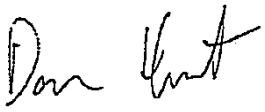
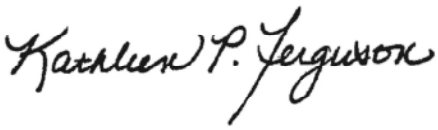
Test Material:	Flutolanil
MRID:	48763101
Title:	Validation of analytical method for flutolanil in soil.
MRID:	48714001
Title:	Independent laboratory validation of the flutolanil analytical method described in Nihon Nohyaku Co., Ltd. Final report no. LSRC-A07-161A (amended), study protocol no. GE-04, 07-0127, entitled "Validation of analytical method for flutolanil in soil."
EPA PC Code:	128975
OCSPP Guideline:	850.6100

For Cambridge Environmental

Primary Reviewer: Dan Hunt **Signature:** 

Date: 1/22/13

Secondary Reviewer: Kathleen Ferguson **Signature:** 

Date: 1/22/13

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

Date: 1/22/13

Analytical method for flutolanil in soil

Reports:	ECM: EPA MRID No. 48763101. Ihara, T. 2007. Validation of analytical method for flutolanil in soil. Report prepared by Nihon Nohyaku Co., Ltd., Osaka, Japan, sponsored and submitted by Nichino America, Inc., Wilmington, Delaware (p. 2); 32 pages. Final report issued December 7, 2007. ILV: EPA MRID No. 48714001. Willoh, J.M. 2011. Independent laboratory validation of the flutolanil analytical method described in Nihon Nohyaku Co., Ltd. Final report no. LSRC-A07-161A (amended), study protocol no. GE-04, 07-0127, entitled "Validation of analytical method for flutolanil in soil." Report prepared by Morse Laboratories, LLC, Sacramento, California, sponsored and submitted by Nihon Nohyaku Co. Ltd., Tokyo, Japan; 134 pages. Final report issued September 21, 2011.
Document No.:	MRIDs 48763101 & 48714001
Guideline:	850.6100
Statements:	ECM: The study was conducted in accordance with the OECD and JMAFF Good Laboratory Practice (GLP) standards (p. 3). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A Certification of Authenticity Statement was provided with the Quality Assurance statement. ILV: The study was conducted in accordance with the USEPA FIFRA Good Laboratory Practice (GLP) standards (40 CFR Part 160; p.3). Signed and dated Data Confidentiality, GLP, Quality Assurance and Certification of Authenticity statements were provided (pp. 2-4, 6).
Classification:	This analytical method is considered supplemental, for residues of flutolanil in soil, at the stated LOQ of 0.01 mg/kg, and 10 LOQ. The determination of the LOD and LOQ were not based on scientifically acceptable procedures; the fortification procedure was not reported; example calculations were incomplete; validation sample sets did not include a reagent blank; and a phone conversation between the ECM and ILV during the trial was not documented.
PC Code:	128975

Final Reviewer:	Jose L. Melendez	**Signature:**	José Luis Meléndez
Title:	Chemist	**Date:**	August 2, 2013

All page citations refer to MRID 48763101 (ECM) unless otherwise noted.

Executive Summary

This analytical method, "Protocol No. GE-04, 07-0127", is designed for the quantitative determination of flutolanil in soil using LC/MS/MS. The method is quantitative for flutolanil at

the stated LOQ of 0.01 mg/kg. The lowest toxicological level of concern in soil was not reported. No major issues were discovered by the independent laboratory.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Flutolanil	48763101	48714001		Soil	12/7/2007	Nichino America, Inc.	LC/MS/MS	0.01 mg/kg

I. Principle of the Method

Soil (20 g) is fortified with a standard solution of flutolanil, in acetonitrile, and extracted by shaking vigorously for 15 minutes with 50 mL of acetonitrile, followed by centrifugation at 2500 rpm for 5 minutes (pp. 14-15; Appendix III, p. 28). The sample was then extracted a second time with 20 mL of acetonitrile and the supernatant was filtered under slight suctioning. The combined extracts were brought to 100 mL with acetonitrile and an aliquot (12.5 mL) of the extract was combined with 3 g NaCl and 12.5 mL phosphate buffer solution (0.1 M, pH 7) and shaken. The upper organic layer was withdrawn, evaporated *in vacuo* and the residue dissolved with 3 mL of acetonitrile:distilled water (4:1, v:v) and loaded onto a carbon graphite/ aminopropyl silica gel cartridge column conditioned with 5 mL each of acetonitrile and acetonitrile:distilled water (4:1, v:v). The cartridge column was washed with 2 mL each of acetonitrile:distilled water (4:1, v:v) and acetonitrile, and the analyte was eluted from the column with 5 mL of acetonitrile:acetic acid (95:5, v:v) and diluted to 25 mL with distilled water.

