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

Reports: ECM: EPA MRID No.: 49653802. Li, F. 2015. Determination of Terbacil and Its Three Metabolites in Water 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; 82 pages. Laboratory Project ID: CPS Method No. 07102014-01, Revision No. 1. Final report issued May 21, 2015.

ILV: EPA MRID No.: 49554801. Malayappan, B. 2014. Independent Laboratory Validation for "Determination of Terbacil and Its Three Metabolites in Water 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; 115 pages. CPS Study No.: 14-CPS-016. Final report issued December 2, 2014.

Document No.: MRIDs 49653802 & 49554801

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 49653802). 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 49554801). 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 water matrices were 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. The LOQ is equal to the lowest toxicological level of concern in water.

PC Code: 012701

EFED Primary Reviewer:	Kristy Crews, Chemist	Signature:	KRISTY CREWS Digitally signed by KRISTY CREWS Date: 2017.08.14 10:06:12 -04'00'
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CDM/CSS-Dynamac JV Reviewers:	Lisa Muto, Environmental Scientist	Signature:	Lisa Muto
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	Kathleen Ferguson, Ph.D., Environmental Scientist	Signature:	Kathleen P. Ferguson
		Date:	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. 07102014-01/07102014-01, 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 water at the stated LOQ of 0.01 µg/mL using HPLC/MS/MS. The LOQ is **equal to** the lowest toxicological level of concern in water (MRID 43909802). 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 a different chromatographic system was used for each validation and that no interactions between the ILV and ECM study directors occurred during the ILV study. The ILV validated the method with the first trial with insignificant modifications to the analytical instrumentation without technical communication between the Study Monitor (Sponsor) and ILV Study Director. The ECM and ILV water matrices were 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 terbacil metabolites, except for the confirmation ion transition of metabolite B and the quantitation ion transition of metabolite C; linearity was not satisfactory for terbacil. All ECM data regarding linearity was satisfactory for all analytes. The LOD of the method was not reported in the ILV.

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	49653802[2]	49554801[3]		Water	21/05/2015	NovaSource/ Tessenderlo Kerley, Inc. (TKI)	LC/MS/MS	0.01 µg/mL
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, tap and surface water were used; the specific water sources and characterization were not reported.

3 In the ILV, the surface water matrix was collected at Upper Merion Township Park, King of Prussia, Pennsylvania; the specific water type and characterization were not reported (p. 10 of MRID 49554801).

I. Principle of the Method

Water samples (3.00 mL) were placed in 15-mL centrifuge tubes and fortified, if necessary, with the mixed fortification solution (pp. 12-13 of MRID 49653802). The samples were mixed well via vortexing for a few seconds; samples were centrifuged for 5 minutes at 4000 rpm, if necessary, then purified by solid phase extraction (SPE). The sample was loaded onto an Agilent C18 cartridge (500 mg, 3 cc; pre-conditioned with 5.00 mL of acetonitrile then 3.00 mL of water). The centrifuge tube was rinsed with 3.00 mL of water which was added to the cartridge. The analytes were eluted with 5.00 mL of acetonitrile into a clean 15-mL centrifuge tube. The volume of the extract was brought to 6.00 mL with acetonitrile. After mixing, 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) mode (pp. 10, 13-14, 16-17 of MRID 49653802). 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*. 3.42, 2.67, 1.99 and 3.28 minutes for terbacil, metabolite A, metabolite B and metabolite C, respectively (Figures 5-36, pp. 25-56).

The ILV performed the ECM method as written with insignificant modifications of the analytical instrumentation (pp. 10, 12-14; Table 3, pp. 21-22 of MRID 49554801). Analyte identification was performed using an Agilent Series 1200 binary pump LC coupled with an Applied Biosystems API 4000 Mass Spectrometer in ESI mode. 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. 28-47).

