Analytical method for terbacil and its transformation products, terbacil metabolite A, terbacil metabolite B and terbacil metabolite C, in sediment

Reports: ECM: EPA MRID No.: 49653801. Li, F. 2015. Determination of Terbacil and Its Three Metabolites in Sediment Using LC-MS/MS. Report prepared by Critical Path Services, LLC (CPS), Garnet Valley, Pennsylvania, and sponsored and submitted by NovaSource/Tessenderlo Kerley, Inc. (TKI), Phoenix, Arizona; 70 pages. Laboratory Project ID: CPS Method No. 07102014-02, Revision No. 1. Final report issued May 21, 2015.

ILV: EPA MRID No.: 49554802. Malayappan, B. 2015. Independent Laboratory Validation for "Determination of Terbacil and Its Three Metabolites in Sediment Using LC-MS/MS". Report prepared by Critical Path Services, LLC (CPS), Garnet Valley, Pennsylvania, and sponsored and submitted by NovaSource/Tessenderlo Kerley, Inc. (TKI), Phoenix, Arizona; 109 pages. CPS Study No.: 14-CPS-017. Final report issued January 5, 2015.

Document No.: MRIDs 49653801 & 49554802

Guideline: 850.6100

Statements: ECM: The study was not conducted in compliance with USEPA FIFRA Good Laboratory Practice (GLP) standards, since it was not an experimental study (p. 3 of MRID 49653801). Signed and dated Data Confidentiality, GLP and Authenticity statements were provided (pp. 2-3). The Quality Assurance statement was not included.

ILV: The study was conducted in compliance with USEPA FIFRA GLP standards (40 CFR 160; p. 3 of MRID 49554802). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). The statement of authenticity was not included.

Classification: This analytical method is classified as **unacceptable**. The study author needed to provide additional information to confirm no interactions and equipment sharing between the ILV and ECM study directors occurred during the ILV study. The ECM and ILV sediment matrices were undescribed and uncharacterized. The LOD of the method was not reported in the ILV. The determination of the LOQ in the ECM and ILV and of the LOD in the ECM were not based on scientifically acceptable procedures.

PC Code: 012701

EFED Primary Reviewer:	Kristy Crews, Chemist	Signature: Date:	KRISTY CREWS Digitally signed by KRISTY CREWS Date: 2017.08.14 10:07:34 -04'00'
EPA Secondary Reviewer:	William Eckel, Senior Scientist Advisor	Signature: Date:	Eckel, William Digitally signed by Eckel, William Date: 2017.08.14 10:24:28 -04'00'
CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, Environmental Scientist	Signature: Date:	Lisa Muto 6/30/17
	Kathleen Ferguson, Ph.D., Environmental Scientist	Signature: Date:	Kathleen P. Ferguson 6/30/17

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel.

Executive Summary

The analytical method, CPS Method No. 07102014-02/07102014-02, Revision No. 1, is designed for the quantitative determination of terbacil and its transformation products, terbacil metabolite A, terbacil metabolite B and terbacil metabolite C, in sediment at the stated LOQ of 0.01 µg/g using HPLC/MS/MS. The LOQ is **less than** the lowest toxicological level of concern in sediment (IUPAC PPDB 7/26/17[1]). Even though the laboratory which performed the ILV was the same as that which performed the ECM, the reviewer believed that the ILV was conducted independently from the ECM; however, the reviewer also believed that the study author needed to provide additional information to confirm that no interactions between the ILV and ECM study directors/authors occurred during the ILV study and that a different chromatographic system was used for each validation. The ILV validated the method with the second trial with insignificant modifications to the analytical instrumentation and sample processing after technical communication between the Study Monitor (Sponsor) and ILV Study Director. The first trial failed due to a sample preparation/processing error by the laboratory technician. The ECM and ILV sediment matrices were undescribed and uncharacterized; it could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. All ECM and ILV data regarding repeatability, accuracy, precision and specificity were satisfactory for all analytes. All ILV data regarding linearity was satisfactory for all analytes, except for the confirmation ion transition of terbacil; all ECM data regarding linearity was satisfactory for all analytes. The LOD of the method was not reported in the ILV.