Samples were analyzed for flutolanil by HPLC (Cadenza CD-C18, 2.0 mm x 50 mm, 3 µm ODS column) using a mobile phase gradient of (A) distilled water containing 0.1% formic acid and (B) methanol containing 0.1% formic acid [percent A:B at 0.0 min. 50:50 (v:v), 0.5 min. 30:70, 5.5 min. 0:100, 7.5 min. 0:100] with MS/MS detection with Multiple Reaction Monitoring (MRM; pp. 13, 15-16).

The LOQ was the same in the ECM and ILV (proposed at 0.01 mg/kg; p. 11; MRID 48714001, p. 12). The LOD was reported as 0.002 mg/kg in the ECM and as 0.003 mg/kg in the ILV (p. 10; Appendix 1, p. 52).

II. Recovery Findings

Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of flutolanil in soil (Table 3, p. 23; MRID 48714001, Table 1, p. 29).

Table 2. Initial Validation Method Recoveries for Analyte in Soil

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Flutolanil	0.01 (LOQ)	5	81.6-97.7	92.0	6.7	7.3
	0.1	5	76.6-92.7	86.6	6.3	7.3

Data were obtained from Table 3, p. 23 in the study report.

Table 3. Independent Validation Method Recoveries for Analyte in Soil

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Flutolanil	0.01 (LOQ)	5	65-84	77	7.4	9.7
	0.1	5	73-87	78	5.5	7.0

Data were obtained from MRID 48714001, Table 1, p. 29.

III. Method Characteristics

For the ECM, the LOQ was set at the low fortification level of the study and the LOD was determined from the lowest calibration level (0.2 µg/L; pp. 10, 19-20). For the ILV, the method LOQ was not independently determined and the LOD was defined as 0.3 x LOQ (MRID 48714001, p. 12; Appendix 1, p. 52). The determination of the LOD/LOQ were not based on scientifically acceptable procedures as defined in 40 CFR Part 136.

Table 4. Method Characteristics

	Flutolanil
Limit of Quantitation (LOQ)	0.01 mg/kg
Limit of Detection (LOD)	0.003 mg/kg
Linearity (calibration curve r^2)	$r^2 = 0.9998$[1]
Linearity Concentration Range	0.2-50 µg/kg
Repeatable	Yes
Reproducible	Yes[2]
Specific	Yes

Data were obtained from MRID 48714001, p. 12; Figure 7, p. 37; Appendix 1, p. 52.
1 Reviewer-calculated from an r value of 0.9999.
2 Mean recovery was satisfactory at the LOQ proposed in the ECM; however, a LOQ was not independently calculated by the ILV.

IV. Method Deficiencies and Reviewer's Comments

1. The estimation of the LOD was not based on scientifically acceptable procedures as defined in 40 CFR Part 136. LOD was estimated from the lowest calibration level (ECM) or as 0.3 x LOQ (ILV) and LOQ was set at the low fortification level of the study (pp. 19-20; MRID 48714001, Appendix 1, p. 52). 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. It is reported that the maximum residue limit (MRL) for flutolanil in soil is proposed to be 0.05 mg/kg and the LOQ was set at 0.01 mg/kg.

2. The sample spiking procedure was not reported.

3. Example calculations were not provided showing how the raw data were converted to a final concentration. The reviewer was able to verify the recoveries for the ECM using example calculations provided in the ILV (MRID 48714001, pp. 22-24).

4. Validation sample sets (ECM or ILV) did not include a reagent blank.

5. Method clarifications were made via email exchange prior to the start of the ILV (MRID 48714001, Appendix 7, pp. 107-130); however, the reviewer notes that the email correspondence indicates a request for a phone conversation during the conduct of the ILV (MRID 48714001, Appendix 7, p. 128). If the requested phone conversation occurred, it was not documented what was discussed. All communications between the developer of the method and the ILV should be logged and it documented that such communication did not compromise the independent evaluation.

6. Data reported for the ILV are the results of a second trial (MRID 48714001, p. 11). The study author stated that the first trial was successful (raw data reported in MRID 48714001, Appendix 8, p. 134); however, was not reported because an in-process QA audit had not yet been performed.

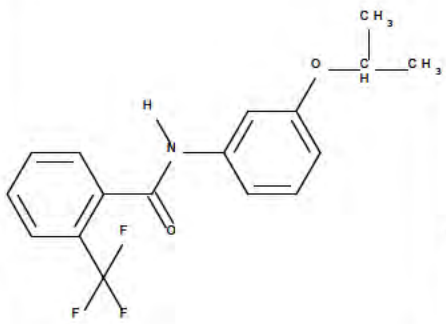
7. It was reported for the ILV that a single analyst completed a sample set consisting of 13 samples in *ca*. 8 hours (MRID 48714001, p. 11).

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.

