterized; soil moisture was determined prior to testing (11.44%; p. 10 of MRID 49477402).
2 Aldicarb was dosed as Temik 15G (14.81% as aldicarb; p. 9 of MRID 49477402). Ion transition monitored was 213.10→89.00 amu.

Table 3. Independent Validation Method Recoveries for Aldicarb in Sandy Loam Soil[1]

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy Loam Soil						
Aldicarb[2]	0.100	5	86.5-99.3	94.6	4.84	5.12
	1.00	5	111-120	114	3.72	3.27

Data (results) were obtained from Table 1, p. 21 of MRID 499477403.
1 Soil matrix was not characterized; soil moisture was determined prior to testing (27.35%; p. 10 of MRID 49477403).
2 Aldicarb was dosed as Temik 15G (14.81% as aldicarb; p. 9 of MRID 49477403). Ion transition monitored was 213.10→89.00 amu.

III. Method Characteristics

In the ECM, the LOQ and LOD were reported as 11.0 µg/kg and 1 µg/kg, respectively (p. 17 of MRID 49477402). The LOQ was calculated from the LOQ of the instrument (using the lowest concentration calibration standard) and the dilution factor of the control samples (pp. 16-17; Appendix 1, p. 29). The LOD was determined by calculating the average noise level in the chromatograms of purified water control solutions and comparing them to the signal of a lowest calibration standard of known concentration. The LOD was calculated as three times the concentration equivalent of the mean noise level of peaks near the retention time of the analyte. In the ILV, the LOQ and LOD were reported as 9.39 µg/kg and 2 µg/kg, respectively (p. 18 of MRID 49477403). The LOQ was calculated in the same manner as that of the ECM (p. 17). The LOD was established by evaluating the signal-to-noise (S:N) ratio from samples of known concentration and blank samples (Appendix 1, p. 32).

Table 4. Method Characteristics

	Aldicarb (dosed as Temik 15G)[1]
Limit of Quantitation (LOQ)	11.0 µg/kg
Limit of Detection (LOD)	1 µg/kg
Linearity (Least squares calibration curve r and concentration range)[2]	Sandy loam
	r^2 = 0.99635 (ECM) r^2 = 0.99902 (ILV) (0.0500-2.0 µg/L)
Repeatable	No; n =3
Reproducible	Yes for 10×LOQ and 100×LOQ; No samples were dosed at LOQ in the ECM or ILV
Specific	No[3]

Data were obtained from p. 17; Table 1, p. 21; Figure 4, p. 25 of MRID 499477402; and p. 18; Table 1, p. 21; Figure 1, p. 22 of MRID 499477403.

1 Aldicarb was dosed as Temik 15G (14.81% as aldicarb; pp. 9, 11 of MRID 49477402; pp. 9, 11 of MRID 49477403). Fortification rates were based on aldicarb active ingredient.

2 Reviewer-calculated r^2 values for the provided ECM calibration curve was 0.9958; reviewer-calculated r^2 values for the provided ILV calibration curve was 0.9989 (DER Attachment 2).

3 No chromatograms were provided for the LOQ in the ECM or ILV since no samples were dosed at the LOQ. In the ECM, chromatograms were only provided for a control sample, one calibration standard and one fortification level (Figures 1-3, pp. 22-24 of MRID 49477402). In the ILV, chromatograms were provided for a control sample, a reagent blank, one calibration standard and both fortification levels (Figures 2-6, pp. 23-27 of MRID 49477403).