[1] http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/620.htm

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						
Terbacil	49653801[2]	49554802[3]		Sediment	21/05/2015	NovaSource/ Tessenderlo Kerley, Inc. (TKI)	LC/MS/MS	0.01 µg/g
Terbacil Metabolite A								
Terbacil Metabolite B								
Terbacil Metabolite C								

1 Terbacil = 3-tert-Butyl-5-chloro-6-methyluracil; Terbacil Metabolite A = Metabolite A; 3-tert-Butyl-5-chloro-6-hydroxymethyl-uracil; Terbacil Metabolite B = Metabolite B; 6-Chloro-7-(hydroxymethyl)-3,3-dimethyl-4-hydro-2H-1,3-oxazolidino[3,2-a]pyrimidin-5-one; Terbacil Metabolite C = Metabolite C; 6-Chloro-3,3,7-trimethyl-4-hydro-2H-1,3-oxazolidino[3,2-a]pyrimidin-5-one.

2 In the ECM, the specific sediment type and characterization were not reported.

3 In the ILV, the specific sediment type and characterization were not reported (p. 10 of MRID 49554802).

I. Principle of the Method

Sediment samples (2.50 g) were placed in 50-mL centrifuge tubes and fortified, if necessary, with the mixed fortification solution (pp. 12-13 of MRID 49653801). The samples were extracted three times with chloroform (35 mL, 20 mL then 20 mL) via shaking by hand for a few seconds then Wrist-Action® Shaker (horizontally) for *ca*. 20 minutes. After centrifugation (5 minutes at 4000 rpm), the supernatant was transferred to a clean 50-mL centrifuge tube though a funnel plugged with glass wool and sodium sulfate. The 50-mL centrifuge tube was attached to an N-EVAP with a water bath set at ≤40°C after each extract, and the evaporation of the solvent was started while the subsequent extracts were performed. When all extracts had been combined into the 50-mL centrifuge tube, the evaporation was continued until the solvent volume was below 1.00 mL or only water was left. The volume of the extract was brought to 5.00 mL with acetonitrile. After vortex and sonication, the sample was filtered through a 0.2-µm nylon syringe filter. An aliquot (1.00 mL) of the sample was transferred to a 1.5-mL centrifuge tube charged with *ca*. 25 mg of Supelclean ENVI-CARB powder. After vortexing for a few seconds and centrifugation (10,000 rpm for 5 minutes), an aliquot (0.600 mL) of the sample was mixed with 0.900 mL of water in an HPLC vial (2.5× dilution). The method noted that the samples should be refrigerated if not analyzed on the same day as extraction.

Samples were analyzed for the analytes using an Agilent Series 1200 LC coupled with a Sciex 4000 Triple Quadrupole Mass Spectrometer in electrospray ionization (ESI; for terbacil and metabolite A) or atmospheric pressure chemical ionization (APCI; for metabolites B and C) mode (pp. 10, 13-15, 17 of MRID 49653801). The method noted that APCI mode was recommended in cases where matrix suppression/enhancement were observed; the method noted that, if APCI mode was used, more sample volume may be injected to obtain the desired sensitivity. The following LC conditions were used: Phenomenex Kinetex C8 column (4.60 mm x 75 mm, 3.0 µ; column temperature 30°C), mobile phase of (A) formic acid:HPLC-grade water (1:1000, v:v) and (B) formic acid:acetonitrile (1:1000, v:v) [Positive MRM: percent A:B (v:v) at 0.00-0.200 min. 60.0:40.0, 1.00-3.00 min. 5.00:95.0, 3.01-5.00 min. 60.0:40.0; Negative MRM: percent A:B (v:v) at 0.00-0.200 min. 55.0:45.0, 1.00-3.00 min. 5.00:95.0, 3.01-5.00 min. 55.0:45.0], injection volume of 10-50 µL, and MRM (550°C) with negative mode (-4500 V) for terbacil and metabolite A and positive mode (5500 V) for metabolites B and C. Two ion pair transitions were monitored for each analyte (quantitation and confirmatory, respectively): *m/z* 215→159 and *m/z* 215→42.1 for terbacil, *m/z* 231→65.9 and *m/z* 231→201 for metabolite A, *m/z* 231→213 and *m/z* 231→185 metabolite B and *m/z* 215→161 and *m/z* 217→163 for metabolite C. Observed retention times were *ca*. 3.39, 2.65, 1.99 and 3.26 minutes for terbacil, metabolite A, metabolite B and metabolite C, respectively (Figures 5-24, pp. 25-44). The method noted that an injection volume of 20 µL was used for analysis of metabolite C with APCI mode to resolve ion enhancement (p. 18).