DER ATTACHMENT 1. Flutolanil and Its Environmental Transformation Products. [A]

Code Name/ Synonym	Chemical Name	Chemical Structure	Study Type	MRID	Maximum %AR (day)	Final %AR (study length)
PARENT						
Flutolanil	**IUPAC:** α,α,α-Trifluoro-3′-isopropoxy-*o*-toluanilide **CAS:** *N*-[3-(1-methylethoxy)phenyl]-2-(trifluoromethyl)benzamide **CAS No.:** 66332-96-5 **Formula:** $C_{17}H_{16}F_3NO_2$ **MW:** 323.3 g/mol **SMILES:** C(F)(F)(F)c1ccccc1C(=O)Nc2cccc(OC(C)C)c2		850.6100 ECM soil	48763101	NA	NA
			850.6100 ILV soil	48714001		
			850.6100 ECM & ILV water	48796401		
MAJOR (>10%) TRANSFORMATION PRODUCTS						
No major transformation products were identified.						
MINOR (<10%) TRANSFORMATION PRODUCTS						
No minor transformation products were identified.						
REFERENCE COMPOUNDS NOT IDENTIFIED						
All compounds used as reference compounds were identified.						

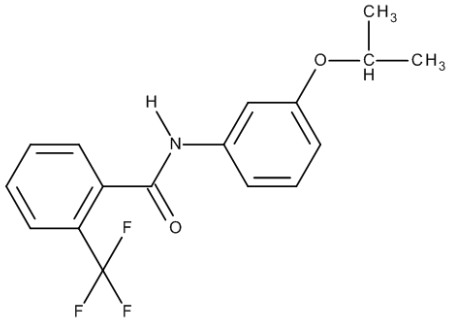
[A] AR means "applied radioactivity". MW means "molecular weight". NA means "not applicable". ECM means "environmental chemical methods". ILV means "independent laboratory validation".

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

ENVIRONMENTAL CHEMISTRY METHOD (ECM)
STANDARD EVALUATION PROCEDURE (SEP) CHECKLIST:
BACKGROUND AND INITIAL REVIEW INFORMATION

All page citations refer to MRID 48763101 unless noted otherwise.

I. Background Information

A.	**Title of Method**	Validation of Analytical Method for Flutolanil in Soil.	
B.	**ECM No.**	[*BEAD ECB*]	
C.	**MRID No.**	48763101	
D.	**Matrix**	Soil	
E.	**Analyte(s) detected**	**Compound:**	
		Common name:	Flutolanil (p. 12; MRID 48714001, p. 13).
		IUPAC name:	α,α,α-trifluoro-3'-isopropoxy-*o*-toluanilide.
		CAS name:	N-[3-(1-methylethoxy)phenyl]-2-(trifluoromethyl) benzamide.
		CAS No:	66332-96-5.
		Synonyms:	Not reported.
			

Information obtained from p. 12 of the study report and MRID 48714001, p. 13.

II. Information about the Laboratory

A.	**Name**	Nihon Nohyaku Co., Ltd. (p. 1).
B.	**Address**	Research Center, Nihon Nohyaku Co., Ltd., 345 Oyamadacho, Kawachinagano, Osaka, Japan.
C.	**Telephone No.**	Not reported.

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

D.	**Name of the Study Director**	T. Ihara (p. 1).
E.	**Name of the Lead Chemist**	A. Nakamura (p. 6).
F.	**Laboratory Validation:**	Yes.

Information obtained from pp. 1, 6 of the study report.

III. Method Summary Information for Analyte(s): **Flutolanil.**

A.	**Statement of Data Confidentiality**	Yes (p. 2).
1.	**Is the Method Classified or Confidential?**	No claim of confidentiality is made for any information contained in the study on the basis of its falling within the scope of FIFRA 10 (d)(1)(A),(B) or (C).
2.	**Submitted Prior to 2008 with a Non-Standard Claim of Confidentiality?**	No.
B.	**Sample Preparation**	Soil (20 g) is fortified with a standard solution of flutolanil, in acetonitrile; no further details provided (p. 14; Appendix III, p. 28).
C.	**Sample Extraction**	50 mL of acetonitrile is added and the sample shaken vigorously for 15 minutes, followed by centrifugation at 2500 rpm for 5 minutes. The sample was then extracted a second time with 20 mL of acetonitrile and the supernatant was filtered under slight suctioning. The combined extracts were brought to 100 mL with acetonitrile (p. 15).
D.	**Sample Cleanup**	An aliquot (12.5 mL) of the extract was combined with 3 g NaCl and 12.5 mL phosphate buffer solution (0.1 M, pH 7) and shaken. The upper organic layer was withdrawn, evaporated *in vacuo* and the residue dissolved with 3 mL of acetonitrile:distilled water (4:1, v:v) and loaded onto a carbon graphite/aminopropyl silica gel cartridge column conditioned with 5 mL each of acetonitrile and acetonitrile: distilled water (4:1, v:v). The cartridge column was washed with 2 mL each of acetonitrile:distilled water (4:1, v:v) and acetonitrile, and the analyte was eluted from the column with 5 mL of acetonitrile:acetic acid (95:5, v:v) and diluted to 25 mL with distilled water (p. 15).
E.	**Sample Derivatization (if applicable)**	None.

