Test Material: Ethaboxam

MRID: 49489941

Title: Ethaboxam: Method Validation for the Determination of Ethaboxam, LGC-32523, LGC-32533 and LGC-32799 in Soil

MRID: 49489921

Title: Ethaboxam Independent Laboratory Validation of Analytical Method RM-49S-1: Ethaboxam: Method Validation for the Determination of Ethaboxam, LGC-32523, LGC-32533 and LGC-32799 in Soil

EPA PC Code: 090205

OCSPP Guideline: 850.6100

For CDM Smith

Primary Reviewer: Lisa Muto **Signature:** Lisa Muto

Date: 4/25/16

Secondary Reviewer: Kathleen Ferguson **Signature:**

Date: 4/25/16

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

Date: 4/25/16

Analytical method for ethaboxam and its metabolites, LGC-32523, LGC-32533 and LGC-32799, in soil

Reports: ECM: EPA MRID No. 49489941. Foster, J. 2015. Ethaboxam: Method Validation for the Determination of Ethaboxam, LGC-32523, LGC-32533 and LGC-32799 in Soil. Laboratory Project ID: VP-38905. Report prepared, sponsored and submitted by Valent Technical Center, Valent U.S.A. Corporation, Dublin, California; 113 pages. Final report issued July 8, 2015.

ILV: EPA MRID No. 49489921. Golich-Moore, R. 2015. Ethaboxam Independent Laboratory Validation of Analytical Method RM-49S-1: Ethaboxam: Method Validation for the Determination of Ethaboxam, LGC-32523, LGC-32533 and LGC-32799 in Soil. Laboratory Project ID: 120.027. Report prepared by North Coast Laboratories, Arcata, California; sponsored and submitted by Valent U.S.A. Corporation, Dublin, California; 171 pages (including page 1i). Final report issued May 1, 2015.

Document No.: MRIDs 49489941 (ECM) & 49489921 (ILV)

Guideline: 850.6100

Statements: ECM: The study was conducted in accordance with the USEPA FIFRA GLP (40 CFR Part 160; p. 3). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-4). A Certification of Authenticity was not provided.

ILV: The study was conducted in accordance with the USEPA FIFRA GLP (40 CFR Part 160), with the exception that there was one instance of a missed audit report (the late entries were cross-checked with other entries in the study raw data for accuracy; p. 3). Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 1i, 2-3). A Certification of Authenticity was not provided.

Classification: This analytical method is classified as supplemental. The determinations of the LOQ and LOD were not based on scientifically acceptable procedures, however, the LOQ far exceeds terrestrial toxicity endpoints for ethaboxam. The ILV did not report LODs. The soil matrices were not characterized. In the ECM, the linear regression coefficient for LGC-32523 was not satisfactory. Ethaboxam recoveries were corrected in the ECM.

PC Code: 090205

Reviewer:

Andrew Shelby, Physical Scientist **Signature:**

Date: December 2, 2016

<u>All referenced page numbers refer to those written in the upper-most, right-hand corner of the pages of the documents</u>.

Executive Summary

This analytical method, Valent Analytical Method RM-49S-1, is designed for the quantitative determination of ethaboxam and its metabolites, LGC-32523, LGC-32533 and LGC-32799, in soil at the stated LOQ of 0.01 mg/kg using LC/MS/MS. The LOQ is less than the lowest toxicological level of concern in soil. The ILV successfully validated the method for all analytes after one trial with insignificant modifications to the ECM. However, the soil matrices of the ECM and ILV were not characterized or classified. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. In the ECM and ILV, analytes were identified and quantified using one ion transition, except for the ILV analysis of ethaboxam which was identified and quantified using two ion transitions. Ethaboxam recoveries were corrected in the ECM for residues quantified in the controls (*ca*. 5% of the LOQ). The LOD was not reported in the ILV.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Ethaboxam	49489941	49489921		Soil*	08/07/2015	Valent U.S.A Corporation	LC/MS/MS	0.01 mg/kg
LGC-32523								
LGC-32533								
LGC-32799								

* The soils were not characterized in the ECM or ILV (see Reviewer's Comment #3).

