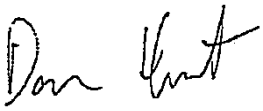
Test Material:	Flutolanil
MRID:	48796401
Title:	Independent laboratory validation of the flutolanil analytical method described in Nihon Nohyaku Co., Ltd. Final report no. LSRC-A11-010A, study protocol no. GE-04, 11-0001, entitled "Validation of analytical method for flutolanil in water."
EPA PC Code:	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 water

Document No.:	EPA MRID 48796401
Reports:	Willoh, J.M. 2012. Independent laboratory validation of the flutolanil analytical method described in Nihon Nohyaku Co., Ltd. Final report no. LSRC-A11-010A, study protocol no. GE-04, 11-0001, entitled "Validation of analytical method for flutolanil in water." Report prepared by Morse Laboratories, LLC, Sacramento, California, sponsored and submitted by Nihon Nohyaku Co. Ltd., Tokyo, Japan; 118 pages. Final report issued April 6, 2012.
Guideline:	850.6100
Statements:	ECM: The study was conducted in accordance with the OECD and JMAFF Good Laboratory Practice (GLP) standards (Appendix 2, p. 59). Signed and dated GLP and Quality Assurance statements were provided (Appendix 2, pp. 59-60). A Certification of Authenticity Statement was provided with the Quality Assurance statement. An unsigned Data Confidentiality statement was provided (Appendix 2, p. 58). 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 classified as supplemental. The determination of the LOD/LOQ was not based on scientifically acceptable procedures; example calculations were incomplete; ECM validation sample sets did not include a reagent blank; and matrix characterization of the river water in the ECM was incomplete. The registrant should provide a separate ECM, with signed and dated Data Confidentiality statement.
PC Code:	128975

Final Reviewer:	Jose L. Melendez	**Signature:**	José Luis Meléndez
Title:	Chemist	**Date:**	08/06/2013

Executive Summary

This analytical method, Protocol No. GE-04, 11-0001, is designed for the quantitative determination of flutolanil in water using LC/MS/MS. The method is quantitative for flutolanil at the stated LOQ of 0.1 µg/L. The lowest toxicological level of concern in water was not reported. Since EU drinking water limit is 0.1 µg/L, the LOQ was set at that level. 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	48796401	48796401		Water	1/31/2011	Nihon Nohyaku Co. Ltd.	LC/MS/MS	0.1 µg/L

I. Principle of the Method

Samples (50 mL) were combined with 5 mL of acetonitrile and loaded to solid phase extraction (SPE) cartridges that were washed with 6 mL of acetonitrile and equilibrated with 6 mL of 10% aqueous acetonitrile prior to use (Appendix 2, p. 71). Cartridges were washed with 6 mL of 10% aqueous acetonitrile and flutolanil was eluted with 9 mL of acetonitrile. Eluate was dried under a stream of nitrogen and reconstituted with 5 mL of 50% acetonitrile in 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) 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, 12 min. 50:50] with MS/MS detection with Multiple Reaction Monitoring (MRM; Appendix 2, pp. 69, 72).

The LOQ was the same in the ECM and ILV (proposed at 0.1 µg/L; p. 12; Appendix 2, p. 69). The LOD was reported as 0.0013 µg/L in the ECM and as 0.03 µg/L in the ILV (Appendix 1, p. 50; Appendix 2, p. 67).

II. Recovery Findings

Mean recoveries and relative standard deviations (RSD) were within guideline requirements (mean 70-120%; RSD ≤20%) for analysis of flutolanil in distilled water (ECM) and river water (ECM and ILV; Table 1, p. 27; Appendix 2, Tables 2-3, pp. 77-78).

Table 2. Initial Validation Method Recoveries for Analyte in Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Distilled Water						
Flutolanil	0.1 (LOQ)	5	90.4-99.7	93.0	3.8	4.1
	1	5	97.6-99.3	98.4	0.7	0.8
River Water						
Flutolanil	0.1 (LOQ)	5	93.5-98.5	94.9	2.1	2.2
	1	5	98.7-103.7	101.4	2.1	2.0

Data were obtained from Appendix 2, Tables 2-3, pp. 77-78; Table IV-1, p. 84; Table IV-2, p. 85 in the study report, and reviewer-calculated.