The ILV performed the ECM method as written with insignificant modifications of the analytical instrumentation, except that the samples were further diluted (1:1) with acetonitrile:water (40:60, v:v) prior to LC/MS/MS analysis (pp. 10, 13-14; Table 3, pp. 23-24 of MRID 49554802). Analyte identification was performed using an Agilent Series 1200 binary pump LC coupled with an Applied Biosystems® API 4000^{TM} Mass Spectrometer in ESI mode for terbacil and metabolite A and in APCI mode for metabolites B and C. The same ion pair transitions were monitored for each analyte as were monitored in the ECM. Observed retention times could not be determined due to the poor resolution of the representative chromatograms (Figures 5-24, pp. 30-49).

In the ECM and ILV, the Limit of Quantification (LOQ) was 0.01 µg/g for terbacil, terbacil metabolite A, terbacil metabolite B and terbacil metabolite C (pp. 8, 18 of MRID 49653801; pp. 8, 16 of MRID 49554802). In the ECM, the Limit of Detection (LOD) was reported as 1.0 ng/g for all analytes; in the ILV, the LOD was not reported.

II. Recovery Findings

ECM (MRID 49653801): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of terbacil, terbacil metabolite A, terbacil metabolite B and terbacil metabolite C at fortification levels of 0.01 µg/g (LOQ) and 0.10 µg/g (10×LOQ) in one sediment matrix (Table 1, p. 20). Two ion pair transitions were monitored for each analyte; quantitation and confirmatory ion analyses were comparable. The specific sediment type and characterization were not reported.

ILV (MRID 49554802): Mean recoveries and RSDs were within guidelines for analysis of terbacil, terbacil metabolite A, terbacil metabolite B and terbacil metabolite C at fortification levels of 0.01 µg/g (LOQ) and 0.10 µg/g (10×LOQ) in one sediment matrix (Tables 1-2, pp. 19-22). Two ion pair transitions were monitored for each analyte; quantitation and confirmatory ion analyses were comparable. The specific sediment type and characterization were not reported (p. 10). The method was validated with the second trial with insignificant modifications to the analytical instrumentation and sample processing after technical communication between the Study Monitor (Sponsor) and ILV Study Director (pp. 8, 10, 13-15; Appendix 5, p. 107). The first trial failed due to a sample preparation/processing error by the laboratory technician.

Table 2. Initial Validation Method Recoveries for Terbacil and Its Transformation Products, Terbacil Metabolite A, Terbacil Metabolite B and Terbacil Metabolite C, in Sediment

