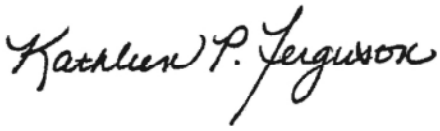
ENVIRONMENTAL CHEMISTRY METHOD REVIEW REPORT

Test Material:	Fluazinam
MRID:	48632401
Title:	Karnik, S.C. 2011. Independent laboratory validation of enforcement method for the analysis of fluazinam and HYPA in soil.
MRID:	48632401 – Appendix A
Title:	Robaugh, E. 2011. Method for the determination of fluazinam and HYPA in soil by LC/MS/MS.
EPA PC Code:	129098
OCSPP Guideline:	850.7100

For Cambridge Environmental

Primary Reviewer: Lynne Binari **Signature:** 

Date: 5/14/12

Secondary Reviewer: Kathleen Ferguson **Signature:** 

Date: 5/14/12

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

Date: 5/14/12

Data Requirement: EPA Guideline: 835.6100
OECD Data Point: IIA 4.4

Test material:
Common name: Fluazinam
Chemical name: 3-Chloro-N-[3-chloro-2,6-dinitro-4-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2-pyridinamine.
IUPAC: 3-Chloro-N-(3-chloro-5-trifluoromethyl-2-pyridyl)-α,α,α-trifluoro-2,6-dinitro-p-toluidine (Appendix B, p. 53).

Primary Reviewer: *See signatures in cover page* **Date** *See above*
Cambridge Environmental

Final Reviewer: José Luis Meléndez **Date** 04/05/2013
U.S. EPA

ANALYTICAL METHOD: EPA MRID No. 48632401 – Appendix A. Robaugh, E. 2011. Method for the determination of fluazinam and HYPA in soil by LC/MS/MS. Report prepared by Pyxant Labs Inc., Colorado Springs, Colorado, sponsored by Ishihara Sangyo Kaisha, Ltd., Osaka, Japan, and submitted by ISK Biosciences Corporation, Concord, Ohio; 8 pages (p. 1A; Appendix A, pp. 45-52). Final report issued October 11, 2011 (Appendix A, p. 45).

INDEPENDENT LABORATORY VALIDATION: EPA MRID No. 48632401. Karnik, S.C. 2011. Independent laboratory validation of enforcement method for the analysis of fluazinam and HYPA in soil. Report prepared by Pyxant Labs Inc., Colorado Springs, Colorado, sponsored by Ishihara Sangyo Kaisha, Ltd., Osaka, Japan, and submitted by ISK Biosciences Corporation, Concord, Ohio; 67 pages. Final report issued October 19, 2011.

EXECUTIVE SUMMARY

This method is designed for the quantitative determination of residues of fluazinam and its product HYPA in soil using an external standardization method. The method was developed Pyxant Labs, for Ishihara Sangyo Kaisha, Ltd.; no regulatory guidelines were cited in the ECM (Appendix A, pp. 45-52). An independent laboratory validation (ILV), performed by Pyxant Labs Inc., was submitted with the method. The Agency finds that this method meets the criteria for a scientifically valid method and is **supplemental** for fluazinam and HYPA. The registrant must provide the method validation results.

Table 1. Analytical Method Summary

Analyte	MRID		EPA Review	Matrix	Method Date	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Fluazinam, HYPA	48632401, Appendix A	48632401		Soil	10/11/11	ISK Biosciences Corp.	LC/MS/ MS-ESI^{+} [1]	10 µg/kg [2]

1. Electrospray ionization in positive ion mode (ESI^{+})
2. The registrant must provide additional data regarding the environmental chemistry method. Available data is incomplete.

Method Summary: Analytes are extracted from soil by sonication with methanol, the extract is diluted with water, then analyzed directly for fluazinam and HYPA using LC/MS/MS (Appendix A, p. 46). The ECM defined a limit of quantitation (LOQ) of 0.01 mg/kg for both analytes in soil, which was supported by the ILV (Appendix A, p. 51). A limit of detection (LOD) was not reported for either analyte.

METHOD ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS

For the ECM, performance data, LOD, chromatograms of standards and method and matrix blank samples, calibration curves and linear regression analyses, results from the confirmatory method, and the source and characterization of the soil matrix were not provided.