Table 3. Independent Validation Method Recoveries for Analyte in River Water

Analyte	Fortification Level (µg/L)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Flutolanil	0.1 (LOQ)	5	79-95	84	6.7	8.0
	1	5	85-95	89	3.8	4.2

Data were obtained from Table 1, p. 27.

III. Method Characteristics

For the ECM, the LOQ was set at 0.1 µg/L (the EU drinking water limit) to satisfy testing requirements and the LOD (0.0013 µg/L) was estimated at 2 x the noise level at the retention time of flutolanil on the chromatogram of the control sample (Appendix 2, pp. 67, 69-70). For the ILV, the method LOQ was not independently determined and the LOD was defined as 0.3 x LOQ (p. 12; Appendix 1, p. 50). The determination of the LOD/LOQ was not based on scientifically acceptable procedures as defined in 40 CFR Part 136.

Table 4. Method Characteristics

	Flutolanil
Limit of Quantitation (LOQ)	0.1 µg/L
Limit of Detection (LOD)	0.03 µg/L
Linearity (calibration curve r^2 and concentration range)	$r^2 = 0.9994$[1] (0.2-50 µg/L)
Repeatable	Yes
Reproducible	Yes[2]
Specific	Yes

Data were obtained from p. 12; Table 1, p. 27; Figure 7, p. 35; Appendix 1, p. 50.
1 Reviewer-calculated from an r value of 0.9997.
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 ECM associated with this ILV was not submitted separately from the ILV. The registrant should submit the ECM with appropriately signed cover pages.

2. The estimation of the LOD was not based on scientifically acceptable procedures as defined in 40 CFR Part 136. In the ECM, LOD was estimated as the concentration that generated response 2 times greater than the noise level at the retention time of the analyte on the chromatogram of the control sample (Appendix 2, p. 70). If using this method, 40 CFR Part 136, Appendix B specifies for the use of 2.5-5 times noise. The LOQ was set at the EU drinking water limit of 0.1 µg/L and was not calculated (Appendix 2, p. 69). For the ILV, the LOD was defined as 0.3 x LOQ, which is also not a scientifically acceptable procedure. Additionally, the lowest toxicological level of concern in water was not reported. An LOQ above toxicological levels of concern results in an unacceptable method classification.

3. Example calculations were not provided showing how the raw data were converted to a final concentration and the reviewer was unable to verify the recoveries for the ECM from equations provided in Appendix 2, p. 74 and data provided in Appendix 2, pp. 84-85 using calibration parameters from Appendix 2 of the study report.

4. For the ECM, validation sample sets did not include a reagent blank.

5. Matrix characterization of the river water in the ECM was incomplete. Only source, pH, appearance and a qualitative assessment of suspended solids were reported (Appendix 2, p. 83).

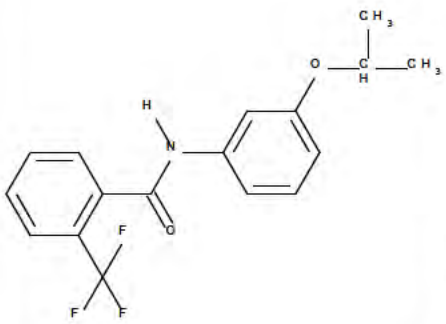
6. It was reported for the ILV that a single analyst completed a sample set consisting of 13 samples in *ca*. 6 hours (p. 11).

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.						

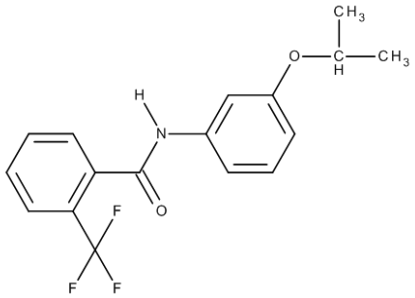
[A] AR means "applied radioactivity". MW means "molecular weight". NA means "not applicable". ECM means "environmental chemical methods". ILV means "independent laboratory validation".

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

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

The pages cited below appear in the bottom right corner of each page of the MRID.

