

Analytical method for fluometuron and its metabolites des-methyl-fluometuron and CGA72903 in soil

Reports: ECM: EPA MRID No.: MRID 50556601. Geschke, S. 2012. Validation of an Analytical Method for Determination of Fluometuron and its Metabolites Des-methyl-Fluometuron and CGA72903 in Soil. Sponsor Study No.: 90014919. Study Code: S12-00098. Lab/Trial Phase Code: S12-00098-L1. Report prepared by Eurofins Agrosience Services, EcoChem GmbH, Niefern-Öschelbronn, Germany, and sponsored and submitted by Agan Chemical Manufacturers Ltd., Ashdod, Israel; 60 pages (including 2A). Final report issued July 12, 2012.

ILV: EPA MRID No.: MRID 50693102. Jutson, J.I. 2018. Independent Laboratory Validation of the Analytical Method for Determination of Fluometuron and its Metabolites (Des-methyl-Fluometuron and CGA 72903) in Soil by LC-MS/MS. Smithers Viscient Study No.: 14090.6107. ADAMA Reference No.: 90020122. Report prepared by Smithers Viscient, Wareham, Massachusetts, and sponsored by ADAMA Agan Ltd., Ashdod, Israel, and submitted by Agan Chemical Manufacturers Ltd., Ashdod, Israel, and Pyxis Registry Consulting, Inc., Gig Harbor, Washington (p. 3; Appendix 3, p. 119); 121 pages. Final report issued September 20, 2018.

Document No.: MRIDs 50556601 & 50693102

Guideline: 850.6100

Statements: ECM: The study was conducted in compliance with German and OECD Good Laboratory Practices (GLP) standards, which are also accepted by the European community, USA (FDA and EPA) and Japan (MHW, MAFF, and METI; p. 2; Appendix F, Figure 19, p. 59 of MRID 50556601). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2, 2A, 3; Appendix F, Figure 19, p. 59). The statement of authenticity was included with the Quality Assurance statement.

ILV: The study was conducted in compliance with USEPA FIFRA and OECD GLP standards, with the exception of the characterization of the test substances (p. 3 of MRID 50693102). Signed and dated Data Confidentiality, GLP and Quality Assurance statements were provided (pp. 2-3, 5). The statement of authenticity was included with the Quality Assurance statement.

Classification: This analytical method is classified as **supplemental**. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method and if the ILV soil matrices covered the range of soils used in the terrestrial field dissipation studies.


PC Code: 035503

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This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

Executive Summary

The analytical method, Agan Chemical Manufacturers, Ltd., Study No. 90014919, is designed for the quantitative determination of fluometuron and its metabolites des-methyl-fluometuron and CGA72903 in soil at the stated LOQ of 0.01 mg/kg. The LOQ is greater than the lowest toxicological level of concern in soil of 1.0×10^{-7} mg/kg for all three analytes. The ECM used one characterized soil; the ILV used two characterized soils. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method and if the ILV soil matrices covered the range of soils used in the terrestrial field dissipation studies. The ILV validated the ECM in the first trial for all three analytes in both soil matrices with the setting of the shaker speed, the minor modification of the LC mobile phase, and insignificant modifications of the MS parameters and analytical equipment. All submitted ILV and ECM data pertaining to precision, repeatability, reproducibility, linearity, and specificity was acceptable at the LOQ and $10 \times$ LOQ for all three analytes in both matrices.

Table 1. Analytical Method Summary

Analyte(s) by Pesticide	MRID		EPA Review	Matrix	Method Date (dd/mm/yyyy)	Registrant	Analysis	Limit of Quantitation (LOQ)
	Environmental Chemistry Method	Independent Laboratory Validation						
Fluometuron	MRID 50556601 ¹	MRID 50693102 ²		Soil	12/07/2012	Agan Chemical Manufacturers, Ltd. ³	LC/MS/MS	0.01 mg/kg
Des-Methyl-Fluometuron								
CGA72903								

1 In the ECM, the sandy loam soil [soil type 2.3; Batch No. F2.31610; pH 6.6 ± 0.5 (in 0.01M CaCl₂); $61.9 \pm 4.2\%$ sand, $29.3 \pm 3.6\%$ silt, $8.8 \pm 1.4\%$ clay; $0.97 \pm 0.07\%$ organic matter (Walkley Black)] was collected from and characterized by LUFA Speyer in Germany (USDA soil texture classification; p. 13; Appendix F, Figure 18, p. 58 of MRID 50556601).