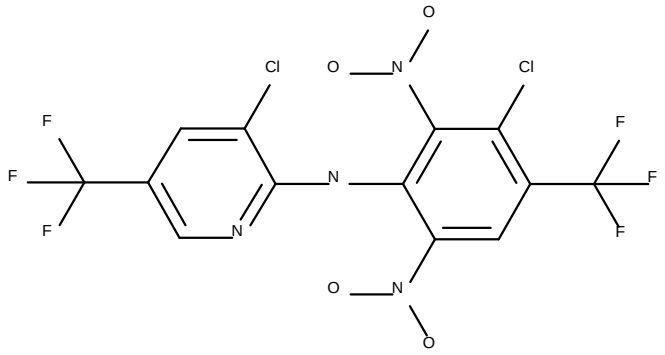
For the ILV, acceptance criteria were met (matrix spike recoveries ranging between 70% to 120% and relative standard deviations of ≤20%) at the LOQ and 10 x LOQ for both analytes, except for one of the five fortifications for HYPA at the LOQ (Tables 1-2, p. 20). Quantitative results from the confirmatory method were not reported and data on the representative chromatograms were illegible (Figures 19-20, pp. 43-44). The soil matrix was not characterized.

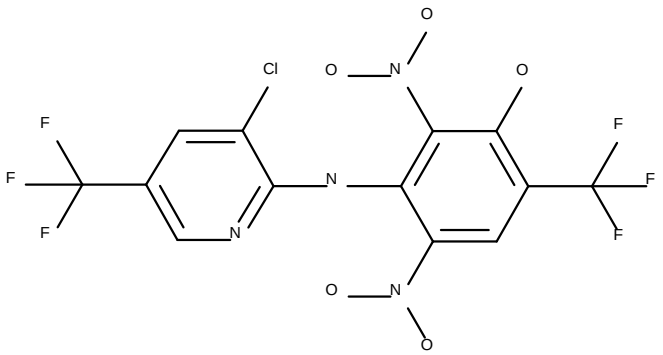
COMPLIANCE

No regulatory guidelines were cited in the ECM.

A. BACKGROUND INFORMATION

TABLE A.1. Test Compound Nomenclature

Parameter	Value
Common name	**Fluazinam**
Company experimental name	IKF-1216 PAI, IKF-1216, B1216, PP192 (p. 1A; Appendix B, p. 53).
IUPAC name	3-Chloro-N-(3-chloro-5-trifluoromethyl-2-pyridyl)-α,α,α-trifluoro-2,6-dinitro-p-toluidine.
CAS Name	3-Chloro-N-[3-chloro-2,6-dinitro-4-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2-pyridinamine.
CAS #	79622-59-6
Structure	
Common name	**HYPA**
Company experimental name	None reported (Appendix B, p. 54).
IUPAC name	5-((3-Chloro-5-(trifluoromethyl)-2-pyridyl)amino)-α,α,α-trifluoro-4,6-dinitro-o-cresol.
CAS Name	3-[[3-Chloro-5-(trifluoromethyl)-2-pyridinyl]amino]-2,4-dinitro-6-(trifluoro-methyl)phenol.
CAS #	79614-99-6

Structure	

Information obtained from p. 1A; Appendix B, pp. 53-54 of the study report. CAS names and compound structures obtained from Fluazinam structures[1].doc, as well as HYPA CAS No.

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound

Parameter	Value
Melting point/range (ºC)	Not reported.
pH	Not reported.
Density (g/cm^3)	Not reported.
Water solubility at 20ºC (mg/L)	Not reported.
Solvent solubility at 20 ºC (mg/L)	Not reported.
Vapor pressure at __ºC (torr)	Not reported.
Dissociation constant (pK_a)	Not reported.
Octanol/water partition coefficient	Not reported.
UV/visible absorption spectrum (nm)	Not reported.

B. MATERIALS AND METHODS

B.1. Principle of Method

Soil is extracted by sonication with methanol, then the extract is diluted with water and analyzed directly for fluazinam and HYPA by LC/MS/MS-ESI$^+$ using a Phenomenex Synergi Polar-RP column (Appendix A, pp. 49-50). For each compound, two ion transitions are monitored for quantitation and confirmation.