I. Background Information

A.	**Title of Method**	Not reported.	
B.	**ECM No.**	[*BEAD ECB*]	
C.	**MRID No.**	48796401	
D.	**Matrix**	Water	
E.	**Analyte(s) detected**	**Compound:**	
		Common name:	Flutolanil (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. 13 of the study report.

II. Information about the Laboratory

A.	**Name**	Nihon Nohyaku Co., Ltd. (Appendix 2, p. 57).
B.	**Address**	Research Center, Nihon Nohyaku Co., Ltd., 345 Oyamadacho, Kawachinagano, Osaka, Japan.
C.	**Telephone No.**	Not reported.
D.	**Name of the Study Director**	Y. Nishimura (Appendix 2, p. 57).

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

E.	**Name of the Lead Chemist**	Y. Nishimura (Appendix 2, p. 62).
F.	**Laboratory Validation:**	Yes.

Information obtained from Appendix 2, pp. 57, 62 of the study report.

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

A.	**Statement of Data Confidentiality**	Yes (Appendix 2, p. 58).
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**	Water (50 mL) is fortified with 0.1 mL, of a standard solution of flutolanil (50 or 500 µg/L) in acetonitrile, to give final flutolanil concentrations of 0.1 and 1.0 µg/L (Appendix 2, p. 71).
C.	**Sample Extraction**	None.
D.	**Sample Cleanup**	Aqueous samples (50 mL) were combined with 5 mL of acetonitrile and loaded to solid phase extraction (SPE) cartridges that were washed with 6 mL of acetonitrile and equilibrated with 6 mL of 10% aqueous acetonitrile prior to use. Cartridges were washed with 6 mL of 10% aqueous acetonitrile and flutolanil was eluted with 9 mL of acetonitrile. Eluate was dried under a stream of nitrogen and reconstituted with 5 mL of 50% acetonitrile in water (Appendix 2, p. 71).
E.	**Sample Derivatization (if applicable)**	None.
F.	**Sample Analysis**	LC/MS/MS (Appendix 2, p. 73).
1.	**Instrumentation**	ECM: Agilent 1200 HPLC with Applied Biosystems/MSD Sciex 3200Q trap triple quadrupole mass spectrometer (Appendix 2, p. 73). ILV: Applied Biosystems/Sciex API 4000 LC/MS/MS System (p. 19).
2.	**Primary Column**	Cadenza CD-C18 (2.0 mm x 50 mm, 3 µm ODS (p. 19; Appendix 2, p. 72).
3.	**Confirmatory Column (if any)**	None.

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

4.	**Detector**	ECM: Multiple Reaction Monitoring (MRM; Appendix 2, p. 69). ILV: Tandem mass spectrometric detection (p. 19).				
5.	**Other Confirmatory Techniques (if any)**	None.				
6.	**Other Relevant Information**	LC conditions: gradient mobile phase combining (A) 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, 12 min. 50:50], column temperature 40ºC, injection volume 20 µL, flow rate 0.20 mL/minute (Appendix 2, p. 72)[1].				
		Compound	Ions monitored (m/z)			Retention time (min., *ca.*)
			Primary	Secondary		
		Flutolanil	324.1 → 262.1[2]	324.1 → 242.0	324.1 → 282.1	ECM: *ca.* 4.6 (Appendix 2, p. 75). ILV: ~2.3 (p. 20)
G.	**Detection and Quantitation Limits**					
1.	**Limit of Quantitation (LOQ)**					
	Claimed in Method	ECM: 0.1 µg/L. ILV: 0.1 µg/L.			ECM: Appendix 2, p. 69. ILV: p. 12.	
2.	**Limit of Detection (LOD)**					
	Claimed in Method	ECM: 0.0013 µg/L. ILV: 0.03 µg/L.			ECM: Appendix 2, p. 67. ILV: Appendix 1, p. 50	

H. Recovery (Accuracy)/Precision Data; expressed as percentage of applied (n = 5)

Spiking Level (µg/L)	Parameter	Flutolanil	
		Distilled Water	River Water[3]
0.1 (LOQ)	Range	90.4-99.7	93.5-98.5
	Mean	93.0	94.9
	SD	3.8	2.1
	RSD	4.1	2.2
1	Range	97.6-99.3	98.7-103.7
	Mean	98.4	101.3
	SD	0.7	2.1
	RSD	0.8	2.0

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

Information obtained from pp. 12, 19-20; Appendix 2, pp. 58, 67, 69, 71-73, 75; Tables 2-3, pp. 77-78; and pp. 84-85 of the study report.
1 For the ILV, the injection volume was 2 µL (p. 20).
2 For the ECM; the reviewer assumed that m/z 262.1 was the primary ion based on Appendix 2, Figure 8, p. 79 of the study report.
2 River water (pH 7.40) was obtained from Ishikawa River, Osaka, Japan on January 19, 2011 (Appendix 2, pp. 69, 83).