2 In the ILV, the sandy loam soil [SMV 15Feb17 Soil-A; pH 7.8 (in 1:1 water:soil ratio); 73% sand, 22% silt, 5% clay; 0.48% organic matter (Walkley Black)] and loam soil [SMV 03Jan18 Soil-A; pH 7.3 (in 1:1 water:soil ratio); 42% sand, 40% silt, 18% clay; 1.7% organic matter (Walkley Black)] were characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification not specified; p. 17 of MRID 50693102). Soil source information was not provided.

3 c/o Pyxis Registry Consulting, Inc., Gig Harbor, Washington (p. 3; Appendix 3, p. 119 of MRID 50693102).

I. Principle of the Method

Soil (10 g dry wt.) was fortified with mixed fortification solutions (10 or 1 µg/mL solution) in 250-mL glass containers (pp. 12, 14-16 of MRID 50556601). The soil samples were extracted with 100 mL of acetonitrile:water (80:20, v:v) via shaking on a horizontal flatbed shaker for *ca.* 2 hours (shaker speed not reported). An aliquot (50 mL) of the extract was transferred to a 50-mL centrifuge bottle. After centrifugation (7800 rpm, equivalent to 6500 x g, for 5 minutes), an aliquot (0.5 mL) of the supernatant was transferred to a glass vial, diluted with 0.5 mL of water, and analyzed by LC/MS/MS.

Samples were analyzed for fluometuron, des-methyl-fluometuron, and CGA72903 using an Agilent HPLC coupled to a Sciex API5000 triple stage quadrupole mass spectrometer equipped with electrospray ionization (ESI) interface in the positive ion, multiple reaction monitoring (MRM) mode (pp. 15-18 of MRID 50556601). The following LC conditions were used: Agilent ZORBAX Eclipse XDB-C18 column (4.6 mm x 150 mm, 5 µm; oven temperature 40°C) + 4 mm guard column, mobile phase of (A) 0.5% formic acid in water and (B) 0.5% formic acid in acetonitrile [mobile gradient phase of percent A:B (v:v) at 0.00-0.50 min. 50:50, 5.00-7.00 min. 5:95, 7.01-9.00 min. 50:50], MS temperature 400°C, and injection volume of 50 µL. Expected retention times were *ca.* 5.2, 4.7, and 5.5 minutes for fluometuron, des-methyl-fluometuron, and CGA72903, respectively. Two ion pair transitions were monitored (quantifier and qualifier, respectively): m/z 233.0→159.9 and m/z 233.0→71.9 for fluometuron, m/z 219.2→162.0 and m/z 219.2→142.0 for des-methyl-fluometuron, and m/z 162.0→142.0 and m/z 162.0→92.9 for CGA72903.

The ILV performed the ECM method as written, except for horizontal flatbed shaker speed set to 300 rpm for *ca.* 2 hours since a shaker speed not reported, the use of 0.25% formic acid in water and 0.25% formic acid in acetonitrile for the LC mobile phase, modifications of the MS parameters, and insignificant modifications to the analytical equipment (pp. 16, 21-25 of MRID 50693102). Samples were analyzed for fluometuron, des-methyl-fluometuron, and CGA72903 using Shimadzu LC-20AD HPLC system coupled with a MDS Sciex API 5000 mass spectrometer equipped with a ESI Turbo V source. The LC/MS/MS parameters were the same as those of the ECM, except that injection volume was reduced to 20.0 µL and MS temperature 600°C. No guard column was specifically reported for the LC. Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 233→160 and m/z 233→72 for fluometuron, m/z 219→162 and m/z 219→142 for des-methyl-fluometuron, and m/z 162→142 and m/z 162→93 for CGA72903. Expected retention times were *ca.* 5.4, 4.8, and 5.7 minutes for fluometuron, des-methyl-fluometuron, and CGA72903, respectively.