Analyte[1]	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Sediment[2]					
	Quantitation ion transition[3]					
Terbacil	0.01 (LOQ)	5	88.0-106.0	96.9	6.77	6.98
	0.10	5	90.5-103.5	96.7	4.70	4.86
Terbacil Metabolite A	0.01 (LOQ)	5	98.5-106.5	102.6	2.92	2.85
	0.10	5	97.5-108.0	103.7	4.34	4.18
Terbacil Metabolite B	0.01 (LOQ)	5	105.5-116.5	110.5	4.06	3.68
	0.10	5	96.0-104.5	100.2	4.07	4.06
Terbacil Metabolite C	0.01 (LOQ)	5	96.0-107.0	102.1	4.75	4.65
	0.10	5	90.5-98.0	93.7	2.80	2.99
	Confirmatory ion transition[3]					
Terbacil	0.01 (LOQ)	5	84.0-103.0	95.2	8.81	9.25
	0.10	5	93.0-103.5	96.3	4.31	4.48
Terbacil Metabolite A	0.01 (LOQ)	5	99.5-112.5	107.4	5.05	4.71
	0.10	5	95.0-111.5	105.0	6.75	6.43
Terbacil Metabolite B	0.01 (LOQ)	5	102.0-114.0	109.0	5.06	4.64
	0.10	5	94.5-104.0	99.5	3.82	3.84
Terbacil Metabolite C	0.01 (LOQ)	5	92.0-107.0	102.5	6.04	5.89
	0.10	5	90.0-97.5	94.4	3.42	3.62

Data (uncorrected recovery results; p. 16) were obtained from Table 1, p. 20 of MRID 49653801.

1 Terbacil = 3-tert-Butyl-5-chloro-6-methyluracil; Terbacil Metabolite A = 3-tert-Butyl-5-chloro-6-hydroxymethyl-uracil; Terbacil Metabolite B = 6-Chloro-7-(hydroxymethyl)-3,3-dimethyl-4-hydro-2H-1,3-oxazolidino[3,2-a]pyrimidin-5-one; Terbacil Metabolite C = 6-Chloro-3,3,7-trimethyl-4-hydro-2H-1,3-oxazolidino[3,2-a]pyrimidin-5-one.

2 The specific sediment type and characterization were not reported.

3 Two ion pair transitions were monitored for each analyte (quantitation and confirmatory, respectively): *m/z* 215→159 and *m/z* 215→42.1 for terbacil, *m/z* 231→65.9 and *m/z* 231→201 for metabolite A, *m/z* 231→213 and *m/z* 231→185 metabolite B and *m/z* 215→161 and *m/z* 217→163 for metabolite C.

Table 3. Independent Validation Method Recoveries for Terbacil and Its Transformation Products, Terbacil Metabolite A, Terbacil Metabolite B and Terbacil Metabolite C, in Sediment

Analyte[1]	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Sediment[2]					
	Quantitation ion transition[3]					
Terbacil	0.01 (LOQ)	5	72.5-89.1	82.5	6.71	8.14
	0.10	5	73.0-80.0	77.4	2.90	3.75
Terbacil Metabolite A	0.01 (LOQ)	5	55.0-88.1	76.5	13.1	17.1
	0.10	5	70.5-82.0	74.9	4.52	6.03
Terbacil Metabolite B	0.01 (LOQ)	5	77.0-92.6	85.2	6.41	7.53
	0.10	5	74.0-84.5	80.2	4.22	5.26
Terbacil Metabolite C	0.01 (LOQ)	5	83.0-90.6	88.0	3.20	3.64
	0.10	5	79.5-86.0	81.8	2.59	3.16
	Confirmatory ion transition[3]					
Terbacil	0.01 (LOQ)	5	77.5-92.1	84.2	6.83	8.12
	0.10	5	73.0-79.5	77.3	2.77	3.59
Terbacil Metabolite A	0.01 (LOQ)	5	57.0-89.1	77.8	13.1	16.8
	0.10	5	72.5-81.5	74.8	3.90	5.21
Terbacil Metabolite B	0.01 (LOQ)	5	79.0-96.1	87.1	7.44	8.53
	0.10	5	76.0-85.0	80.2	3.49	4.36
Terbacil Metabolite C	0.01 (LOQ)	5	78.5-88.1	83.5	4.06	4.86
	0.10	5	79.0-87.0	83.8	3.11	3.72

Data (uncorrected recovery results; Appendix 2, p. 99) were obtained from Tables 1-2, pp. 19-22 of MRID 49554802.