In the ECM and ILV, the Limit of Quantification (LOQ) was 0.01 µg/mL for terbacil, terbacil metabolite A, terbacil metabolite B and terbacil metabolite C (pp. 8, 17 of MRID 49653802; pp. 8, 15 of MRID 49554801). In the ECM, the Limit of Detection (LOD) was reported as 1.0 ng/mL for all analytes; in the ILV, the LOD was not reported.

II. Recovery Findings

ECM (MRID 49653802): 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/mL (LOQ) and 0.10 µg/mL (10×LOQ) in two water matrices (Tables 1-2, pp. 19-20). Two ion pair transitions were monitored for each analyte; quantitation and confirmatory ion analyses were comparable. Surface and tap water matrices were used; the specific water sources and characterization were not reported.

ILV (MRID 49554801): 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/mL (LOQ) and 0.10 µg/mL (10×LOQ) in one water matrix (Tables 1-2, pp. 17-20). Two ion pair transitions were monitored for each analyte; quantitation and confirmatory ion analyses were comparable. The surface water matrix was collected at Upper Merion Township Park, King of Prussia, Pennsylvania; the specific water type and characterization were not reported (p. 10). The method was validated with the first trial with insignificant modifications to the analytical instrumentation (pp. 13-15). Technical communication between the Study Monitor (Sponsor) and ILV Study Director was not required and did not occur.

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

Analyte[1]	Fortification Level (µg/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Tap Water[2]					
	Quantitation ion transition[3]					
Terbacil	0.01 (LOQ)	5	103.5-108.5	106.4	2.43	2.29
	0.10	5	105.0-111.5	108.6	2.39	2.19
Terbacil Metabolite A	0.01 (LOQ)	5	77.0-103.5	94.9	10.5	11.0
	0.10	5	102.5-106.0	104.8	1.68	1.60
Terbacil Metabolite B	0.01 (LOQ)	5	89.0-107.5	97.7	7.02	7.19
	0.10	5	98.0-106.5	102.3	3.09	3.02
Terbacil Metabolite C	0.01 (LOQ)	5	93.5-106.5	102.3	5.07	4.96
	0.10	5	101.0-109.0	105.6	3.16	2.98
	Confirmatory ion transition[3]					
Terbacil	0.01 (LOQ)	5	105.5-112.5	108.9	2.58	2.37
	0.10	5	101.5-110.5	105.9	3.19	3.01
Terbacil Metabolite A	0.01 (LOQ)	5	81.0-103.0	93.1	8.61	9.24
	0.10	5	101.5-109.5	105.0	3.30	3.14
Terbacil Metabolite B	0.01 (LOQ)	5	90.5-110.5	98.1	8.23	8.39
	0.10	5	101.0-104.5	102.4	1.39	1.35
Terbacil Metabolite C	0.01 (LOQ)	5	91.0-108.0	102.5	6.78	6.62
	0.10	5	105.0-107.5	106.0	1.37	1.29

Analyte[1]	Fortification Level (µg/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Surface Water[2]					
	Quantitation ion transition[3]					
Terbacil	0.01 (LOQ)	5	95.0-101.5	99.5	267	2.68
	0.10	5	105.0-112.0	107.6	2.70	2.51
Terbacil Metabolite A	0.01 (LOQ)	5	94.5-101.0	98.2	3.17	3.23
	0.10	5	106.0-112.0	109.2	3.01	2.76
Terbacil Metabolite B	0.01 (LOQ)	5	94.0-110.5	103.2	6.48	6.28
	0.10	5	105.0-115.5	110.0	4.26	3.87
Terbacil Metabolite C	0.01 (LOQ)	5	90.0-100.0	94.2	4.25	4.51
	0.10	5	104.0-111.5	106.7	3.21	3.01
	Confirmatory ion transition[3]					
Terbacil	0.01 (LOQ)	5	92.0-96.5	94.4	1.85	1.96
	0.10	5	102.0-110.0	105.2	2.93	2.78
Terbacil Metabolite A	0.01 (LOQ)	5	95.5-105.0	102.5	4.05	3.95
	0.10	5	103.0-110.5	106.9	2.95	2.76
Terbacil Metabolite B	0.01 (LOQ)	5	88.5-104.0	98.5	6.92	7.02
	0.10	5	101.5-110.0	106.3	3.62	3.32
Terbacil Metabolite C	0.01 (LOQ)	5	87.0-104.5	95.4	5.58	5.85
	0.10	5	102.0-110.5	105.8	3.17	3.00

Data (uncorrected recovery results; p. 16) were obtained from Tables 1-2, pp. 19-20 of MRID 49653802.