III. Method Deficiencies and Reviewer's Comments

1. The ECM cannot be validated with the study's stated LOQ of 11.0 µg/kg because the lowest level of soil fortification in the study is at 100 µg/kg, which is 10X the LOQ. The study is upgradable when an acceptable performance data of soil fortification at the LOQ of 11.0 µg/kg is submitted for both ECM and ILV. The reviewer reported the LOQ for the method based on the ECM report. The LOQ was reported as 11.0 µg/kg in the ECM (p. 17 of MRID 49477402). In the ILV, the LOQ was reported as 0.00939 mg/kg in a table of study results; this value confirmed the value reported by the ECM (p. 18 of MRID 49477403). However, the LOQ was reported as "approximately 0.10 mg/kg" in the Introduction Section (p. 8) of the ILV study report and "approximately 0.100 mg/kg" in the last sentence of the Results and Discussion Section (p. 18). Table 1 of the ILV identified the 0.100 mg/kg samples as LOQ A-E (p. 21). Figure 3 of the ILV reported the 0.100 mg/kg recovery sample with a Sample ID of "LOQ C" (p. 24). In the sponsor-approved Study Protocol of the ILV, the LOQ was also reported as 0.100 ppm (Appendix 1, p. 32). In the ECM, this value (0.100 mg/kg) corresponded to the "Low Concentration" however, the LOQ was never reported as 0.100 mg/kg or 100 µg/kg in the Tables or the Results section of the ECM (pp. 13, 17-18; Table 1, p. 21; Appendix 1, p. 29 of MRID 49477402).

2. The levels of LOD and LOQ cannot be verified and validated due to the lack of performance data. In the ECM and ILV, no performance data was provided for the LOQ to validate the method. A validation sample set should consist of, at a minimum, a reagent blank, two unspiked matrix control samples, five matrix control samples spike at the LOQ, and five matrix control samples spiked at 10×LOQ for each analyte and matrix. Samples were only prepared at 10×LOQ, 100×LOQ and 700×LOQ (ECM only; Table 1, p. 21 of MRID 49477402; Table 1, p. 21 of MRID 49477403).

3. The laboratory which performed the ECM, Smithers Viscient, was the same laboratory which performed the ILV (p. 1 of MRID 49477402; p. 1 of MRID 49477403). OCSPP guidelines state "If the laboratory that conducted the validation belonged to the same organization as the originating laboratory, the analysts, study director, equipment, instruments, and supplies of the two laboratories must have been distinct and operated separately and without collusion. 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." Based on the information reported in the ECM, ILV and Addendum MRIDs (MRIDs 49477402, 49477403 and 49513501), the reviewer concluded that insufficient information was provided to verify that the ECM laboratory was distinct from the ILV laboratory.

Regarding analysts and study director: In the lists of Key Study Personnel for the ECM and ILV, the study directors were different (p. 5 of MRID 49477402; p. 5 of MRID 49477403). In addition, the ECM listed two analysts (chemists) while the ILV listed no analysts. However, the Technical Report Writer and Director of Chemistry were identical between the ECM and ILV. OCSPP guidelines specify "the personnel conducting the independent validation should not report to the same study director who was involved in developing the original method or who may have used the method to develop data for laboratory field studies to support pesticide registration or reregistration actions."

Regarding equipment, instruments and supplies: In Section 2.0 Materials and Methods of the ECM and ILV, the test and reference substances, reagents, laboratory equipment and analytical instruments were identical, although not always listed in the identical order (pp. 8-10, 14 of MRID 49477402; pp. 9-10, 14-15 of MRID 49477403). In EPA MRID No. 49513501 which was a Technical Response Letter prepared by Smithers Viscient (written by the ILV study author) in response to comments from Susan Bartow, Chemical Review Manager at the EPA, the ILV study author "confirmed" that the analytical instruments were "totally different systems, each with its own HPLC and MS/MS detector" (p. 2 of MRID 49513501). The ILV study author reported that the **Sciex 5000** system (Smithers Viscient EQP No. **5368**) was used for the ECM while the **Sciex W-5000** system (Smithers Viscient EQP No. **5297**) was used for the ILV. The reviewer could not validate the identity of the Sciex W-5000 system from the AB Sciex website (www.absciex.com). In the Technical Response Letter, the Equipment Lists of the ECM and ILV were also provided. The reviewer observed the following issues with these lists (pp. 4, 6 of MRID 49513501):

1) The ECM system was entitled "**SPS** Equipment Number: EQP5368", not "**SMV** Equipment Number: EQP5368" or "**Smithers Viscient** EQP No. 5368". The ILV system was entitled "**SMV** Equipment Number: EQP5297", as expected.

2) The Manufacturer/Model/Part Number of the Mass Spectrometer of the ILV was reported as "AB Sciex/ API 5000". The designation "**Sciex W-5000"** was not indicated anywhere on the ILV equipment list. However, the reviewer noted that the Serial Numbers differed for the Mass Spectrometers of the ECM and ILV.