I. Principle of the Method

Method RM-49S-1 (Appendix 2, pp. 25-49 of MRID 49489941):

Soil (5.0 ± 0.1 g) in a 50 mL polypropylene centrifuge tube was fortified, if necessary, then extracted twice with *ca*. 20 mL of acetone:water (3:1, v:v) via reciprocal shaker for 30 minutes (Appendix 2, p. 34). After centrifugation (5 minutes at ca. 4000 rpm), the supernatant was decanted into a fresh 50 mL polypropylene centrifuge tube. The volume of the combined extracts was adjusted to 45 mL using methanol. An aliquot (0.25 mL) of the extract was mixed with 0.25 mL of internal standard (0.01 mg/L concentration) and 3.5 mL of HPLC-grade water (the method noted that different volumes could be used as long as the same proportions were maintained). The mixture was filtered using a 0.2µ PTFE syringe filter (more than one filter may be used) then at least 1 mL of the sample was transferred into two separate autosampler vials so that samples may be analyzed in LC/MS/MS positive and negative ion modes.

Samples are analyzed for ethaboxam, LGC-32523 and LGC-32533 using an Agilent Technologies 1200 series LC coupled with an Applied Biosystems API 4000 MS with positive electrospray ionization (pp. 9-11). The following LC conditions were used: Phenomenex C8 guard column (2.0 x 4.0 mm), Agilent Eclipse XDB-C18 column (50 mm x 4.6 mm, 5 µm, column temperature 25°C) using a mobile phase of (A) 0.05% formic acid in HPLC-grade water and (B) 0.05% formic acid in methanol [percent A:B (v:v) at 0.0-1.0 min. 65:35, 5.0-7.5 min. 10:90, 8.0-11.0 min 65:35]. Injection volume was 25 µL. The following MS/MS conditions were used: ESI in positive ion mode detection and multiple reaction monitoring (MRM). Analytes were identified with one ion transition: *m/z* 321.1→200.0 for ethaboxam, *m/z* 200.0→129.0 for LGC-32523 and *m/z* 310.3→201.0 for LGC-32533. A confirmatory ion transition was reported, but not monitored, for ethaboxam: *m/z* 321.1→183.0 for “ethaboxam 2”. The internal standard was monitored with one ion transition: *m/z* 326.1→205.0 for *d5*-ethaboxam. Expected retention times were *ca*. 6.8, 2.0 and 5.9 minutes for ethaboxam, LGC-32523 and LGC-32533, respectively.

Samples are analyzed for LGC-32799 using an Agilent Technologies 1200 series LC coupled with an Applied Biosystems API 4000 MS with negative electrospray ionization (pp. 9, 11-12). The following LC conditions were used: Phenomenex C8 guard column (2.0 x 4.0 mm), Agilent Eclipse XDB-C18 column (50 mm x 4.6 mm, 5 µm, column temperature 25°C) using a mobile phase of (A) 5mM ammonium acetate in HPLC-grade water and (B) 0.05% formic acid in methanol [percent A:B (v:v) at 0.0-1.0 min. 50:50, 3.0-7.0 min. 10:90, 7.5-10.0 min 50:50]. Injection volume was 25 µL. The following MS/MS conditions were used: ESI in negative ion mode detection and multiple reaction monitoring (MRM). LGC-32799 was identified with one ion transition: *m/z* 321.8→107.8. Expected retention time was *ca*. 6.4 minutes.