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

<table>
<tr><td>F.</td><td>Sample Analysis</td><td colspan="5">LC/MS/MS (p. 10).</td></tr>
<tr><td>1.</td><td>Instrumentation</td><td colspan="5">ECM: Agilent 1200 HPLC with Applied Biosystems/MSD Sciex 3200Q trap triple quadrupole mass spectrometer (pp. 15-17).
ILV: Shimadzu LC-20AD HPLC system with Applied Biosystems API 4000 mass spectrometer (MRID 48714001, p. 15).</td></tr>
<tr><td>2.</td><td>Primary Column</td><td colspan="5">Cadenza CD-C18 (2.0 mm x 50 mm, 3 µm ODS (p. 15 and MRID 48714001, p. 20).</td></tr>
<tr><td>3.</td><td>Confirmatory Column (if any)</td><td colspan="5">None.</td></tr>
<tr><td>4.</td><td>Detector</td><td colspan="5">Multiple Reaction Monitoring (MRM; p. 13).</td></tr>
<tr><td>5.</td><td>Other Confirmatory Techniques (if any)</td><td colspan="5">None.</td></tr>
<tr><td>6.</td><td>Other Relevant Information</td><td colspan="5">LC conditions: gradient mobile phase combining (A) distilled water containing 0.1% formic acid and (B) methanol containing 0.1% formic acid [percent A:B at 0.0 min. 50:50 (v:v), 0.5 min. 30:70, 5.5 min. 0:100, 7.5 min. 0:100], column temperature 40ºC, injection volume 10 µL, flow rate 0.20 mL/minute (pp. 15-16).[1]
<table>
<tr><td rowspan="2">Compound</td><td colspan="3">Ions monitored (m/z)</td><td rowspan="2">Retention time (min., ca.)</td></tr>
<tr><td>Primary</td><td colspan="2">Secondary</td></tr>
<tr><td>Flutolanil</td><td>324→ 262</td><td>324→ 242</td><td>324→ 282</td><td>ECM: ca. 5.9 (Figure 3, p. 26).
ILV: 4.3 (MRID 48714001, p. 21)</td></tr>
</table></td></tr>
<tr><td>G.</td><td>Detection and Quantitation Limits</td><td colspan="5"></td></tr>
<tr><td>1.</td><td>Limit of Quantitation (LOQ)</td><td colspan="5"></td></tr>
<tr><td></td><td>Claimed in Method</td><td colspan="2">ECM: 0.01 mg/kg.
ILV: 0.01 mg/kg.</td><td>Estimated</td><td colspan="2">ECM: p. 20.
ILV: MRID 48714001, p. 26.</td></tr>
<tr><td>2.</td><td>Limit of Detection (LOD)</td><td colspan="5"></td></tr>
</table>

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

	Claimed in Method	ECM: 0.002 mg/kg. ILV: 0.003 mg/kg.	**Estimated**	ECM: pp. 10, 20. ILV: MRID 48714001, Appendix 1, p. 52	
H.	**Recovery (Accuracy)/Precision Data; expressed as percentage of applied (n = 5)[2]**				
	Spiking Level (mg a i./kg)	**Parameter**	**Flutolanil**		
	0.01 (LOQ)	**Range**	81.6-97.7		
		Mean	92.0		
		SD	6.7		
		RSD	7.3		
	0.1	**Range**	76.6-92.7		
		Mean	86.6		
		SD	6.3		
		RSD	7.3		

Information obtained from pp. 2, 10, 20; Table 2, p. 11; pp. 13-17; Appendix III, p. 28; and Figure 3, p. 26 of the study report and MRID 48714001, pp. 15, 20-21, 26.
1 The ILV extended the mobile phase gradient to 8.0-11.0 min, with A:B at 50:50 (MRID 48714001, p. 21).
2 Soil obtained from Kochi Station, Kochi, Japan and sieved through 3360 µm sieve (pp. 12-13).

IV. Detailed Information about the Method

		YES	NO	REVIEW FURTHER
A.	**Does the method require spiking with the analytes(s) of interest?**	x		p. 14
B.	**If the method requires explosive or carcinogenic reagents, are proper precautions explained?**			Not applicable.
C.	**Is the following information supplied?**			
1.	**Detailed stepwise description of:**			
a.	**The sample preparation procedure?**	x		pp. 12-13
b.	**The sample spiking procedure?**		x	p. 14
c.	**The extraction procedure?**	x		p. 15
d.	**The derivatization procedure?**			Not applicable.
e.	**The clean-up procedure?**	x		p. 15
f.	**The analysis procedure?**	x		pp. 15-16