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 Tap and surface water were used; the specific water sources 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 Water

Analyte[1]	Fortification Level (µg/mL)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Surface Water[2]					
	Quantitation ion transition[3]					
Terbacil	0.01 (LOQ)	5	103-107	105	1.40	1.33
	0.10	5	93.5-95.5	94.7	0.76	0.80
Terbacil Metabolite A	0.01 (LOQ)	5	103-109	106	2.27	2.14
	0.10	5	97.0-99.5	97.7	1.52	1.56
Terbacil Metabolite B	0.01 (LOQ)	5	91.0-104	96.3	4.58	4.75
	0.10	5	96.0-99.5	98.1	1.39	1.41
Terbacil Metabolite C	0.01 (LOQ)	5	103-110	107	3.09	2.90
	0.10	5	97.0-99.0	98.1	1.04	1.02
	Confirmatory ion transition[3]					
Terbacil	0.01 (LOQ)	5	99.0-108	105	4.75	4.52
	0.10	5	92.0-96.0	94.7	1.64	1.74
Terbacil Metabolite A	0.01 (LOQ)	5	101-109	105	3.49	3.32
	0.10	5	96.5-100	98.1	1.43	1.46
Terbacil Metabolite B	0.01 (LOQ)	5	96.0-104	99.5	3.04	3.06
	0.10	5	95.5-102	98.4	2.19	2.23
Terbacil Metabolite C	0.01 (LOQ)	5	101-110	106	3.23	3.06
	0.10	5	96.0-99.0	97.8	1.25	1.28

Data (uncorrected recovery results; Appendix 2, p. 99) were obtained from Tables 1-2, pp. 17-20 of MRID 49554801.

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 surface water matrix was collected at Upper Merion Township Park, King of Prussia, Pennsylvania; the specific water 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/mL for terbacil, terbacil metabolite A, terbacil metabolite B and terbacil metabolite C (pp. 8, 17 of MRID 49653802; pp. 8, 15 of MRID 49554801). 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/mL 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 Water

Analyte[1]		**Terbacil**	**Terbacil Metabolite A**	**Terbacil Metabolite B**	**Terbacil Metabolite C**
Limit of Quantitation (LOQ)		0.01 µg/mL			
Limit of Detection (LOD)	ECM	1.0 ng/mL			
	ILV	**Not reported**			
Linearity (calibration curve r^2 and concentration range)	ECM[2]	r^2 = 0.9990 (Q) r^2 = 0.9992 (C)	r^2 = 0.9990 (Q) r^2 = 0.9984 (C)	r^2 = 0.9982 (Q) r^2 = 0.9978 (C)	r^2 = 0.9972 (Q) r^2 = 0.9974 (C)
	ILV[3]	r^2 = **0.9928** (Q) r^2 = **0.9946** (C)	r^2 = 0.9992 (Q) r^2 = 0.9984 (C)	r^2 = 0.9970 (Q) r^2 = **0.9932** (C)	r^2 = **0.9910** (Q) r^2 = 0.9960 (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. Minor baseline noise which interfered with peak integration was noted in many chromatograms.			
	ILV				

Data were obtained from pp. 8, 12, 17; Tables 1-2, pp. 19-20 (recovery results); Figures 1-4, pp. 21-24 (calibration curves); Figures 5-36, pp. 25-56 (chromatograms) of MRID 49653802; pp. 8, 12, 15; Tables 1-2, pp. 17-20 (recovery results); Figures 1-4, pp. 24-27 (calibration curves); Figures 5-24, pp. 28-47 (chromatograms) of MRID 49554801. 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 49653802; 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. 24-27 of MRID 49554801; DER Attachment 2). The reviewer noted that r values were difficult to read due to poor resolution of the calibration plot outputs.