1 Terbacil = 3-tert-Butyl-5-chloro-6-methyluracil; Terbacil Metabolite A = 3-tert-Butyl-5-chloro-6-hydroxymethyl-uracil; Terbacil Metabolite B = 6-Chloro-7-(hydroxymethyl)-3,3-dimethyl-4-hydro-2H-1,3-oxazolidino[3,2-a]pyrimidin-5-one; Terbacil Metabolite C = 6-Chloro-3,3,7-trimethyl-4-hydro-2H-1,3-oxazolidino[3,2-a]pyrimidin-5-one.

2 The specific sediment type and characterization were not reported (p. 10).

3 Two ion pair transitions were monitored for each analyte (quantitation and confirmatory, respectively): *m/z* 215→159 and *m/z* 215→42.1 for terbacil, *m/z* 231→65.9 and *m/z* 231→201 for metabolite A, *m/z* 231→213 and *m/z* 231→185 metabolite B and *m/z* 215→161 and *m/z* 217→163 for metabolite C.

III. Method Characteristics

In the ECM and ILV, the LOQ was 0.01 µg/g for terbacil, terbacil metabolite A, terbacil metabolite B and terbacil metabolite C (pp. 8, 18 of MRID 49653801; pp. 8, 16 of MRID 49554802). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated. Also, the analyte peak response at the LOQ should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. In the ECM, the LOD was reported as 1.0 ng/g for all analytes; in the ILV, the LOD was not reported. In the ECM, the LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time, as well as ≤50% of the concentration in the final extract for the LOQ sample. Also, an estimate of the LOD can be taken as four times the background noise. The

ECM study author additionally noted that the LOD can vary between runs and from instrument to instrument. No calculations were reported to support the method LOQ and LOD.

Table 4. Method Characteristics for Terbacil and Its Transformation Products, Terbacil Metabolite A, Terbacil Metabolite B and Terbacil Metabolite C, in Sediment

Analyte[1]		**Terbacil**	**Terbacil Metabolite A**	**Terbacil Metabolite B**	**Terbacil Metabolite C**
Limit of Quantitation (LOQ)		0.01 µg/g			
Limit of Detection (LOD)	ECM	1.0 ng/g			
	ILV	**Not reported**			
Linearity (calibration curve r^2 and concentration range)	ECM[2]	r^2 = 0.9990 (Q) r^2 = 0.9984 (C)	r^2 = 0.9986 (Q) r^2 = 0.9984 (C)	r^2 = 0.9992 (Q) r^2 = 0.9996 (C)	r^2 = 0.9990 (Q) r^2 = 0.9994 (C)
	ILV[3]	r^2 = 0.9962 (Q) r^2 = **0.9946** (C)	r^2 = 0.9984 (Q) r^2 = 0.9992 (C)	r^2 = 0.9968 (Q) r^2 = 0.9986 (C)	r^2 = 0.9962 (Q) r^2 = 0.9980 (C)
	Range:	1.00-50.0 ng/mL			
Repeatable	ECM[4]	Yes at LOQ and 10×LOQ.			
	ILV[5,6]				
Reproducible		Yes at LOQ and 10×LOQ.			
Specific	ECM	No matrix interferences were observed.			
	ILV	No matrix interferences were observed.	Minor matrix interferences (< 5% of the LOQ, based on peak height) were observed and interfered with peak integration of the Q ion at the LOQ.	No matrix interferences were observed, but minor baseline noise interfered with peak integration of the C ion at the LOQ.	No matrix interferences were observed, but non-uniform peak integration was noted at the LOQ.

Data were obtained from pp. 8, 12, 18; Table 1, p. 20 (recovery results); Figures 1-4, pp. 21-24 (calibration curves); Figures 5-24, pp. 25-44 (chromatograms) of MRID 49653801; pp. 8, 12, 16; Tables 1-2, pp. 19-22 (recovery results); Figures 1-4, pp. 26-29 (calibration curves); Figures 5-24, pp. 30-49 (chromatograms) of MRID 49554802. Q = quantitation ion transition; C = confirmation ion transition.