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

		YES	NO	REVIEW FURTHER
2.	**Procedures for:**			
a.	**Preparation of standards?**	x		p. 14
b.	**Calibration of instrument?**	x		p. 14
3.	**List of glassware and chemicals**	x		p. 17
a.	**Are sources recommended?**		x	
b.	**Are they commercially available?**	x		
4.	**Name, model, *etc.*, of the instrument, column, detector, *etc.*, used?**	x		pp. 15-17
a.	**Are sources recommended?**	x		
b.	**Are they commercially available?**	x		
5.	**LOD**			
a.	**Is there an explanation of how it was calculated?**	ECM ILV		ECM: Determined from lowest calibration level (p. 20). ILV: Defined as 0.3 x LOQ (MRID 48714001, Appendix 1, p. 52).
b.	**Is it a scientifically accepted procedure?**		x	
c.	**Is the matrix blank free of interference(s) at the retention time, wavelength, *etc.*, of the analyte(s) of interest?**	ECM ILV		ECM: Figure 3, p. 26; Appendix V, p. 31. ILV: MRID 48714001, Figure 8, p. 38.
6.	**LOQ**			
a.	**Is there an explanation of how it was calculated?**	x		Lowest fortification level (p. 19).
b.	**Is it a scientifically accepted procedure?**		x	
7.	**Precision and accuracy data**			
a.	**Were there an adequate number of spiked samples analyzed?**	x		Table 3, p. 23

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

		YES	NO	REVIEW FURTHER
b.	**Are the mean recoveries between 70-120%?**	x		Table 3, p. 23
c.	**Are the RSDs of the replicates 20% or less at or above the LOQ?**	x		Table 3, p. 23
8.	**Description and/or explanation of:**			
a.	**Areas where problems may be encountered?**		x	
b.	**Steps that are critical?**		x	
c.	**Interferences that may be encountered?**		x	
9.	**Characterization of the Matrix(ces)?**	ECM ILV		ECM: Table 1, p. 22. ILV: MRID 48714001, p. 14.

Information obtained from pp. 12-16, 19-20; Table 3, p. 23; Figure 3, p. 26; and Appendix V, p. 31 of the study report and MRID 48714001, p. 14; Figure 8, p. 38; Appendix 1, p. 52.

V. Representative Chromatograms

		YES	NO	REVIEW FURTHER
A.	**Are there representative chromatograms for:**			
1.	**Analyte(s) in each matrix at the LOQ and 10 x LOQ?**	ECM ILV		ECM: Figure 3, p. 26. ILV: MRID 48714001, Figures 9-10, pp. 39-40.
2.	**Method blanks?**		ECM ILV	
3.	**Matrix blanks?**	ECM ILV		ECM: Figure 3, p. 26. ILV: MRID 48714001, Figure 8, p. 38.

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

4.	**Standard curves?**	ECM ILV		ECM: Figure 2, p. 25. ILV: MRID 48714001, Figure 7, p. 37
a.	**Do the standard curves have acceptable linearity?**	ECM ILV		ECM: r = 0.9999; r^2 = 0.9998 (Table 2, p. 23; Figure 2, p. 25). ILV: r = 0.9999; r^2 = 0.9998 (MRID 48714001, Figure 7, p. 37).
5.	**Standards that can be used to recalculate some of the values for analyte(s) in the sample chromatograms?**	ECM[1] ILV		ECM: Appendix VI, p. 32. ILV: MRID 48714001, pp. 22-24; Appendix 4, p. 98.
B.	**Can the responses of the analytes(s) in the chromatograms of the lowest spiking level be accurately measured?**	ECM ILV		ECM: Figure 3, p. 26. ILV: MRID 48714001, Figure 9, p. 39.

Information obtained from Table 2, p. 23; Figures 2-3, pp. 25-26; and Appendix VI, Table VI, p. 32 of the study report and MRID 48714001, Figures 7-10, pp. 37-40.
1 Could not be verified using the calculations reported on pp. 17-18 of the study report; however, were verified using calculations reported in the ILV (MRID 48714001, pp. 22-24).

VI. Good Laboratory Practice (GLP) Standards

		YES	**NO**	**REVIEW FURTHER**
A.	**Is there a statement of adherence to the FIFRA GLP standards?**	ILV	ECM	ECM: JMAFF and OECD GLP (p. 3).

Information obtained from p. 3 of the study report and MRID 48714001, p. 3

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

VII. Independent Lab Validation (ILV)

		YES	NO	REVIEW FURTHER
A.	**Was an ILV performed?**	x		
B.	**Was the validation independent?**			See comment (MRID 48714001, p. 25; Appendix 7, pp. 107-132).
C.	**Did the ILV's precision/accuracy data meet the criteria established in OPPTS Guideline 850.6100?**	x		MRID 48714001, pp. 25, 29.
D.	**Were recommendations of major or minor modifications to the method made by the independent lab performing the ILV? If major modifications were suggested, what were they?**		x	

E.	**Recovery (Accuracy)/Precision Data; expressed as percentage of applied (n = 5)**		
	Spiking Level (ppm)	**Analyte**	**Flutolanil**
	0.01 (LOQ)	**Range**	65-84
		Mean	77
		SD	7.4
		RSD	9.7
	0.1	**Range**	73-87
		Mean	78
		SD	5.5
		RSD	7.0

Information obtained from MRID 48714001, pp. 25, 29.