TABLE B.1. Summary Parameters for the Analytical Method Used for the Quantitation of Chemical Residues in Matrices Studied	
Parameter	**Value**
Method ID	Method for the determination of fluazinam and HYPA in soil by LC/MS/MS. Pyxant Labs Inc. Method Number STM2356.03 (Appendix A, p. 45).
Analyte(s)	Fluazinam and HYPA.
Extraction solvent/technique	Soil (10 g) is transferred to a glass tube, extracted twice with methanol (30 mL, 20 mL) using a digital sonifier, with cup horn, for 10 minutes; pulse signal 50 seconds on/10 seconds off/80% capacity (Appendix A, p. 49).
Cleanup strategies	Extract and soil separated by centrifugation and decanted. Extracts combined, brought to volume (50 mL) with methanol, then diluted 1:10 (v:v) with water for analysis (Appendix A, p. 50).
Instrument/Detector	ECM: Symbiosis HPLC system with Phenomenex Synergi Polar-RP column (2 x 50 mm, 4-µm) and Applied Biosystems API5000 LC/MS/MS equipped with Turbo Spray electrospray ionization in positive ion mode (ESI^+) and multiple reaction monitoring (MRM; Appendix A, pp. 47, 50). ILV: same as ECM except Shimadzu HPLC system (pp. 13, 17).

Information obtained from pp. 13, 17; Appendix A, pp. 45, 47, 49-50 of the study report.

C. RESULTS AND DISCUSSION

C.1. Recovery Results Summary

TABLE C.1. Recovery Results from Method Validation for the Determination of Fluazinam and HYPA in Soil			
Analyte	**Spiking Level (mg a.i./kg)**	**Recoveries Obtained (%)**	**Relative Standard Deviation**
Fluazinam	0.01 (LOQ)	--	--
	0.1	--	--
HYPA	0.01 (LOQ)	--	--
	0.1	--	--

Results (Spiking Level) from Appendix A, p. 51 of the study report.
-- = Not reported.

Results from the confirmatory method were not reported.

C.1.1. Method Characteristics

TABLE C.2. Method Characteristics	
Parameter	**Value**
Analyte(s)	Fluazinam and HYPA.
Limit of Quantitation (LOQ)	0.01 mg a.i./kg (Appendix A, p. 51).
Limit of Detection (LOD)	Not reported.
Accuracy/Precision at LOQ	ECM: Performance data were not reported.
Reliability of the Method/[ILV]	ILV: Acceptance criteria were met at the LOQ for both analytes, except for one HYPA fortification (LOQ-2 123%), with matrix spike recoveries ranging between 70% to 120% and relative standard deviations of ≤20% (Tables 1-2, p. 20). The method was validated in one trial (p. 16).
Linearity	ECM: not reported. ILV: Linear regression: r = 0.9969-0.9986 (p. 14; Figures 1-2, pp. 25-26).
Specificity	ECM: Could not be determined because quantitative results and chromatograms for standards and method and matrix blank samples were not provided. ILV: Comparison of chromatograms produced for standards and control and fortified samples demonstrates that the method, based on LC/MS/MS, is highly specific for the analysis of fluazinam and its product HYPA (Figures 3-18, pp. 27-42). Method and matrix blank controls showed no significant interferences (<0.0011 mg/kg) at the retention times of the two analytes (p. 14; Tables1-2, p. 20; Figures 7-10, pp. 31-34).

Information obtained from pp. 14, 16; Tables 1-2, p. 20; Figures 1-18, pp. 25-42; Appendix A, p. 51 of the study report.

C.2. Independent Laboratory Validation (ILV)

The ILV was conducted in compliance with USEPA GLP Standards 40 CFR, Part 160, OPPTS 850.7100 guidelines, and PR Notice 96-1 (pp. 3, 10, 18-19).

TABLE C.3. Recovery Results of the Method Obtained by an Independent Laboratory Validation for the Determination of Residues in Soil (n = 5)

Analyte	Spiking Level (mg a.i./kg)	Mean Recoveries Obtained (%)	Relative Standard Deviation
Fluazinam	0.01 (LOQ)	111	3
	0.1	86	10
HYPA	0.01 (LOQ)	115	5
	0.1	92	11

Results obtained from Tables 1-2, p. 20; reported results verified by reviewer (DER Attachment 2).