In the ECM and ILV, the Limit of Quantification (LOQ) was 0.01 mg/kg for fluometuron, des-methyl-fluometuron and CGA72903 in soil (p. 22 of MRID 50556601; pp. 26-30 of MRID 50693102). In the ECM, the Limit of Detection (LOD) was defined as 0.003 mg/kg (30% of the LOQ). In the ILV, the LOD was calculated to be 0.002 mg/kg for all three analytes.

II. Recovery Findings

ECM (MRID 50556601): Mean recoveries and RSDs were within guidelines (mean 70-120%; RSD $\leq 20\%$) for analysis of fluometuron, des-methyl-fluometuron, and CGA72903 at fortification levels of 0.01 mg/kg (LOQ) and 0.10 mg/kg (10 \times LOQ) in one soil matrix (Tables 4-6, pp. 21-22). Two ion pair transitions were monitored for fluometuron, des-methyl-fluometuron and CGA72903 using LC/MS/MS in positive mode; the quantification and confirmation ion data was comparable for all analytes/matrices. The sandy loam soil [soil type 2.3; Batch No. F2.31610; pH 6.6 ± 0.5 (in 0.01M CaCl₂); $61.9 \pm 4.2\%$ sand, $29.3 \pm 3.6\%$ silt, $8.8 \pm 1.4\%$ clay; $0.97 \pm 0.07\%$ organic matter (Walkley Black)] was collected from and characterized by LUFA Speyer in Germany (USDA soil texture classification; p. 13; Appendix F, Figure 18, p. 58).

ILV (MRID 50693102): Mean recoveries and RSDs were within guidelines (mean 70-120%; RSD $\leq 20\%$) for analysis of fluometuron, des-methyl-fluometuron, and CGA72903 at fortification levels of 0.01 mg/kg (LOQ) and 0.10 mg/kg (10 \times LOQ) in two soil matrices (Tables 1-12, pp. 35-46). Two ion pair transitions were monitored for fluometuron, des-methyl-fluometuron and CGA72903 using LC/MS/MS in positive mode; the quantification and confirmation ion data was comparable. The sandy loam soil [SMV 15Feb17 Soil-A; pH 7.8 (in 1:1 water:soil ratio); 73% sand, 22% silt, 5% clay; 0.48% organic matter (Walkley Black)] and loam soil [SMV 03Jan18 Soil-A; pH 7.3 (in 1:1 water:soil ratio); 42% sand, 40% silt, 18% clay; 1.7% organic matter (Walkley Black)] were characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification not specified; p. 17). Soil source information was not provided. The ILV validated the ECM in the first trial for all three analytes in both soil matrices with the horizontal flatbed shaker speed set to 300 rpm for *ca.* 2 hours since a shaker speed not reported, the substitution of 0.25% formic acid in water and 0.25% formic acid in acetonitrile for the LC mobile phase, and insignificant modifications of the MS parameters and analytical equipment (pp. 16, 21-25, 29).

Table 2. Initial Validation Method Recoveries for Fluometuron, Des-Methyl-Fluometuron and CGA72903 in Soil

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%) ¹	Relative Standard Deviation (%)
Sandy Loam Soil²						
Quantitation ion ³						
Fluometuron	0.01 (LOQ)	5	92-98	96	2	2
	0.10	5	90-95	93	2	2
Des-Methyl-Fluometuron	0.01 (LOQ)	5	91-92	92	1	1
	0.10	5	92-96	94	1	2
CGA72903	0.01 (LOQ)	5	95-98	96	2	2
	0.10	5	93-97	95	2	2
Confirmation (Qualifier) ion ³						
Fluometuron	0.01 (LOQ)	5	95-91	92	2	2
	0.10	5	93-97	96	2	2
Des-Methyl-Fluometuron	0.01 (LOQ)	5	94-99	97	2	2
	0.10	5	93-94	94	1	1
CGA72903	0.01 (LOQ)	5	91-95	93	2	2
	0.10	5	94-98	96	2	2

Data (uncorrected recovery results; pp. 26-27) were obtained from Tables 4-6, pp. 21-22 of MRID 50556601 and DER Attachment 2.