VIII. Completeness

		YES	NO	REVIEW FURTHER
A.	**Has enough information been supplied to do a proper review?**	x		

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

		YES	NO	REVIEW FURTHER
B.	**Has enough information been supplied to do a laboratory evaluation, if requested?** [*BEAD ECB.*]			
C.	**Are all steps in the method scientifically sound?**	x		
D.	**Is a confirmatory method or technique provided?**			LC/MS is used as primary method.
E.	**Check the category below which best describes this ECM.**			
1.	**Satisfactory** [*Agency determination*]			Supplemental
2.	**Major Deficiencies**	x		See section ***IX. Recommendations*** below.
3.	**Minor Deficiencies**	x		See section ***IX. Recommendations*** below.

The study is considered supplemental for residues at the reported LOQ of 0.01 mg/kg and 10 LOQ.

IX. Recommendations

1. For the ECM:

   a) The estimation of the LOD was not based on scientifically acceptable procedures as defined in 40 CFR Part 136. LOD was estimated from the lowest calibration level and LOQ was set at the low fortification level of the study (pp. 19-20). Detection limits should not be based on the arbitrarily selected lowest concentration in the spiked samples.

   b) Example calculations were not provided showing how the raw data were converted to a final concentration. The reviewer was able to verify the recoveries from the raw data using the equations reported in the ILV (MRID 48714001, pp. 22-24).

   c) The validation sample set did not include a reagent blank.

   d) The sample spiking procedure was not reported.

2. For the ILV:

Data reported for the ILV are the results of a second trial (MRID 48714001, p. 11). The study author stated that the first trial was successful (raw data reported in MRID 48714001, Appendix 8, p. 134); however, was not reported because an in-process QA audit had not yet been performed.

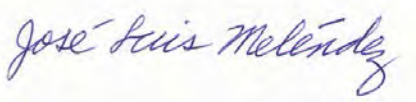
a) The estimation of the LOD was not based on scientifically acceptable procedures as defined in 40 CFR Part 136 and no justification for selection of the LOQ was provided. LOD was estimated at 0.3 x LOQ (MRID 48714001, Appendix 1, p. 52).

b) Method clarifications were made via email exchange prior to the start of the ILV (MRID 48714001, Appendix 7, pp. 107-130); however, the reviewer notes that the email correspondence indicates a request for a phone conversation during the conduct of the ILV (MRID 48714001, Appendix 7, p. 128). If the requested phone conversation occurred, it was not documented what was discussed. All communications between the developer of the method and the ILV should be logged and it documented that such communication did not compromise the independent evaluation.

c) The validation sample set did not include a reagent blank.

See signatures in the cover page of the DER.

Primary Reviewer: Cambridge Environmental



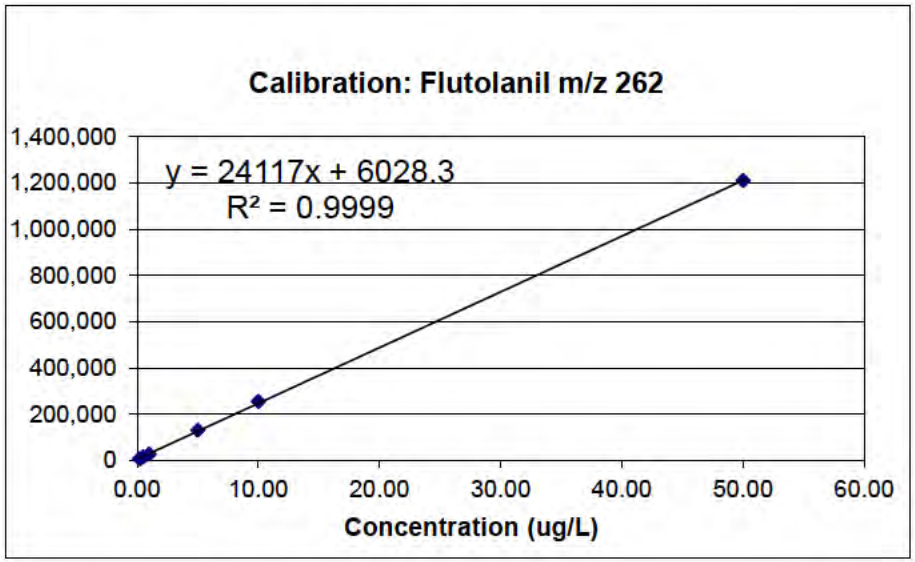
Secondary Reviewer: José L. Meléndez, Chemist

Chemical: Flutolanil **MRID: 48763101 and 48714001**
PC: 128975 **Guideline: 850.6100**

ECM calibration curve.