Quantitative results from the confirmatory method were not reported and data on representative chromatograms were illegible (Figures 19-20, pp. 43-44).

D. CONCLUSION

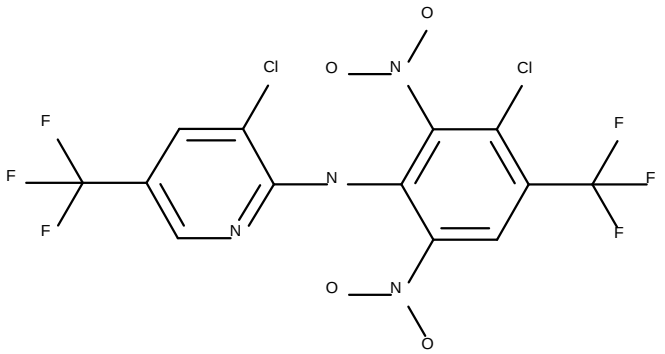
This method is designed for the quantitative determination of residues of fluazinam and its product and HYPA in soil. The Agency finds that this method meets the criteria for a scientifically valid method and is **supplemental** for fluazinam and HYPA. However, for HYPA in the ILV, one of the five fortifications at the LOQ was not within acceptance criteria (*i.e.*, <70% or >120% recovery; EFED-ECM 2, Version 1, December 2010, p. 5). Further, the registrant must provide additional data regarding the environmental chemistry method.

[Refer to the review checklist attached to this review.]

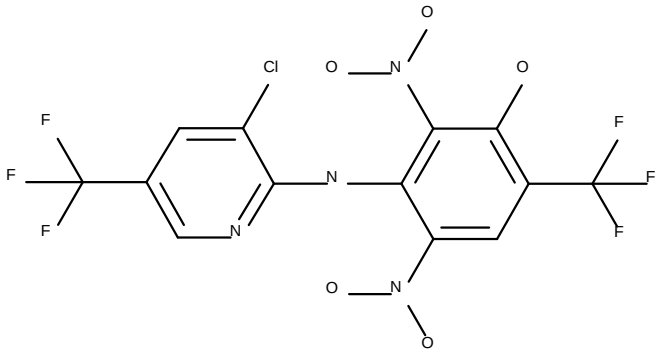
ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

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

I. Background Information

A.	**Title of Method**	Method for the determination of fluazinam and HYPA in soil by LC/MS/MS (Appendix A, p. 45).	
B.	**ECM No.** [ECB use]		
C.	**MRID No.**	48632401	
D.	**Matrix**	Soil	
E.	**Analyte(s) detected**	**Compound:**	
		Common name:	Fluazinam
		IUPAC name:	3-Chloro-N-(3-chloro-5-trifluoromethyl-2-pyridyl)-α,α,α-trifluoro-2,6-dinitro-p-toluidine (Appendix B, p. 53).
		CAS name:	3-Chloro-N-[3-chloro-2,6-dinitro-4-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2-pyridinamine.
		CAS No:	79622-59-6
		Synonyms:	IKF-1216 PAI, IKF-1216, B1216, PP192 (p. 1A; Appendix B, p. 53).
			
		Compound:	
		Common name:	HYPA

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

IUPAC name:	5-((3-Chloro-5-(trifluoromethyl)-2-pyridyl)amino)-α,α,α-trifluoro-4,6-dinitro-o-cresol (Appendix B, p. 54).
CAS name:	3-[[3-Chloro-5-(trifluoromethyl)-2-pyridinyl]amino]-2,4-dinitro-6-(trifluoro-methyl)phenol.
CAS No:	79614-99-6
Synonyms:	None reported.
	

Information obtained from Appendix A, p. 45; Appendix B, pp. 53-54 of the study report. CAS names and compound structures obtained from Fluazinam structures[1].doc, as well as HYPA CAS No.

II. Information about the Laboratory

A.	**Name**	Pyxant Labs Inc. (Appendix A, p. 45).
B.	**Address**	4720 Forge Road, Suite 108, Colorado Springs, Colorado 80907.
C.	**Telephone No.**	719-593-1165
D.	**Name of the Study Director**	Not reported.
E.	**Name of the Lead Chemist**	Erin Robaugh.
F.	**Laboratory Validation:**	Not provided.