1 Reported values for standard deviation were reviewer-calculated because the study author did not provide these values (see DER Attachment 2). Rules of significant figures were followed.

2 The sandy loam soil [soil type 2.3; Batch No. F2.31610; pH 6.6 ± 0.5 (in 0.01M CaCl₂); $61.9 \pm 4.2\%$ sand, $29.3 \pm 3.6\%$ silt, $8.8 \pm 1.4\%$ clay; $0.97 \pm 0.07\%$ organic matter (Walkley Black)] was collected from and characterized by LUFA Speyer in Germany (USDA soil texture classification; p. 13; Appendix F, Figure 18, p. 58).

3 Two ion pair transitions were monitored (quantifier and qualifier, respectively): m/z 233.0→159.9 and m/z 233.0→71.9 for fluometuron, m/z 219.2→162.0 and m/z 219.2→142.0 for des-methyl-fluometuron, and m/z 162.0→142.0 and m/z 162.0→92.9 for CGA72903.

Table 3. Independent Validation Method Recoveries for Fluometuron, Des-Methyl-Fluometuron, and CGA72903 in Soil

Analyte	Fortification Level (mg/kg)	Number of Tests	Recovery Range (%)	Mean Recovery (%)	Standard Deviation (%)	Relative Standard Deviation (%)
Sandy Loam Soil¹						
Quantitation ion ²						
Fluometuron	0.01 (LOQ)	5	93.5-97.5	95.7	1.69	1.77
	0.10	5	88.4-99.5	93.4	4.48	4.79
Des-Methyl-Fluometuron	0.01 (LOQ)	5	93.3-99.9	96.9	2.94	3.03
	0.10	5	90.9-97.0	93.6	2.82	3.02
CGA72903	0.01 (LOQ)	5	94.4-97.1	96.1	1.05	1.09
	0.10	5	87.7-98.8	94.2	4.02	4.27
Confirmation ion ²						
Fluometuron	0.01 (LOQ)	5	94.7-107	103	4.84	4.71
	0.10	5	93.6-101	97.2	2.91	2.99
Des-Methyl-Fluometuron	0.01 (LOQ)	5	92.7-98.9	96.6	2.49	2.58
	0.10	5	89.9-96.1	93.6	2.45	2.62
CGA72903	0.01 (LOQ)	5	96.0-98.4	96.9	0.920	0.950
	0.10	5	88.8-99.6	94.5	3.84	4.06
Loam Soil¹						
Quantitation ion ²						
Fluometuron	0.01 (LOQ)	5	94.8-98.5	97.0	1.44	1.49
	0.10	5	95.3-98.4	96.7	1.23	1.27
Des-Methyl-Fluometuron	0.01 (LOQ)	5	92.7-97.8	96.0	2.13	2.22
	0.10	5	96.0-100	97.8	1.48	1.51
CGA72903	0.01 (LOQ)	5	92.1-95.2	93.7	1.28	1.36
	0.10	5	95.1-98.1	96.8	1.14	1.17
Confirmation ion ²						
Fluometuron	0.01 (LOQ)	5	96.6-101	98.3	1.82	1.85
	0.10	5	98.9-99.5	99.1	0.230	0.232
Des-Methyl-Fluometuron	0.01 (LOQ)	5	94.0-98.6	96.9	1.94	2.01
	0.10	5	96.0-97.9	97.1	0.702	0.723
CGA72903	0.01 (LOQ)	5	93.5-97.5	95.8	1.68	1.76
	0.10	5	96.0-97.8	96.9	0.657	0.678

Data (uncorrected recovery results; pp. 20-21) were obtained from Tables 1-12, pp. 35-46 of MRID 50693102.