1 Terbacil = 3-tert-Butyl-5-chloro-6-methyluracil; Terbacil Metabolite A = 3-tert-Butyl-5-chloro-6-hydroxymethyl-uracil; Terbacil Metabolite B = 6-Chloro-7-(hydroxymethyl)-3,3-dimethyl-4-hydro-2H-1,3-oxazolidino[3,2-a]pyrimidin-5-one; Terbacil Metabolite C = 6-Chloro-3,3,7-trimethyl-4-hydro-2H-1,3-oxazolidino[3,2-a]pyrimidin-5-one.

2 Correlation coefficients (r^2) were reviewer-calculated based on r values (1/x weighted linear regression analysis) reported in the study report; solvent standards were used (p. 12; Figures 1-4, pp. 21-24 of MRID 49653801; DER Attachment 2).

3 Correlation coefficients (r^2) were reviewer-calculated based on r values (1/x weighted linear regression analysis) reported in the study report; solvent standards were used (p. 12; Figures 1-4, pp. 26-29 of MRID 49554802; DER Attachment 2).

4 In the ECM, the specific sediment type and characterization were not reported.

5 In the ILV, the specific sediment type and characterization were not reported (p. 10 of MRID 49554802).

6 The method was validated with the second trial with insignificant modifications to the analytical instrumentation and sample processing after technical communication between the Study Monitor (Sponsor) and ILV Study Director (pp. 8, 10, 13-14, 15; Appendix 5, p. 107 of MRID 49554802). The first trial failed due to a sample preparation/processing error by the laboratory technician.

A confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

IV. Method Deficiencies and Reviewer's Comments

1. Even though the laboratory which performed the ILV was the same as that which performed the ECM [Critical Path Services, LLC (CPS), Garnet Valley, Pennsylvania], the reviewer believed that the ILV was conducted independently from the ECM; however, the reviewer also believed that the study author needed to provide additional information to confirm that no interactions between the ILV and ECM study directors/authors occurred during the course of the ILV study and that a different chromatographic system was used for each validation. According to OCSPP guidelines, 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. Furthermore, 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.

   In order to support their independence claim, the ILV included a summary of and some details of the communication between the Sponsor and ILV Study Director (p. 15; Appendix 5, pp. 106-108 of MRID 49554802). The ILV reported that the ILV was successfully completed with technical communication between the Study Monitor (Sponsor) and ILV Study Director. In Appendix 5 of the ILV, some of the email communication was provided for review: the communication which involved the ILV Study Director describing the technical problems found in the failed first trial. The reviewer noted that the response of the Sponsor to the ILV Study Director included the fact that the Sponsor advised the ILV Study Monitor to talk to "Fenn" (the ECM Study Director) before proceeding to the second trial. This advice of the Sponsor is an infringement of the OCSPP guidelines for satisfactory independence of the ILV from the ECM. Subsequent emails/communications were not provided by the ILV to ensure that no direct or indirect communication occurred between the ECM and ILV Study Directors (see Appendix 5, p. 106). In the Revisions of the ECM, the ECM Study Director/Author (L. Fenn) reported that no interactions between staff and no sharing of equipment occurred even though both validations occurred at the same address (pp. 5, 8 of MRID 49653801). The ECM Study Director noted that the study directors for the ECM and ILV reported to the same supervisor, but the execution and performance of the method was not discussed.

   According to the ECM Study Director, a different chromatographic system was used for each validation; however, the ECM used an Agilent Series 1200 LC (or equivalent) coupled with a Sciex 4000 Triple Quadrupole MS (or equivalent) while the ILV used an Agilent Series 1200 binary pump LC coupled with an Applied Biosystems® API 4000™ MS (p. 10 of MRID 49653801; p. 10 of MRID 49554802). The reviewer noted that MDS Sciex and Applied Biosystems have a joint venture in the production of LC/MS instruments. More information, such as instrument laboratory ID numbers, should be provided to ensure that the two chromatographic systems were distinct.

2. The specific type and characterization of the ECM and ILV test sediment matrices were not reported. Also, the sources of the sediments were not reported. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method.