ILV

The samples were processed using the same procedure as that of the ECM, except that the volume of the final extract sample was increased (45 mL to 50 mL, pp. 18-22; Appendix 2, Appendix 2, pp. 146-149). LC/MS/MS conditions were the similar to those in the ECM. The LC conditions which differed from the ECM were Phenomenex P/N AJO-4287 guard column (3.0 x

4 mm), the injection volume (30 µL) and the positive MS analysis mobile phase gradient [percent A:B (v:v) at 0.0-0.3 min. 80:20, 1.0 min. 65:35, 2.00-3.00 min. 50:50, 6.80 min. 15:85, 10.80 min. 10:90, 11.30 min. 80:20]. The monitored ions were the same as those of the ECM (± 1 *m/z*). Expected retention times were *ca*. 6.5, 2.7, 6.0, 4.1 and 6.4 minutes for ethaboxam, LGC-32523, LGC-32533, LGC-32799 and *d5*-ethaboxam, respectively

In the ECM and ILV, the LOQ was 0.01 mg/kg (0.01 µg/g; 0.01 ppm) for ethaboxam, LGC-32523, LGC-32533 and LGC-32799 (pp. 14-15; Appendix 2, p. 37 of MRID 49489941; pp. 12, 25, 28 of MRID 49489921). In the ECM, the LOD was reported as 0.005 mg/kg (0.005 ppm) for all four analytes; the LOD was not reported in the ILV.

II. Recovery Findings

ECM (MRID 49489941): Mean recoveries and relative standard deviations (RSDs) were within guidelines (mean 70-120%; RSD ≤20%) for analysis of ethaboxam, LGC-32523, LGC-32533 and LGC-32799 in soil at the LOQ (0.01 ppm) and 10x LOQ (0.1 ppm; pp. 14-15; Appendix 5, pp. 110-113). All analytes were identified and quantified using one ion transition (pp. 9-12). Therefore, quantitation ion and confirmation ion recovery results could not be compared. Ethaboxam recovery results were corrected for residues (average peak area ratio) found in the controls (*ca*. 5% of the LOQ; Appendix 5, p. 110). Recovery results of the other analytes were not corrected since no residues were found in the controls (Appendix 5, pp. 111-113). The soil was a control soil from Valent study V-13-38327, collected at Madera County, California, but it was not characterized or further described in this study (p. 9).

ILV (MRID 49489921): Mean recoveries and RSDs were within guideline requirements for analysis of ethaboxam, LGC-32523, LGC-32533 and LGC-32799 in soil at the LOQ and 10x LOQ (Tables 1-5, pp. 30-34). All analytes, except ethaboxam, were identified and quantified using one ion transition. Ethaboxam was identified and quantified using two ion transitions. Therefore, quantitation ion and confirmation ion recovery results could only be compared for ethaboxam. Quantitation ion and confirmation ion recovery results of ethaboxam were comparable. The soil was reported as V-38327CA Bulk Soil sent from the sponsor, but it was not characterized or further described (p. 16). The method was validated with the first trial using insignificant modifications (p. 12).

Table 2. Initial Validation Method Recoveries for Ethaboxam, LGC-32523, LGC-32533 and LGC-32799 in Soil[1]

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)[2]
	Quantitation Ion					
Ethaboxam[3] *m/z* 321.1→200.0	0.01 (LOQ)	5	93.70-97.50	96.2	0.015	1.59
	0.1	5	92.60-101.20	97.9	0.035	3.60
LGC-32523 *m/z* 200.0→129.0	0.01 (LOQ)	5	89.90-101.20	96.6	0.056	5.76
	0.1	5	93.30-113	102.5	0.085	8.30
LGC-32533 *m/z* 310.3→201.0	0.01 (LOQ)	5	86.40-98.00	91.9	0.042	4.62
	0.1	5	81.60-94.30	90.7	0.052	5.74
LGC-32799 *m/z* 321.8→107.8	0.01 (LOQ)	5	90.00-115	103.1	0.092	8.92
	0.1	5	72.40-86.90	83.4	0.062	7.42
	Confirmation Ion					
Ethaboxam *m/z* 321.1→183.0	0.01 (LOQ)	5	Not evaluated			
	0.1	5				

Data (results) were obtained from pp. 14-15 and Appendix 5, pp. 110-113 of MRID 49489941.

1 The soil was a control soil from Valent study V-13-38327, collected at Madera County, California, but it was not characterized or further described in this study (p. 9 of MRID 49489941).

2 Coefficient of Variance in study tables.

3 Recovery results were corrected for residues (average peak area ratio) found in the controls (*ca*. 5% of the LOQ; Appendix 5, p. 110).