1 The sandy loam soil [SMV 15Feb17 Soil-A; pH 7.8 (in 1:1 water:soil ratio); 73% sand, 22% silt, 5% clay; 0.48% organic matter (Walkley Black)] and loam soil [SMV 03Jan18 Soil-A; pH 7.3 (in 1:1 water:soil ratio); 42% sand, 40% silt, 18% clay; 1.7% organic matter (Walkley Black)] were characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification not specified; p. 17). Soil source information was not provided.

2 Two ion pair transitions were monitored (quantitation and confirmation, respectively): m/z 233→160 and m/z 233→72 for fluometuron, m/z 219→162 and m/z 219→142 for des-methyl-fluometuron, and m/z 162→142 and m/z 162→93 for CGA72903; these were similar to those of the ECM.

III. Method Characteristics

In the ECM and ILV, the LOQ was 0.01 mg/kg for fluometuron, des-methyl-fluometuron and CGA72903 in soil (p. 22 of MRID 50556601; pp. 26-30 of MRID 50693102). In the ECM and ILV, the LOQ was defined as the lowest fortification level for which mean recoveries were 70-120%, RSD was $\leq 20\%$, and blanks did not exceed 30%. No calculations were provided for the LOQ in the ECM or ILV. In the ECM, the LOD was defined as 0.003 mg/kg (30% of the LOQ). In the ILV, the LOD was calculated using three times the signal-to-noise value of the control samples (equation and values not reported). In the ILV, the LOD was also defined as the lowest concentration in test samples which can be detected based on the concentration of the low calibration standard and the dilution factor of the control solutions using the following equation:

$$\text{LOD} = \text{LOD}_{\text{LCAL}} \times \text{DF}_{\text{CTRL}}$$

Where, LOD is the limit of detection of the analysis, LOD_{LCAL} is the lowest concentration calibration standard (0.100 $\mu\text{g/L}$), and DF_{CTRL} is the dilution factor of the control samples (smallest dilution factor used, i.e., 20.0). In the ILV, the LOD was calculated to be 0.002 mg/kg for all three analytes.

Table 4. Method Characteristics for Fluometuron, Des-Methyl-Fluometuron and CGA72903 in Soil

Parameter		Fluometuron	Des-Methyl-Fluometuron	CGA72903
Limit of Quantitation (LOQ)	ECM	0.01 mg/kg		
	ILV			
Limit of Detection (LOD)	ECM (Method)	0.003 mg/kg (30% of the LOQ)		
	ILV (Calculated)	0.002 mg/kg		
Linearity (calibration curve r^2 and concentration range) ¹	ECM	$r^2 = 0.9984$ (Q) $r^2 = 0.9972$ (C)	$r^2 = 0.9990$ (Q) $r^2 = 0.9984$ (C)	$r^2 = 0.9986$ (Q) $r^2 = 0.9988$ (C)
	ILV	$r^2 = 0.9980$ (Q & C)	$r^2 = 1.0000$ (Q & C)	$r^2 = 1.0000$ (Q & C)
	Range	0.1-10 ng/mL		
Repeatable	ECM ²	Yes at LOQ and 10×LOQ. (one characterized soil matrix used)		
	ILV ^{3,4}	Yes at LOQ and 10×LOQ. (two characterized soil matrices used)		
Reproducible		Yes at LOQ and 10×LOQ.		
Specific	ECM	Yes, no matrix interferences were observed.		Yes, no matrix interferences were observed; elevated baseline noted.
	ILV	Yes, no matrix interferences were observed, but some minor contaminants were observed. Some non-uniform peak integration noted in the C ion.	Yes, no matrix interferences were observed, but some minor contaminants were observed.	