IV. Detailed Information about the Method

		YES	NO	REVIEW FURTHER
A.	**Does the method require spiking with the analytes(s) of interest?**	x		Appendix 2, p. 71
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?**			Not applicable.
b.	**The sample spiking procedure?**		x	Appendix 2, p. 71
c.	**The extraction procedure?**	x		Appendix 2, p. 71
d.	**The derivatization procedure?**			Not applicable.
e.	**The clean-up procedure?**	x		Appendix 2, p. 71
f.	**The analysis procedure?**	x		Appendix 2, pp. 72-73
2.	**Procedures for:**			
a.	**Preparation of standards?**	x		Appendix 2, p. 71
b.	**Calibration of instrument?**	x		Appendix 2, p. 71
3.	**List of glassware and chemicals**	x		Appendix 2, p. 73
a.	**Are sources recommended?**		x	
b.	**Are they commercially available?**	x		
4.	**Name, model, *etc.*, of the instrument, column, detector, *etc.*, used?**	x		Appendix 2, pp. 72-73
a.	**Are sources recommended?**	x		
b.	**Are they commercially available?**	x		

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

		YES	NO	REVIEW FURTHER
5.	**LOD**			
a.	**Is there an explanation of how it was calculated?**	ECM ILV		ECM: 2 x noise of the control sample (Appendix 2, p. 70). ILV: Defined as 0.3 x LOQ (Appendix 1, p. 50).
b.	**Is it a scientifically accepted procedure?**		ECM ILV	
c.	**Is the matrix blank free of interference(s) at the retention time, wavelength, *etc.*, of the analyte(s) of interest?**	ECM ILV		ECM: Appendix 2, Figures 3-4, p. 81. ILV: Figure 8, p. 36.
6.	**LOQ**			
a.	**Is there an explanation of how it was calculated?**			Set at the EU drinking water limit of 0.1 µg/L (Appendix 2, p. 69).
b.	**Is it a scientifically accepted procedure?**			Not applicable.
7.	**Precision and accuracy data**			
a.	**Were there an adequate number of spiked samples analyzed?**	x		Appendix 2, Tables 2-3, pp. 77-78
b.	**Are the mean recoveries between 70-120%?**	x		Appendix 2, Tables 2-3, pp. 77-78
c.	**Are the RSDs of the replicates 20% or less at or above the LOQ?**	x		Appendix 2, Tables 2-3, pp. 77-78
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	

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

		YES	NO	REVIEW FURTHER
9.	**Characterization of the Matrix(ces)?**	ECM[1] ILV		ECM: Appendix 2, p. 83. ILV: Appendix 4, p. 92.

Information obtained from Figure 8, p. 36; Appendix 1, p. 50; Appendix 2, pp. 69, 71; Tables 2-3, pp. 77-78; Figures 3-4, p. 81; and Appendix 4, p. 92 of the study report.
1 For the ECM, characterization of river water was incomplete. Parameters reported were: pH, appearance, suspended solids (Appendix 2, p. 83).

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: Appendix 2, Figures 3-4, p. 81. ILV: Figures 9-10, pp. 37-38.
2.	**Method blanks?**	ILV	ECM	ILV: Figure 11, p. 39.
3.	**Matrix blanks?**	ECM ILV		ECM: Appendix 2, Figures 3-4, p. 81. ILV: Figure 8, p. 36.
4.	**Standard curves?**	ECM ILV		ECM: Appendix 2, Figure 1, p. 79. Figure 7, p. 35.

a.	**Do the standard curves have acceptable linearity?**	ECM
		ILV
				ECM:
				r = 0.9998;
				r^2 = 0.9996 (Appendix 2, p. 77).
				ILV:
				r = 0.9997;
				r^2 = 0.9994 (Figure 7, p. 35).