Information obtained from Appendix A, p. 45 of the study report.

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

III. Method Summary Information for Analyte(s): **Fluazinam and its product HYPA.**

A.	**Statement of Data Confidentiality**	Yes (p. 2).
1.	**Is the Method Classified or Confidential?**	No.
2.	**Submitted Prior to 2008 with a Non-Standard Claim of Confidentiality?**	No.
B.	**Sample Preparation**	Soil (10 g) was fortified with a mixed standard solution of fluazinam and HYPA, in acetonitrile, at 0.01 and 0.1 mg a.i./kg (Appendix A, pp. 48-49, 51). Application solution volumes were not reported.
C.	**Sample Extraction**	Soil (10 g) extracted twice with methanol (30 mL, 20 mL) using a digital sonifier, with cup horn, for 10 minutes; pulse signal 50 seconds on/10 seconds off/80% capacity (Appendix A, p. 49).
D.	**Sample Cleanup**	Extract and soil separated by centrifugation and decanted. Extracts combined, brought to volume (50 mL) with methanol, then diluted 1:10 (v:v) with water for analysis (Appendix A, p. 50).
E.	**Sample Derivatization (if applicable)**	None reported.
F.	**Sample Analysis**	LC/MS/MS (Appendix A, p. 46).
1.	**Instrumentation**	Symbiosis Pharma HPLC System and Applied Biosystems API5000 LC/MS/MS equipped with Turbo Spray electrospray ionization in positive ion mode (ESI^+; Appendix A, p. 50).
2.	**Primary Column**	Phenomenex Synergi Polar-RP column (2 x 50 mm, 4 µm) with Phenomenex C18 guard column (optional; Appendix A, p. 50).
3.	**Confirmatory Column (if any)**	None reported.
4.	**Detector**	Multiple Reaction Monitoring (MRM; Appendix A, p. 50).
5.	**Other Confirmatory Techniques (if any)**	For each compound, two ion transitions were monitored for quantitation and confirmation (Appendix A, p. 50).

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

6.	**Other Relevant Information**	Compound	Ions monitored (m/z)		Retention time (min.)[1]
			Quantitation	Confirmation	
		Fluazinam	465.2 > 373.0	465.2 > 338.0	*ca*. 5.10
		HYPA	447.0 > 355.0	447.0 > 382.9	*ca*. 4.70
G.	**Detection and Quantitation Limits**				
1.	**Limit of Quantitation (LOQ)**				
	Claimed in Method	0.01 mg/kg (Appendix A, p. 51).	**Estimated**	No justification for selected LOQ was provided.	
2.	**Limit of Detection (LOD)**				
	Claimed in Method	Not reported.	**Estimated**		

H.	**Recovery (Accuracy)/Precision Data; expressed as percentage of applied**			
	Spiking Level (mg a.i./kg)	**Parameter**	**Fluazinam**	**HYPA**
	0.01 (LOQ)	**Range**	--	--
		Mean	--	--
		SD	--	--
		RSD	--	--
	0.1	**Range**	--	--
		Mean	--	--
		SD	--	--
		RSD	--	--

Information obtained from pp. 2, 14; Appendix A, pp. 46, 48-51 of the study report.
-- = Not reported.
1 Obtained from ILV (p. 14); HPLC retention times not reported in ECM.

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

IV. Detailed Information about the Method

		YES	NO	REVIEW FURTHER
A.	**Does the method require spiking with the analytes(s) of interest?**	x		Appendix A, p. 49.
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		Appendix A, p. 49.
b.	**The sample spiking procedure?**		x	
c.	**The extraction procedure?**	x		Appendix A, p. 49.
d.	**The derivatization procedure?**			Not applicable.
e.	**The clean-up procedure?**	x		Appendix A, p. 50.
f.	**The analysis procedure?**	x		Appendix A, p. 50.
2.	**Procedures for:**			
a.	**Preparation of standards?**	x		Appendix A, p. 49.
b.	**Calibration of instrument?**	x		Appendix A, p. 51.
3.	**List of glassware and chemicals**	x		Appendix A, pp. 46-47.
a.	**Are sources recommended?**	Extraction tubes, HPLC vials	Chemicals Additional glassware	
b.	**Are they commercially available?**	x		
4.	**Name, model, *etc.*, of the instrument, column, detector, *etc.*, used?**	x		Appendix A, pp. 47, 50.
a.	**Are sources recommended?**	x		
b.	**Are they commercially available?**	x		
5.	**LOD**			
a.	**Is there an explanation of how it was calculated?**		x	LOD not reported.