Table 3. Independent Validation Method Recoveries for Ethaboxam, LGC-32523, LGC-32533 and LGC-32799 in Soil[1]

Analyte	Fortification Level (µg/g)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
	Quantitation Ion					
Ethaboxam *m/z* 321.0→200.1	0.01 (LOQ)	5	99.1-105	101	2.3	2.2
	0.1	5	100-102	101	0.59	0.58
LGC-32523 *m/z* 200.0→129.1	0.01 (LOQ)	5	95.2-102	99.9	3.8	3.8
	0.1	5	95.3-98.7	97.2	1.3	1.3
LGC-32533 *m/z* 310.0→201.0	0.01 (LOQ)	5	87.4-96.3	93.5	3.5	3.7
	0.1	5	94.1-99.6	97.4	2.3	2.3
LGC-32799 *m/z* 320.0→107.9	0.01 (LOQ)	5	96.7-105	102	3.5	3.4
	0.1	5	98.3-102	100	1.6	1.6
	Confirmation Ion					
Ethaboxam *m/z* 321.0→182.9	0.01 (LOQ)	5	101-106	104	1.9	1.9
	0.1	5	100-102	101	1.0	1.0

Data (uncorrected recovery results, pp. 25-26) were obtained from Tables 1-5, pp. 30-34 of MRID 49489921.

1 The soil was reported as V-38327CA Bulk Soil sent from the sponsor, but it was not characterized or further described (p. 16 of MRID 49489921).

III. Method Characteristics

In the ECM and ILV, ECM and ILV, the LOQ was 0.01 mg/kg (0.01 µg/g; 0.01 ppm) for ethaboxam, LGC-32523, LGC-32533 and LGC-32799 (pp. 14-15; Appendix 2, p. 37 of MRID 49489941; pp. 12, 25, 28 of MRID 49489921). No justification or calculation was provided for the LOQ. No comparison was made to chromatogram background levels. In the ECM, the LOD was reported as 0.005 mg/kg (0.005 ppm) for all four analytes; the LOD was not reported in the ILV. The LOD was calculated in the ECM based on the 5.0 g sample weight, a 0.25-mL aliquot (of the 45-mL extract volume), a 4-mL final volume, and the lowest calibration standard, 0.0375 µg/L. The LOD was calculated using the following equation:

$$\text{LOD} = \{[(4\text{ mL Final Vol.}) \times (0.0375\ \mu\text{g/L Stnd})]/(5.0\text{ g Sample Wt.})\} \times (1\text{ L}/1000\text{ mL}) \times [(45\text{ mL Extract Vol.})/(0.25\text{ mL Aliquot})]$$

Table 4. Method Characteristics in Soil

			Ethaboxam	**LGC-32523**	**LGC-32533**	**LGC-32799**
Limit of Quantitation (LOQ)			0.01 µg/g			
Limit of Detection (LOD)			0.005 µg/g			
Linearity (calibration curve r^2 and concentration range)	ECM:	Polynomial[1]	$r^2 = 0.9999$	$r^2 = 0.9998$	$r^2 = 0.9996$	$r^2 = 0.9998$
		Linear[2]	$r^2 = 0.9998$	$r^2 = 0.9900$	$r^2 = 0.9997$	$r^2 = 0.9999$
	Range:		0.0375-2.0 mg/L			
	ILV (Linear)[3]:		$r^2 = 1.0000$	$r^2 = 1.0000$	$r^2 = 0.9998$	$r^2 = 0.9994$
	Range:		0.007-0.32 µg/g			
Repeatable			Yes at LOQ and 10x LOQ (uncharacterized soil).[4]			
Reproducible			Yes at LOQ and 10x LOQ (uncharacterized soil).[5]			
Specific	ECM:		Yes; interferences at the analyte retention times were ≤5% (based on peak height) of the LOQ.[6]	Yes, no matrix interferences were observed.		
	ILV:		Yes, no matrix interferences were observed.[7]			

Data were obtained from pp. 9, 14-15; Appendix 2, p. 37; Figures 1-76, pp. 60-108; Appendix 5, pp. 110-113 of MRID 49489941; pp. 12, 25, 28; Tables 1-5, pp. 30-34; Figures 1-84, pp. 37-120 of MRID 49489921; DER Attachment 2.