Data were obtained from p. 22 (LOQ/LOD); Tables 4-6, pp. 21-22 (recovery results); p. 22; Appendix C, Figures 1-6, pp. 32-37 (calibration data & curves); Appendix E, Figures 10-14, pp. 40-54 (chromatograms) of MRID 50556601; pp. 26-30 (LOQ/LOD); Tables 1-12, pp. 35-46 (recovery results); p. 30 (calibration data); Figures 1-42, pp. 59-100 (calibration curves & chromatograms) of MRID 50693102; DER Attachment 2. Q = quantitation ion; C = confirmation ion. All results reported for Q and C ions unless specified otherwise. All results reported for both test matrices in each study unless specified otherwise.

1 Reported r^2 values were reviewer-calculated from r values provided in the study report (Appendix C, Figures 1-6, pp. 32-37 of MRID 50556601; p. 30 of MRID 50693102; DER Attachment 2). Reported ILV values include sample set correlation data from both soils.

2 In the ECM, the sandy loam soil [soil type 2.3; Batch No. F2.31610; pH 6.6 ± 0.5 (in 0.01M CaCl₂); $61.9 \pm 4.2\%$ sand, $29.3 \pm 3.6\%$ silt, $8.8 \pm 1.4\%$ clay; $0.97 \pm 0.07\%$ organic matter (Walkley Black)] was collected from and characterized by LUFA Speyer in Germany (USDA soil texture classification; p. 13; Appendix F, Figure 18, p. 58 of MRID 50556601).

3 In the ILV, the sandy loam soil [SMV 15Feb17 Soil-A; pH 7.8 (in 1:1 water:soil ratio); 73% sand, 22% silt, 5% clay; 0.48% organic matter (Walkley Black)] and loam soil [SMV 03Jan18 Soil-A; pH 7.3 (in 1:1 water:soil ratio); 42% sand, 40% silt, 18% clay; 1.7% organic matter (Walkley Black)] were characterized by Agvise Laboratories, Northwood, North Dakota (USDA soil texture classification not specified; p. 17 of MRID 50693102). Soil source information was not provided.

4 The ILV validated the ECM in the first trial for all three analytes in both soil matrices with the horizontal flatbed shaker speed set to 300 rpm for *ca.* 2 hours since a shaker speed not reported, the substitution of 0.25% formic acid in water and 0.25% formic acid in acetonitrile for the LC mobile phase, and insignificant modifications of the MS parameters and analytical equipment (pp. 16, 21-25, 29 of MRID 50693102).

IV. Method Deficiencies and Reviewer's Comments

1. It could not be determined if the ILV was provided with the most difficult matrix with which to validate the method. While the ILV soil matrices were fairly diverse in sand/silt/clay ratios, the percent organic matter was similar between the two ILV soils (sandy loam soil, 0.48% organic matter; loam soil, 1.7% organic matter; p. 17 of MRID 50693102). Additionally, soil source information was not provided. It could not be determined if the ILV soil matrices covered the range of soils used in the terrestrial field dissipation studies since no fluometuron terrestrial field dissipation studies were submitted.
2. Minor contaminants were observed in ILV representative chromatograms (Figures 1-42, pp. 59-100 of MRID 50693102).
3. The communications between the ILV and Study Monitor (Janelle Kay of Pyxis Registry Consulting, Inc., Gig Harbor, Washington, as an agent for ADAMA Agan Ltd.) were reportedly limited to approval of the protocol and method, timing updates, and the results of the first attempt of the ILV (p. 27; Appendix 3, pp. 115-121 of MRID 50693102). A complete summary list of communication was provided.
4. The reviewer reported the texture of the ILV "loamy sand" soil and loam soil based on the soil texture calculator (42% sand, 40% silt, 18% clay; p. 17 of MRID 50693102). The reviewer noted that, although the GLP soil characterization was performed by Agvise Laboratories, Northwood, North Dakota, USDA soil texture classification not specified.
5. The reviewer considered all ILV modifications to be optimization of the LC/MS/MS parameters, which is usually considered to be adjusted from laboratory to laboratory, unless the ILV stated that the original LC/MS/MS parameters were defective. The only other ILV modification was the choice of shaking speed since the speed was not reported in the ECM. The reviewer determined that an updated ECM was not required.
6. The determinations of the LOD and LOQ in the ECM and ILV were not based on scientifically acceptable procedures as defined in 40 CFR Part 136 (p. 22 of MRID 50556601; pp. 26-30 of MRID 50693102). In the ECM and ILV, the LOQ was defined as the lowest fortification level for which mean recoveries were 70-120%, RSD was $\leq 20\%$, and blanks did not exceed 30%. No calculations were provided for the LOQ in the ECM or ILV. In the ECM, the LOD was defined as 0.003 mg/kg (30% of the LOQ). In the ILV, the LOD was calculated using three times the signal-to-noise value of the control samples (equation and values not reported). In the ILV, the LOD was also defined as the lowest concentration in test samples which can be detected based on the concentration of the low calibration standard and the dilution factor of the control solutions using the following equation: $LOD = LOD_{LCAL} \times DF_{CTRL}$, where, LOD is the limit of detection of the analysis, LOD_{LCAL} is the lowest concentration calibration standard (0.100 $\mu\text{g/L}$), and DF_{CTRL} is the dilution factor of the control samples (smallest dilution factor used, i.e., 20.0). In the ILV, the LOD was calculated to be 0.002 mg/kg for all three analytes. Detection limits should not be based on arbitrary values.
7. In the ILV and ECM, it was reported that matrix effects were insignificant ($< 20\%$