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

		YES	NO	REVIEW FURTHER
b.	**Is it a scientifically accepted procedure?**			
c.	**Is the matrix blank free of interference(s) at the retention time, wavelength, *etc.*, of the analyte(s) of interest?**			ECM: Results from matrix blanks not reported.
6.	**LOQ**			
a.	**Is there an explanation of how it was calculated?**		x	
b.	**Is it a scientifically accepted procedure?**			
7.	**Precision and accuracy data**			
a.	**Were there an adequate number of spiked samples analyzed?**			For the ECM: No performance data.
b.	**Are the mean recoveries between 70-120%?**			
c.	**Are the RSDs of the replicates 20% or less at or above the LOQ?**			
8.	**Description and/or explanation of:**			
a.	**Areas where problems may be encountered?**			None reported.
b.	**Steps that are critical?**			None specified.
c.	**Interferences that may be encountered?**			None reported.
9.	**Characterization of the Matrix?**		x	

Information obtained from Appendix A, pp. 46-47, 49-51 of the study report.

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

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?**	ILV	ECM	Figures 11-18, pp. 35-42.
2.	**Method blanks?**	ILV	ECM	Figures 7-8, pp. 31-32.
3.	**Matrix blanks?**	ILV	ECM	Figures 9-10, pp. 33-34.
4.	**Standard curves?**	ILV	ECM	Figures 1-2, pp. 25-26.
a.	**Do the standard curves have acceptable linearity?**	x		r = 0.9969-0.9986
5.	**Standards that can be used to recalculate some of the values for analyte(s) in the sample chromatograms?**	ILV	ECM	DER Attachment 2
B.	**Can the responses of the analytes(s) in the chromatograms of the lowest spiking level be accurately measured?**	x		Tables 3-4, pp. 21-22.

Information obtained from Tables 3-4, pp. 21-22; Figures 1-2, pp. 25-26; Figures 7-18, pp. 31-42 of the study report.

VI. Good Laboratory Practice (GLP) Standards

		YES	NO	REVIEW FURTHER
A.	**Is there a statement of adherence to the FIFRA GLP standards?**	ILV	ECM	

Information obtained from p. 3 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		The ILV was reported as being conducted in a different department and by personnel who had no prior experience or knowledge of the ECM procedures (pp. 9-10).
C.	**Did the ILV's precision/accuracy data meet the criteria established in OCSPP Guideline 850.6100?**	x		
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		Minor (p. 17).

E. Recovery (Accuracy)/Precision Data; expressed as percentage of applied (n = 5)[1]

Spiking Level (mg a.i./kg)	Parameter	Fluazinam	HYPA
0.01 (LOQ)	Range	107-115	108-123
	Mean	111	115
	SD	3	6
	RSD	3	5
0.1	Range	76-96	84-109
	Mean	86	92
	SD	9	10
	RSD	10	11

Information obtained from pp. 9-10 of the study report.
1 Results obtained from Tables 1-2, p. 20 of the study report; reported results verified by reviewer (DER Attachment 2).

ENVIRONMENTAL CHEMISTRY METHOD REVIEW CHECKLIST

VIII. Completeness

		YES	NO	REVIEW FURTHER
A.	**Has enough information been supplied to do a proper review?**		x	See section ***IX. Recommendations*** (below).
B.	**Has enough information been supplied to do a laboratory evaluation, if requested?** [*This may be left blank, as it is a determination made by BEAD ECB.*]			
C.	**Are all steps in the method scientifically sound?**	x		
D.	**Is a confirmatory method or technique provided?**	x		However, adequate supporting results were not provided.
E.	**Check the category below which best describes this ECM.**			
1.	**Satisfactory** [*Agency determination*]		x	Study may be upgraded with the submission of additional data.
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) No performance data were provided.

   b) No chromatograms were provided.

c) The limit of detection was not reported.

d) The source and characterization of the soil matrix were not reported.

e) HPLC retention times for the two analytes were not reported.

f) No results from the confirmatory method were provided.

g) No justification for selection of the LOQ concentration was provided.

h) No regulatory guidelines were cited in the ECM (Appendix A, pp. 45-52).