4 In the ECM, tap and surface water were used; the specific water sources and characterization were not reported.

5 In the ILV, the surface water matrix was collected at Upper Merion Township Park, King of Prussia, Pennsylvania; the specific water type and characterization were not reported (p. 10 of MRID 49554801).

6 The method was validated with the first trial with insignificant modifications to the analytical instrumentation (pp. 13-15 of MRID 49554801). Technical communication between the Study Monitor (Sponsor) and ILV Study Director was not required and did not occur.

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 a different chromatographic system was used for each validation and that no interactions between the ILV and ECM study authors/directors occurred during the course of the ILV study. 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.

   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^{TM} MS (p. 10 of MRID 49653802; p. 10 of MRID 49554801). 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.

   In order to support their independence claim, the ILV included a summary of the communication between the Sponsor and ILV Study Director (pp. 14-15 of MRID 49554801). The ILV Study Author reported that the ILV was successfully completed without technical communication between the Study Monitor (Sponsor) and ILV Study Director/Author, and the only communication was the notification of the successful completion of the trial; however, the detailed communication was not provided for review. 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 49653802). 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.

2. The characterization of the ECM and ILV test water matrices were not reported. Also, the sources of the ECM waters 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 terbacil [$r^2 = 0.9928$ (Q), $r^2 = 0.9946$ (C)], the quantitation ion transition of terbacil metabolite B ($r^2 = 0.9910$) and the confirmation ion transition of terbacil metabolite C ($r^2 = 0.9932$); linearity is satisfactory when $r^2 \geq 0.995$ (Figures 1-4, pp. 24-27 of MRID 49554801; 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, 17 of MRID 49653802; pp. 8, 15 of MRID 49554801). 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 ECM and ILV, many of the representative chromatograms of terbacil and terbacil metabolites A, B and C showed irregular peak integration along the baseline, most notable in the LOQ chromatograms, due to minor baseline noise (Figures 5-36, pp. 25-56 of MRID 49653802; Figures 5-24, pp. 28-47 of MRID 49554801). This did not affect the specificity of the method.

6. The ILV was provided CPS Method No. 07102014-01 as the ECM (Appendix 1, Appendix 1, pp. 55-103 of MRID 49554801). The ECM was revised in CPS Method No. 07102014-01, Revision No. 1 to include a typographical error correction and information about the ILV (pp. 5, 8 of MRID 49653802).

7. The communications between the Sponsor and ILV Study Monitor included a summary of the communication between the Sponsor and ILV Study Director (pp. 14-15 of MRID 49554801).

8. In the ILV, the total time required to complete one set of 13 samples (one reagent blank, two unfortified matrix control samples and 10 fortified samples) was reported as *ca*. 1.5 days to complete, where extraction and analysis time were each *ca*. 6 hours (p. 14 of MRID 49554801).

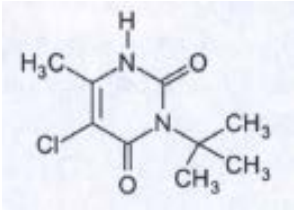
V. References

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.

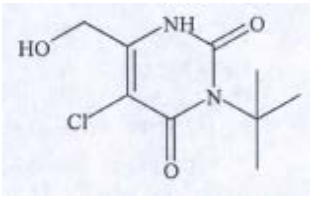
40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures

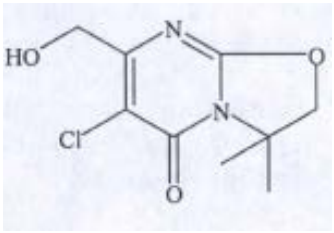
Terbacil
IUPAC Name: Not reported
CAS Name: 3-tert-Butyl-5-chloro-6-methyluracil
CAS Number: 5902-51-2
SMILES String: Not found



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