3. In the ILV, the linearity was not satisfactory for the confirmation ion transition of terbacil (r^2 = 0.9946); linearity is satisfactory when $r^2 \geq 0.995$ (Figure 1, p. 26 of MRID 49554802; DER Attachment 2). The reviewer noted that a confirmatory method is not usually required when LC/MS and GC/MS is the primary method.

4. The determination of the LOQ in the ECM and ILV and of the LOD in the ECM were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (pp. 8, 18 of MRID 49653801; pp. 8, 16 of MRID 49554802). In the ECM, the LOQ was defined as the lowest analyte concentration in a sample at which the methodology has been validated. Also, the analyte peak response at the LOQ should be no lower than four times the mean amplitude of the background noise in an untreated sample at the corresponding retention time. In the ECM, the LOD was defined as the lowest analyte concentration detectable above the mean amplitude of the background noise in an untreated sample at the corresponding retention time, as well as ≤50% of the concentration in the final extract for the LOQ sample. Also, an estimate of the LOD can be taken as four times the background noise. The ECM study author additionally noted that the LOD can vary between runs and from instrument to instrument. No calculations were reported to support the method LOQ and LOD. No method LOD was reported in the ILV.

5. In the ILV, representative chromatograms of terbacil metabolites A, B and C showed irregular peak integration along the baseline, most notable in the LOQ chromatograms, due to either minor matrix interferences or minor baseline noise (Figure 13, p. 38; Figure 18, p. 43; Figure 23, p. 48 of MRID 49554802). This did not affect the specificity of the method.

6. The ILV was provided CPS Method No. 07102014-02 as the ECM (Appendix 1, Appendix 1, pp. 57-97 of MRID 49554802). The ECM was revised in CPS Method No. 07102014-02, Revision No. 1 to include a typographical error correction and information about the ILV (pp. 5, 8 of MRID 49653801).

7. The communications between the Sponsor and ILV Study Monitor included a summary of and some details of the communication between the Sponsor and ILV Study Director (see Reviewer's Comment #1 for more information; p. 15; Appendix 5, pp. 106-108 of MRID 49554802).

8. In the ILV, the total time required to complete one set of up to 20 samples was reported as *ca*. 1 day (8 working hours) to complete, excluding instrument rum time (p. 15 of MRID 49554802).

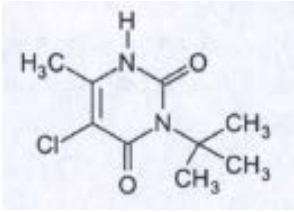
V. References

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.

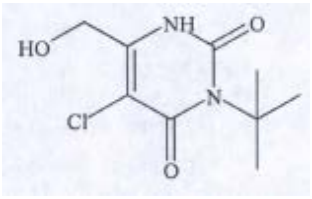
40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

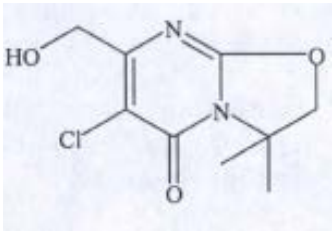
Terbacil
IUPAC Name: Not reported
CAS Name: 3-tert-Butyl-5-chloro-6-methyluracil
CAS Number: 5902-51-2
SMILES String: Not found



Terbacil Metabolite A (Metabolite A)
IUPAC Name: Not reported
CAS Name: 3-tert-Butyl-5-chloro-6-hydroxymethyl-uracil
CAS Number: 25546-02-5
SMILES String: Not found



Terbacil Metabolite B (Metabolite B)
IUPAC Name: Not reported
CAS Name: 6-Chloro-7-(hydroxymethyl)-3,3-dimethyl-4-hydro-2H-1,3-oxazolidino[3,2-a]pyrimidin-5-one
CAS Number: 34138-55-1
SMILES String: Not found



Terbacil Metabolite C (Metabolite C)

IUPAC Name:	Not reported
CAS Name:	6-Chloro-3,3,7-trimethyl-4-hydro-2H-1,3-oxazolidino[3,2-a]pyrimidin-5-one
CAS Number:	34112-90-8
SMILES String:	Not found