2. For the ILV:

a) For HYPA, one of the five fortifications at the LOQ (LOQ-2 123%) was not within acceptance criteria (*i.e.*, <70% or >120% recovery; EFED-ECM 2, Version 1, December 2010, p. 5).

b) Quantitative results from the confirmatory method were not reported and data on the representative chromatograms were illegible (Figures 19-20, pp. 43-44).

c) Data on all chromatograms were for the most part illegible; therefore, verification of results using the chromatograms was done using Peak Area counts reported in Table 3, p. 21, Table 4, p. 23 of the study report (DER Attachment 2).

d) Except for being described as "acquired locally", the soil matrix was not characterized (p. 11).

Cambridge Environmental see signatures in the cover page of the DER

Primary Reviewer

José Luis Meléndez 04/05/2013

Secondary Reviewer: José L. Meléndez, Chemist

Chemical: Fluazinam
PC: 129098
MRID: 48632401
Guideline: 850.6100

Independent laboratory validation for determination of fluazinam and its product HYPA in soil.

Fortified (mg a.i./kg)	Fluazinam					HYPA				
	Measured (mg/kg)	Recovery (%)	Mean (%)	SD[1] (%)	RSD[2] (%)	Measured (mg/kg)	Recovery (%)	Mean (%)	SD (%)	RSD (%)
0.01	0.0107	107				0.0112	112			
	0.0115	115				0.0123	123			
	0.0113	113				0.0108	108			
	0.0111	111				0.0119	119			
	0.0112	112	112	3	3	0.0112	112	115	6	5
0.1	0.0925	93				0.1090	109			
	0.0870	87				0.0930	93			
	0.0775	78				0.0835	84			
	0.0960	96				0.0885	89			
	0.0760	76	86	9	10	0.0840	84	92	10	11
Overall mean		99					103			
SD		15					15			
RSD		15					14			
Max		115					123			
Min		76					84			
n =		10					10			

Chemical: Fluazinam
PC: 129098
MRID: 48632401
Guideline: 850.6100
Verification of ILV recoveries in fortified soil using chromatogram "Area" and calibration curve regression equations.

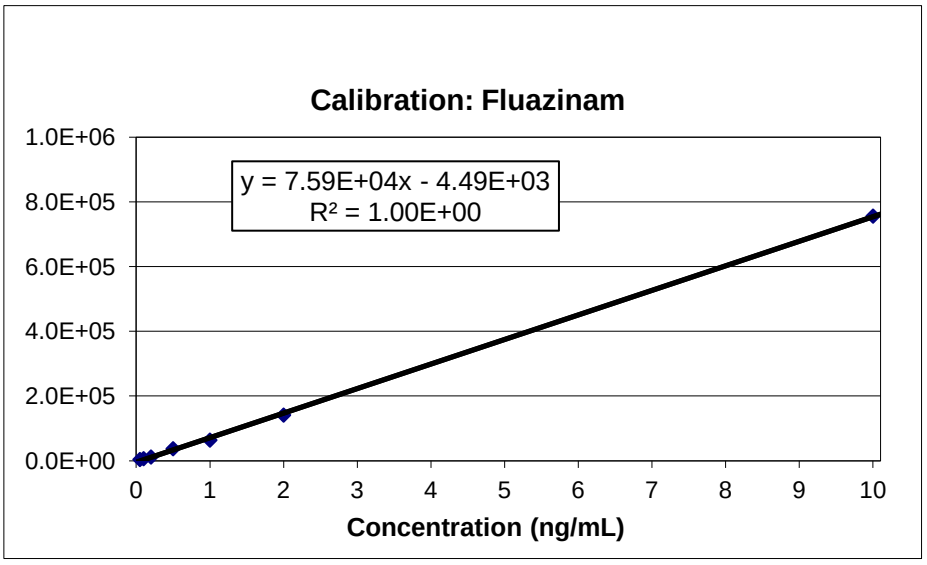
Fortified (mg a.i./kg)	Analyte	Sample	Peak Area (counts)	Reviewer		Reported	
				Measured (mg/kg)	Recovery (%)	Measured (mg/kg)	Recovery (%)
0.01	Fluazinam	LOQ-1	14800	0.0107	107	0.0107	107
		LOQ-2	16000	0.0115	115	0.0115	115
	HYPA	LOQ-2	4310	0.0112	112	0.0112	112
		LOQ-3	4140	0.0107	107	0.0108	108
0.1	Fluazinam	LOQ-1	136000	0.0924	92	0.0925	92
		LOQ-2	128000	0.0871	87	0.0870	87
	HYPA	LOQ-1	44200	0.1084	108	0.1090	109
		LOQ-2	37800	0.0928	93	0.0930	93