difference from non-matrix standards) for fluometuron, CGA72903 and des-methyl-fluometuron for each mass transition for the soils tested (p. 23; Appendix B, Tables 10-15, pp. 29-31 of MRID 50556601; p. 30; Tables 13-24, pp. 47-58 of MRID 50693102). Solvent-based calibration standards were used.

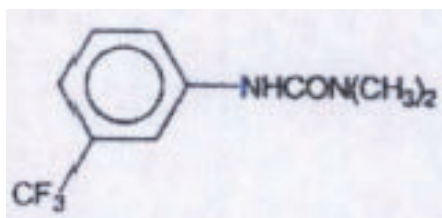
8. In the ECM, it was stated that storage stability was not tested (p. 23 of MRID 50556601).
9. The time required to complete the method for a set of 26 samples (ten fortified samples, two unfortified samples, one reagent blank, six matrix effects standards, and seven calibration standards) was reported as one working day (eight hours) in the ILV (p. 27 of MRID 50693102).

V. References

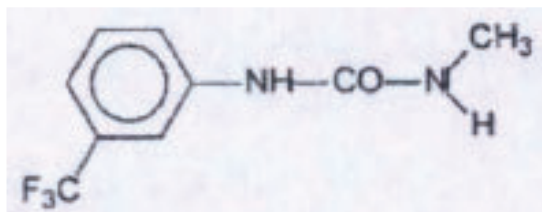
- U.S. Environmental Protection Agency. 2012. Ecological Effects Test Guidelines, OCSPP 850.6100, Environmental Chemistry Methods and Associated Independent Laboratory Validation. Office of Chemical Safety and Pollution Prevention, Washington, DC. EPA 712-C-001.
- 40 CFR Part 136. Appendix B. Definition and Procedure for the Determination of the Method Detection Limit-Revision 1.11, pp. 317-319.

Attachment 1: Chemical Names and Structures**Fluometuron**

IUPAC Name: Not reported
CAS Name: N,N-dimethyl-N'-[3-(trifluoromethyl)phenyl]urea
CAS Number: 2164-17-2
SMILES String: Not found

**Des-Methyl-Fluometuron**

IUPAC Name: Not reported
CAS Name: 1-Methyl-1-hydrogen-3-(a,a,a-trifluoro-m-tolyl)urea
CAS Number: 3032-404
SMILES String: Not found

**CGA72903**

IUPAC Name: Not reported
CAS Name: 3-Trifluoromethyl aniline (TFMA)
CAS Number: 98-16-8
SMILES String: Not found