Linearity is satisfactory when $r^2 \geq 0.995$.

1 The ECM study author provided polynomial-fit calibration curves (Figure 1, p. 60, Figure 20, p. 79, Figure 39, p. 89 and Figure 58, p. 99 of MRID 49489941).

2 The reviewer calculated ECM coefficient of determination (r^2) values using reviewer-generated linear regression calibration curves of the peak areas provided in the study report (Figures 2-63, pp. 61-102 of MRID 49489941; DER Attachment 2).

3 The reviewer calculated ILV coefficient of determination (r^2) values from the provided r values (Figure 1, p. 37, Figure 22, p. 58, Figure 43, p. 79 and Figure 64, p. 100 of MRID 49489921; DER Attachment 2).

4 In the ECM, all analytes were identified and quantified using one ion transition (pp. 11-12, 14-15 of MRID 49489941). The soil was a control soil from Valent study V-13-38327, collected at Madera County, California, but it was not characterized or further described in this study (p. 9).

5 In the ILV, all analytes, except ethaboxam, were identified and quantified using one ion transition (Table 1, p. 19 of MRID 49489921). Ethaboxam was identified and quantified using two ion transitions (quantitation and confirmation). The soil was reported as V-38327CA Bulk Soil sent from the sponsor, but it was not characterized or further described (p. 16).

6 Ethaboxam recovery results were corrected for residues (average peak area ratio) found in the controls (*ca*. 5% of the LOQ; Appendix 5, p. 110).

7 Chromatograms of the confirmation ion were not provided in the ILV. Typically, a confirmatory method is not required where GC/MS and LC/MS methods are used as the primary method(s) to generate study data.

Linearity is satisfactory when $r^2 \geq 0.995$.

IV. Method Deficiencies and Reviewer's Comments

1. The original ECM method was Valent Method RM-49S-1 (Foster, J. 2015. Ethaboxam: Determination of Ethaboxam, LGC-32523, LGC-32533 and LGC-32799 in Soil. Method ID: RM-49S-1. Report prepared, sponsored and submitted by Valent Technical Center, Valent U.S.A. Corporation, Dublin, California; 25 pages. Final report issued January 5, 2015.) which was contained in Appendix 2 of ECM MRID 49489941 (Appendix 2, pp. 25-49 of MRID 49489941). Valent Method RM-49S-1 was a method only with no validation data.

2. The determination of the LOQ and LOD were not based on scientifically acceptable procedures as defined in 40 CFR Part 136, Appendix B. No justification or calculation was provided for the LOQ (pp. 14-15; Appendix 2, p. 37 of MRID 49489941). No comparison was made to chromatogram background levels. The LOD was reported in the ECM based on the lowest concentration standard. 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, however, the LOQ far exceeds terrestrial toxicity endpoints for ethaboxam. An LOQ above toxicological levels of concern results in an unacceptable method classification. The LOD was not reported in the ILV.

3. The soil matrices were not characterized or classified by soil texture in the ECM or ILV. The soil matrix of the ECM was a control soil from Valent study V-13-38327, collected at Madera County, California, but it was not characterized or further described in this study (p. 9 of MRID 49489941). The soil matrix of the ILV was reported as V-38327CA Bulk Soil sent from the sponsor, but it was not characterized or further described (p. 16 of MRID 49489921). The reviewer concluded that the soil of the ILV was probably the same soil used in the ECM, based on the soil identification numbers. Without soil texture classifications of the ECM and ILV soil matrices, it could not be determined if the ILV was provided with the most difficult matrix with which to validate the method.

4. In the ECM, the linear regression coefficient for LGC-32523 was not satisfactory ($r^2 = 0.9900$). Linearity is satisfactory when $r^2 \geq 0.995$. The reviewer calculated ECM coefficient of determination (r^2) values using reviewer-generated linear regression calibration curves of the peak areas provided in the study report since the r^2 values of the ECM were based on polynomial-fit regression equations (Figures 2-63, pp. 61-102 of MRID 49489941; DER Attachment 2).