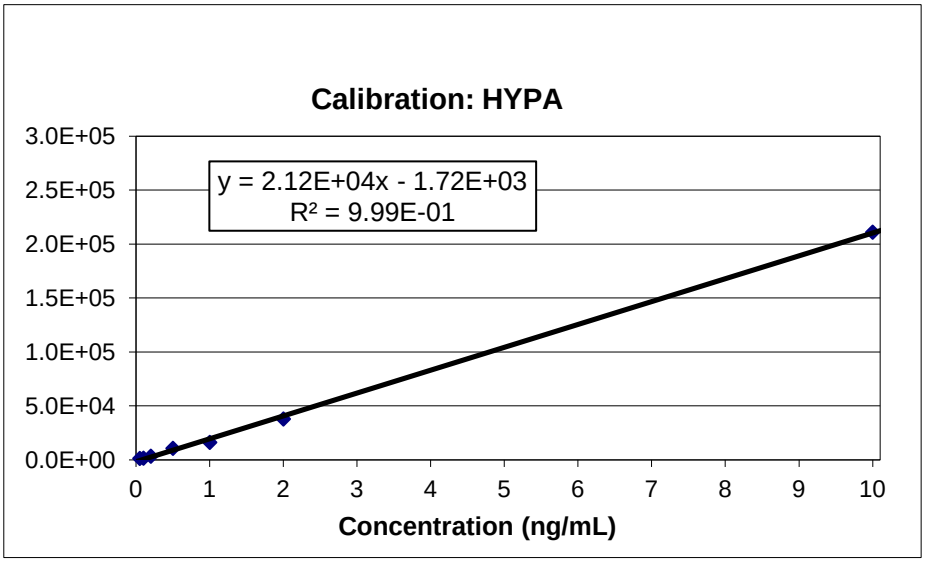
Peak Area from Table 3, p. 21; Table 4, p. 23 for Figures 11-18, pp. 35-42 of the study report.
Linear regression coefficients from Figures 1-2, pp. 25-26 of the study report.
Reported Measured and Recovery from Tables 1-2, p. 20 of the study report.
Measured calculated as using reported equations (pp. 15-16).

Chemical: Fluazinam
PC: 129098
MRID: 48632401
Guideline: 850.6100
ILV calibration curves.

Concentration (ng/mL)	Fluazinam Peak Area (counts)	HYPA Peak Area (counts)
0.05	3.77E+03	1.23E+03
0.10	6.32E+03	1.46E+03
0.20	1.15E+04	3.29E+03
0.50	3.71E+04	1.06E+04
1.00	6.37E+04	1.61E+04
2.00	1.41E+05	3.78E+04
10.0	7.56E+05	2.11E+05

Results from Table 3, p. 21; Table 4, p. 23 of the study report.





Chemical: Fluazinam
PC: 129098
MRID: 48632401
Guideline: 850.6100
ILV method (reagent) and matrix blank samples.

Analyte	Sample	Peak Area (counts)	Measured (mg/kg)	Reported (mg/kg)
Fluazinam	Reagent blank	610	0.00109	0.00109
	Matrix blank	592	0.00108	0.00108
	Matrix blank	450	0.000985	0.000985
HYPA	Reagent blank	0		ND
	Matrix blank	0		ND
	Matrix blank	0		ND

Peak Area from Table 3, p. 21; Table 4, p. 23 of the study report.
Reported (Calculated Concentration, mg/kg) from Tables 1-2, p. 20 of the study report.
Linear regression coefficients from Figure 1, p. 33 of the study report.
Measured calculated as using reported equations (pp. 15-16).