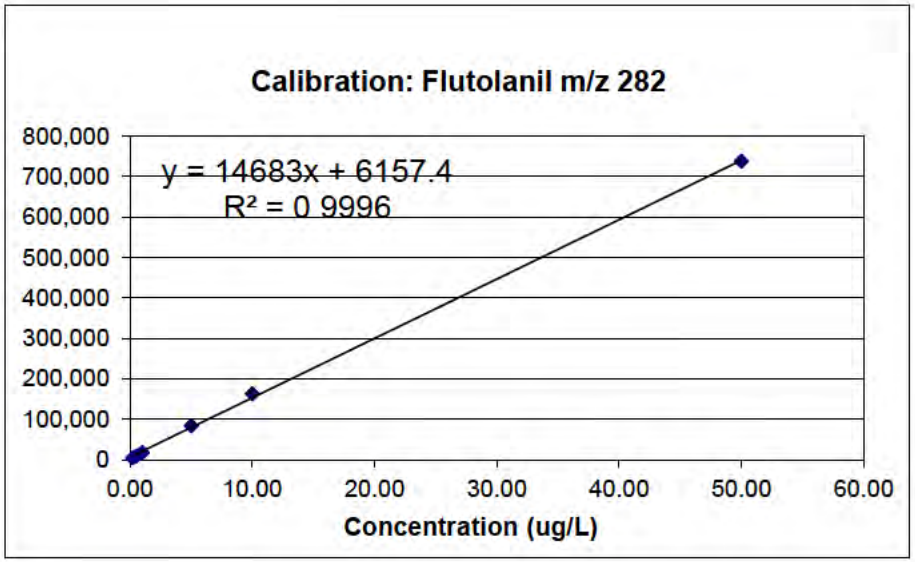
Concentration (ug/L)	Flutolanil 262 (Peak Area)	Flutolanil 242 (Peak Area)	Flutolanil 282 (Peak Area)
0.2	6590	7080	4220
0.5	15100	14500	9290
1	27100	28600	17900
5	131000	139000	83900
10	255000	256000	163000
50	1210000	1240000	738000

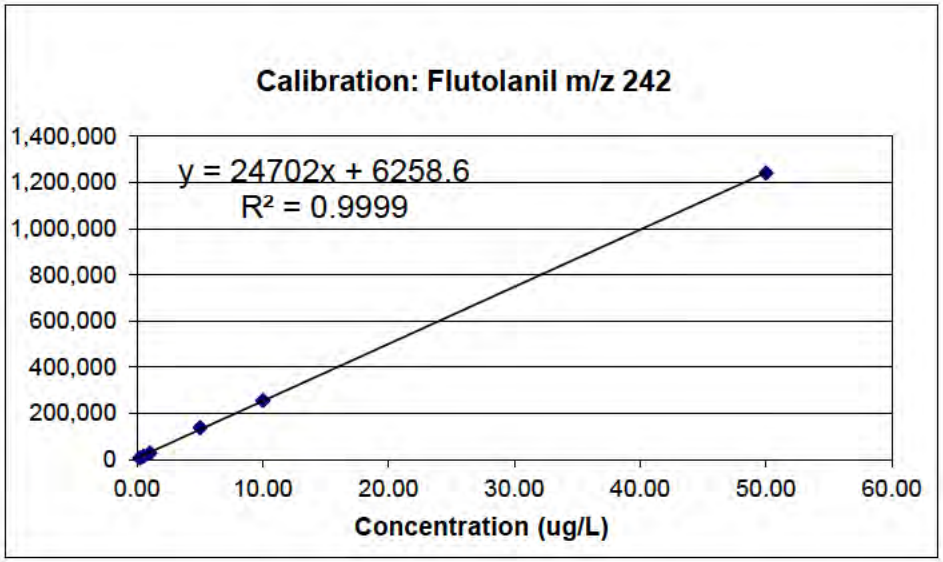
Results from Table 2, p. 23 of the study report.



Chemical: Flutolanil
PC: 128975

MRID: 48763101 and 48714001
Guideline: 850.6100





Chemical: Flutolanil **MRID: 48763101 and 48714001**
PC: 128975 **Guideline: 850.6100**

ECM validation for determination of flutolanil in soil.

ECM Recoveries

Analyte	Fortified (mg/kg)	Recovery (%)	Mean (%)	SD1 (%)	RSD2 (%)
Flutolanil	0.01	97.7			
		89.4			
		97.7			
		93.5			
		81.6	92.0	6.7	7.3
	0.1	91.5			
		92.7			
		76.6			
		85.7			
		86.5	86.6	6.4	7.3

Results from Table 3, p. 23 of the study report.
Means and standard deviations calculated using Microsoft program functions =AVERAGE(A1:A2) and =STDEV(A1:A2).
1 SD = Standard Deviation; determined using the “unbiased” or “n-1” method.
2 RSD = Relative Standard Deviation; calculated as (SD/mean) x 100.

Chemical: Flutolanil **MRID: 48763101 and 48714001**
PC: 128975 **Guideline: 850.6100**

ECM: Verification of recoveries using chromatogram Peak Area and calibration curve regression equations (quantitation ion).

Fortified (ug/kg)	Analyte	Ion m/z	Sample	Peak Area counts	Reviewer		Reported Recovery (%)
					Measured (ug/kg)	Recovery (%)	
10	Flutolanil	262	L1	23700	9.765021	97.7	97.7
10			L2	21700	8.940969	89.4	89.4
10			L3	23700	9.765021	97.7	97.7
10			L4	22700	9.352995	93.5	93.5
10			L5	19800	8.158119	81.6	81.6
100		262	H1	222000	91.469821	91.5	91.5
100			H2	225000	92.705900	92.7	92.7
100			H3	186000	76.636877	76.6	76.6
100			H4	208000	85.701454	85.7	85.7
100			H5	210000	86.525506	86.5	86.5

Chromatogram Peak Area counts and reported recovery from Appendix VI, Table VI, p. 32 of the study report.
Calibration parameters (slope and y-intercept) reported in Table 2, p. 23.
Recoveries could not be verified using example calculations reported in p. 17;
however, were verified using the example calculations provided in the ILV (MRID 48714001, pp. 22-24).