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

5.	**Standards that can be used to recalculate some of the values for analyte(s) in the sample chromatograms?**	ILV	ECM[1]	ILV: pp. 20-22; Appendix 3, p. 90.
B.	**Can the responses of the analytes(s) in the chromatograms of the lowest spiking level be accurately measured?**	ECM ILV		ECM: Appendix 2, Figures 3-4, p. 81. ILV: Figure 9, p. 37.

Information obtained from pp. 20-22; Figures 7-11, pp. 35-39; and Appendix 2, Figure 1, p. 79; Figures 3-4, p. 81; and Appendix 3, p. 90 of the study report.
1 For the ECM, flutolanil recoveries could not be verified from the equations provided in Appendix 2, p. 74 and data provided in Appendix 2, pp. 84-85 using calibration parameters from Appendix 2, Figure 8, p. 79.

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 (Appendix 2, p. 59).

Information obtained from p. 3; Appendix 2, p. 59 of the study report.

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?**	x		Appendix 6, pp. 96-116).
C.	**Did the ILV's precision/accuracy data meet the criteria established in OPPTS Guideline 850.6100?**	x		Table 1, p. 27.
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)[1]

Spiking Level (µg/L)	Analyte	Flutolanil
0.1 (LOQ)	Range	79-95
	Mean	84
	SD	6.7
	RSD	8.0
1	Range	85-95
	Mean	89
	SD	3.8
	RSD	4.2

Information obtained from Table 1, p. 27 and Appendix 6, pp. 96-116 in the study report.
1 River water (pH 7.2; Ca: 5.4 ppm; Mg: 1.7 ppm; Na: 2.1 ppm; hardness: 21 mg eq. $CaCO_3$/L; conductivity: 0.05 mmhos/cm; TDS: 58 ppm; turbidity: 1.13 NTU; Appendix 4, p. 92).

VIII. Completeness

		YES	NO	REVIEW FURTHER
A.	**Has enough information been supplied to do a proper review?**	x		The ECM and ILV were submitted in the same document.

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**			Supplemental
2.	**Major Deficiencies**	x		See section ***IX. Recommendations*** below.
3.	**Minor Deficiencies**	x		See section ***IX. Recommendations*** below.

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 as the concentration that generated response 2 times greater than the noise level at the retention time of the analyte on the chromatogram of the control sample (Appendix 2, p. 70). If using this method, 40 CFR Part 136, Appendix B specifies for the use of 2.5-5 times noise. The LOQ was set at the EU drinking water limit of 0.1 µg/L and was not calculated (Appendix 2, p. 69).

   b) Example calculations were not provided showing how the raw data were converted to a final concentration and the reviewer was unable to verify the recoveries from the chromatograms and raw data.

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

   d) Matrix characterization of the river water was incomplete. Only source, pH, appearance and a qualitative assessment of suspended solids were reported (Appendix 2, p. 83).

e) A separate ECM and ILV should be provided to the Agency when performing such studies.

2. For the ILV:

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 (Appendix 1, p. 50).

See signatures in the cover page of the DER.

Primary Reviewer: Cambridge Environmental

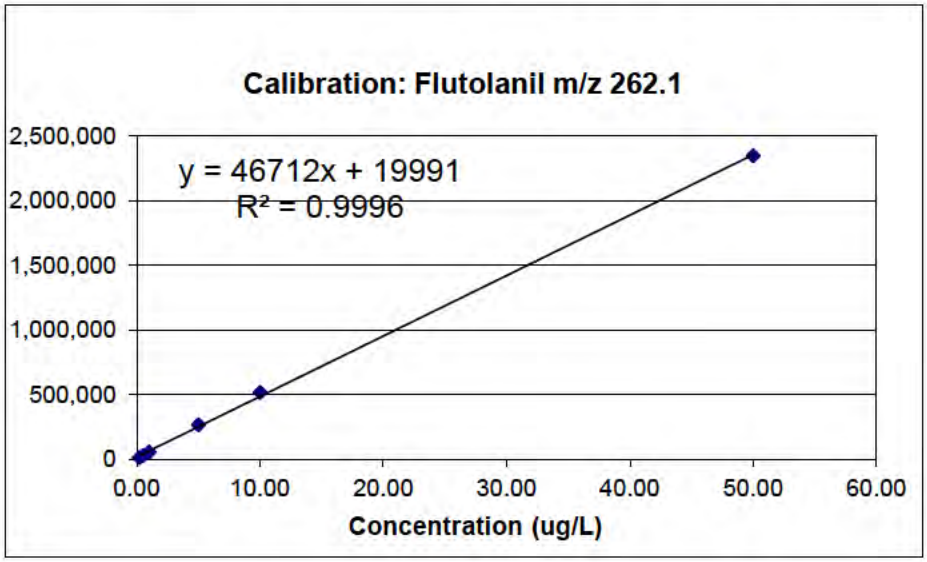
Secondary Reviewer: José L. Meléndez, Chemist