3) The Serial Numbers of the MDS Sciex APPI Sources were identical in the ECM and ILV, "EQP 5273".

4) The Software of the ECM was reported as "Analyst **1.4.2**" in the equipment list, but was reported as "Analyst **1.6**" in the ECM MRID No. 49477402 (p. 10 of MRID 49477402). "Analyst 1.6" was the Software used in the ILV.

5) The ILV study author reported on p. 2 of the Technical Response Letter that the information detailing that supplies and equipment used in the studies was "also included in the ILV report on pages 69 and 70" (p. 2 of MRID 49513501). However, the ILV MRID contained only 38 pages.

The communication between the ECM and ILV was also questioned by Susan Bartow, Chemical Review Manager at the EPA. The ILV report stated that "communications occurred with the Sponsor Monitor" and that the communications would be included with the raw data; however, no communications were included in the ILV (p. 16 of MRID 49477403; p. 1 of MRID 49513501). The ILV report stated that communication included protocol and method clarification, acquisition of the test materials and test matrix, and pre-validation evaluation and method establishment (p. 16 of MRID 49477403). In the Technical Response Letter, the ILV study author "confirmed that there were no project related communications between the study personnel who performed the ILV and the sponsor monitor...[or]…the study personnel who performed method development/validation" (p. 1 of MRID 49513501). Furthermore, the ILV study author explained that communications were not included in the ILV report because no communications occurred.

Overall, the reviewer determined that the information provided in EPA MRID No. 49513501 (a Technical Response Letter prepared by Smithers Viscient, written by the ILV study author) did not provide valid, conclusive responses to comments from Susan Bartow, Chemical Review Manager at the EPA, since the instrument lists and summary of communication in the Technical Response Letter contained inconsistencies with information provided in the original ECM and ILV reports.

4. In the ECM, only three samples were prepared at each fortification level (10×LOQ, 100×LOQ and 700×LOQ; Table 1, p. 21 of MRID 49477402). The OCSPP guidelines specify that five matrix control samples are prepared at each fortification level.

5. In the ECM, chromatograms were only provided for a control sample, one calibration standard and one fortification level (Figures 1-3, pp. 22-24 of MRID 49477402). Chromatograms were not provided for the 100 µg/kg or 7000 µg/kg fortification levels. A reagent blank was not included. In the ECM and ILV, no chromatograms were provided for samples dosed at the LOQ since they were not included in the studies.

6. In the Study Protocol, the directed test concentrations for the ILV were "approximately 0.1, 1 and 7 mg (a.i.)/kg" (Appendix 1, p. 29 of MRID 49477403). The omission of the 7 mg/kg test concentration was not included in the Protocol Deviations section of the ILV (p. 19).

7. The soil matrix was the same in the ECM and ILV (p. 10 of MRID 49477402; p. 10 of MRID 49477403). The Lot No. and Sample ID was the same, but the soil moisture content differed between the two studies.

8. It was reported for the ILV that one set of nineteen samples (10 fortified and 2 unfortified samples, 1 reagent blank and 6 solvent standards) required 8 hours (one working day) to complete the preparation and extraction (p. 16 of MRID 49477403). Subsequent LC/MS analysis was performed overnight.

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

Aldicarb

IUPAC Name: (EZ)-2-Methyl-2-(methylthio)propionaldehyde *O*-methylcarbamoyloxime
CAS Name: 2-Methyl-2-(methylthio)propanal *O*-[(methylamino)carbonyl]oxine
CAS Number: 116-06-3
SMILES String: O=C(O\N=C\C(SC)(C)C)NC

Structure not available

Test Material:	Aldicarb
MRID:	49477402
Title:	Temik 15G (Aldicarb) – Validation of the Analytical Method for the Determination of a Test Substance in Soil
MRID:	49477403
Title:	Independent Laboratory Validation (ILV) of the Analytical Method: Temik 15G (Aldicarb) – Validation of the Analytical Method for the Determination of a Test Substance in Soil
EPA PC Code:	098301
OCSPP Guideline:	850.6100

For CDM Smith

Primary Reviewer: Lisa Muto **Signature:** 

Date: 2/9/15

Secondary Reviewer: Lynne Binari **Signature:** 

Date: 2/9/15

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

Date: 2/9/15