5. In the ECM, ethaboxam recovery results were corrected for residues (average peak area ratio) found in the controls (*ca*. 5% of the LOQ; Appendix 5, p. 110). OCSPP guidelines specify that recoveries should not be corrected for reagent blanks, matrix blanks, or other recoveries.

6. In the ILV, ethaboxam was identified using two ion transitions; however, representative chromatograms of the confirmation ion of ethaboxam were not provided in the ILV (Table 1, p. 19 of MRID 49489921). Therefore, the confirmatory method in the ILV was

incompletely validated. All other analytes were only identified using one ion transition. In the ECM, all analytes were only identified using one ion transition, although the method described a primary and confirmatory ion pair transition for ethaboxam (pp. 11-12, 14-15 of MRID 49489941). Typically, a confirmatory method is not required where GC/MS and LC/MS methods are used as the primary method(s) to generate study data.

7. The minor ILV modifications of the ECM to the extraction procedure (volume increase of the final sample) and optimization of the analytical parameters were considered insignificant modifications of the ECM and had no effect on the outcome of the study (pp. 18-22; Appendix 2, Appendix 2, pp. 146-149 of MRID 49489921). The ECM method noted that different volumes could be used in the final extract as long as the same proportions were maintained (Appendix 2, p. 34 of MRID 49489941).

8. The ILV reported that communication with the study sponsor was only required during method development regarding the acceptability of preparation of different standards and calibration standards (p. 24 of MRID 49489921).

9. It was reported for the ILV that a complete sample set consisting of 13 samples required *ca*. 6 hours for laboratory personnel (p. 24 of MRID 49489921).

V. References

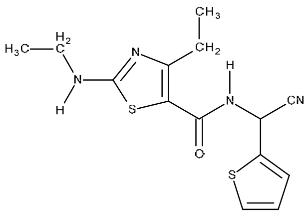
U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.

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

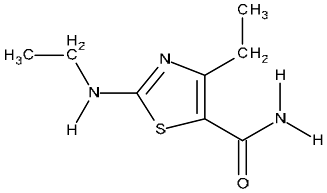
Ethaboxam (LGC-30473)

IUPAC Name:	(RS)-N-(α-cyano-2-thenyl)-4-ethyl-2-(ethylamino)-1,3-thiazole-5-carboxamide
CAS Name:	N-(cyano-2-thienylmethyl)-4-ethyl-2-(ethylamino)-5-thiazolecarboxamide
CAS Number:	162650-77-3
SMILES String:	CCc1c(sc(n1)NCC)C(=O)NC(C#N)c2cccs2



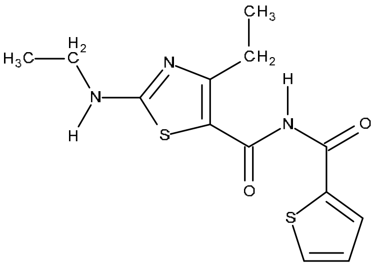
LGC-32523 (Thiazole carboxamide)

IUPAC Name:	4-Ethyl-2-(ethylamino)-1,3-thiazole-5-carboxamide
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	CCc1c(sc(n1)NCC)C(=O)N



LGC-32533 (α-carbonyl LGC-30473)

IUPAC Name:	4-Ethyl-2-(ethylamino-N-(2-thienylcarbonyl)-1,3-thiazole-5-carboxamide
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	CCc1c(sc(n1)NCC)C(=O)NC(=O)c2cccs2



LGC-32799 (Di-hydro-amido LGC-30473)

IUPAC Name:	N-[cyano(2-thienyl)methylene]-4-ethyl-2-(ethylamino)-1,3-thiazole-5-carboxamide
CAS Name:	Not reported
CAS Number:	Not reported
SMILES String:	CCc1c(sc(n1)NCC)C(=O)/N=C(/C#N)\c2cccs2