Chemical: Flutolanil **MRID: 48796401**
PC: 128975 **Guideline: 850.6100**

ECM calibration curve.

Concentration (ug/L)	Flutolanil 262.1 (Peak Area)
0.2	14050.95
0.5	29924.67
1	57509.21
5	267835.10
10	517943.70
50	2348377.00

Results from Appendix 2, Table 1, p. 77; Figure 8, p. 79 of the study report.



Chemical: Flutolanil **MRID: 48796401**
PC: 128975 **Guideline: 850.6100**

ECM validation for determination of flutolanil in water.

ECM Recoveries		**Distilled Water**				**River Water**			
Analyte	Fortified (ug/L)	Recovery (%)	Mean (%)	SD1 (%)	RSD2 (%)	Recovery (%)	Mean (%)	SD1 (%)	RSD2 (%)
Flutolanil	0.1	90.4				93.5			
		91.4				94.0			
		91.7				94.6			
		99.7				93.9			
		91.7	93.0	3.8	4.1	98.5	94.9	2.1	2.2
	1	97.6				98.7			
		99.0				103.0			
		98.0				103.7			
		97.9				101.4			
		99.3	98.4	0.7	0.8	100.0	101.4	2.1	2.0

Results from Appendix 2, Tables IV-1 to IV-2, pp. 84-85 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: 48796401**
PC: 128975 **Guideline: 850.6100**

ILV validation for determination of flutolanil in water.

ILV Recoveries

Analyte	Fortified (ug/L)	Recovery (%)	Mean (%)	SD1 (%)	RSD2 (%)
Flutolanil	0.1	80			
		86			
		80			
		95			
		79	84.0	6.7	8.0
	1	88			
		87			
		89			
		85			
		95	88.8	3.8	4.2

Results from Table 1, p. 27 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: 48796401**
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ILV calibration curve.

Set 3 - Method Validation Trial

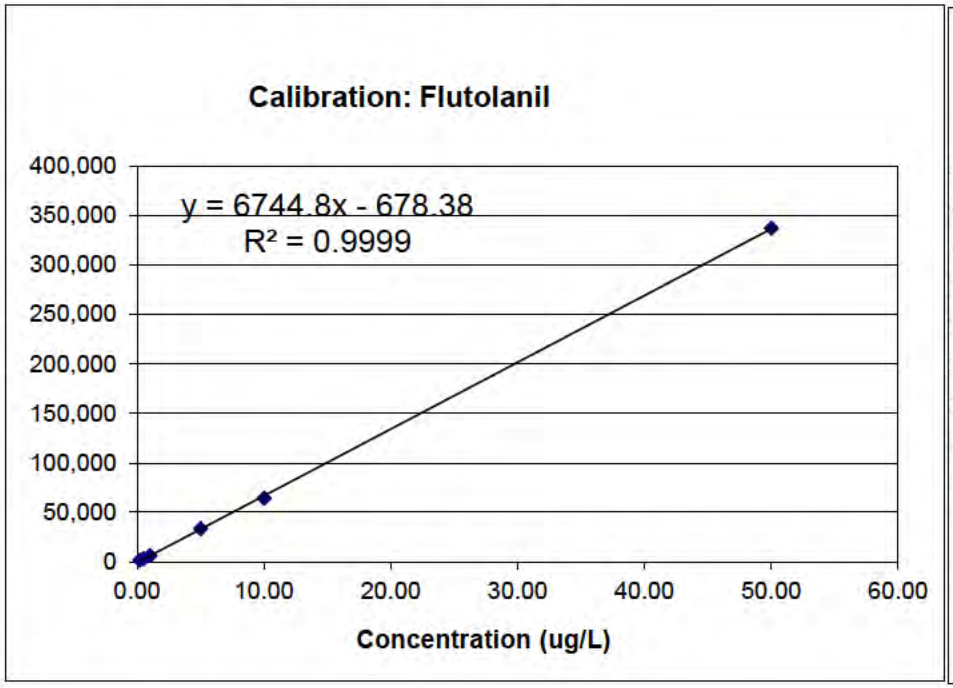
Concentration (ug/L)	Flutolanil (Peak Area)
0.2	1,660
0.5	3,090
1	6,260
5	33,500
10	64,300
50	337,000