Chemical: Flutolanil **MRID: 48763101 and 48714001**
PC: 128975 **Guideline: 850.6100**

ILV validation for determination of flutolanil in soil.

ILV Recoveries

Analyte	Fortified (ppm)	Recovery (%)	Mean (%)	SD1 (%)	RSD2 (%)
Flutolanil	0.01	81			
		65			
		84			
		79			
		74	76.6	7.4	9.7
	0.1	87			
		77			
		75			
		80			
		73	78.4	5.5	7.0

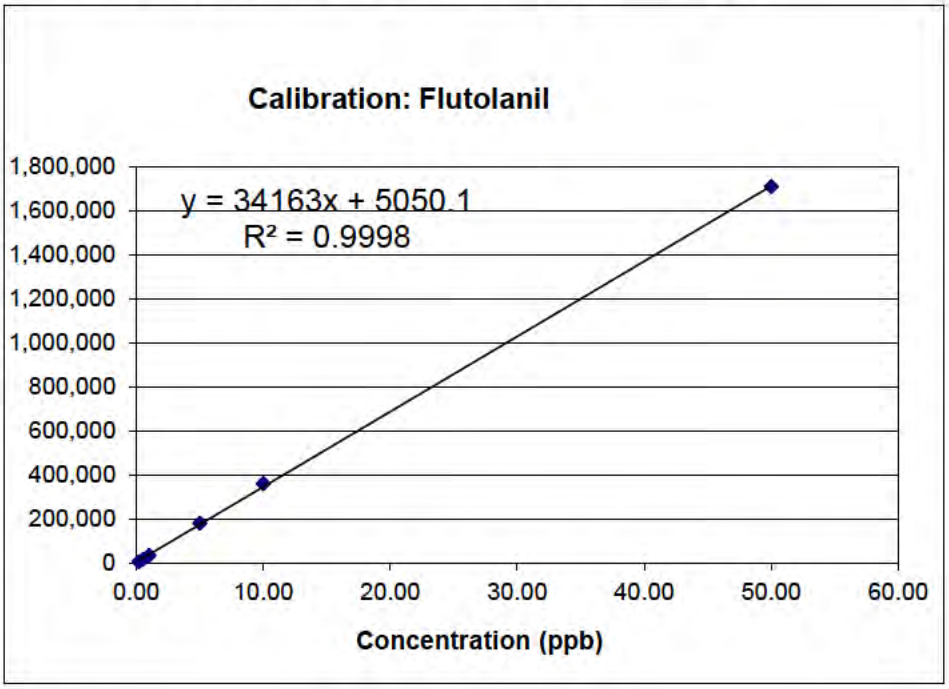
Results from MRID 48714001, Table 1, p. 29 of the study report.
Means and standard deviations calculated using Microsoft program functions =AVERAGE(A1:A2) and =STDEV(A1:A2).
1 SD = Standard Deviation; determined using the “unbiased” or “n-1” method.
2 RSD = Relative Standard Deviation; calculated as (SD/mean) x 100.
Value outside the recommended range of 70-12%.

Chemical: Flutolanil **MRID: 48763101 and 48714001**
PC: 128975 **Guideline: 850.6100**

ILV calibration curve.

Concentration (ppb)	Flutolanil (Peak Area)
0.2	5,880
0.5	16,400
1	34,700
5	181,000
10	361,000
50	1,710,000

Results from MRID 48714001, Figure 7, p. 37 of the study report.



Chemical: Flutolanil **MRID: 48763101 and 48714001**
PC: 128975 **Guideline: 850.6100**

ILV: Verification of recoveries using chromatogram Peak Area and calibration curve regression equations.

Fortified (ug/kg)	Analyte	Sample	Peak Area counts	Reviewer		Reported Recovery (%)
				Measured (ug/kg)	Recovery (%)	
10	Flutolanil	11	27700	8.099415	81.0	81.0
10		12	22300	6.520468	65.2	65.0
10		13	28600	8.362573	83.6	84.0
10		14	27100	7.923977	79.2	79.0
10		15	25200	7.368421	73.7	74.0
100		16	297000	86.842105	86.8	87.0
100		17	262000	76.608187	76.6	77.0
100		18	258000	75.438596	75.4	75.0
100		19	275000	80.409357	80.4	80.0
100		20	251000	73.391813	73.4	73.0

Based on the calculations reported in pp. 22-24, calibration parameters reported in Figure 7, p. 37 and raw data reported in Appendix 4, p. 98 of the study report.

Peak area counts confirmed by chromatograms (Figures 9-10, pp. 39-40).