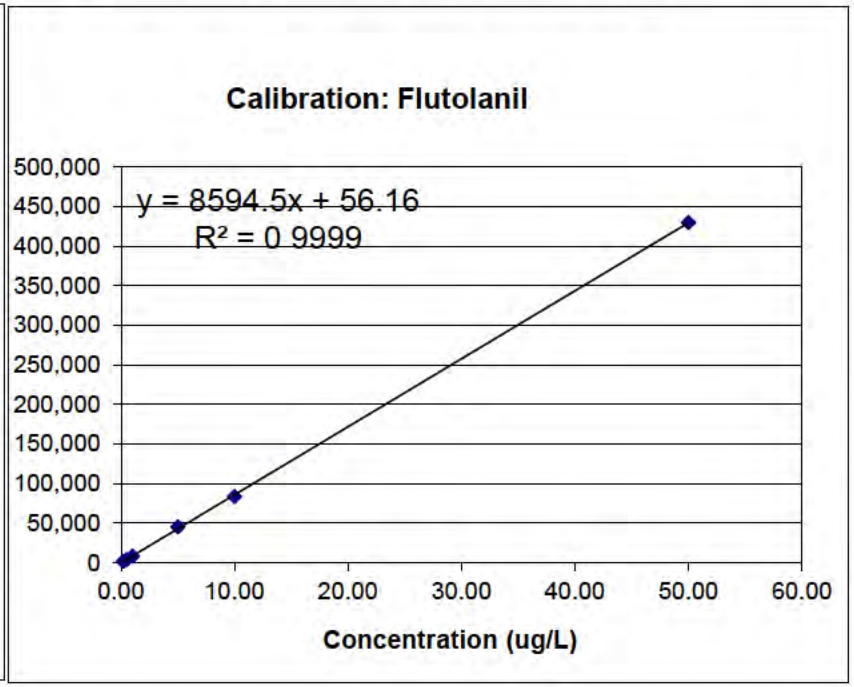
Results from Figure 7, p. 35 of the study report.

Set 2 - Control suitability

Concentration (ug/L)	Flutolanil (Peak Area)
0.2	1,930
0.5	4,110
1	8,450
5	45,300
10	83,800
50	430,000

Results from Appendix 3, p. 89 of the study report.





Chemical: Flutolanil **MRID: 48796401**
PC: 128975 **Guideline: 850.6100**

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

River Water

Fortified (ug/L)	Analyte	Sample	Peak Area counts	Reviewer		Reported Recovery (%)
				Measured (ug/kg)	Recovery (%)	
0.1	Flutolanil	1	5380	0.079660	79.7	80.0
0.1		2	5830	0.086406	86.4	86.0
0.1		3	5430	0.080409	80.4	80.0
0.1		4	6380	0.094652	94.7	95.0
0.1		5	5350	0.079210	79.2	79.0
1		6	58600	0.877561	87.8	88.0
1		7	58200	0.871564	87.2	87.0
1		8	59700	0.894052	89.4	89.0
1		9	57000	0.853573	85.4	85.0
1		10	63200	0.946526	94.7	95.0

Based on the calculations reported in pp. 20-22, calibration parameters reported in Figure 7, p. 35 and raw data reported in Appendix 3, p. 90 of the study report.

Peak area counts confirmed by chromatograms (Figures 9-10, pp. 37-38).