Addendum No. 1 to MRIDs 46902209 and 46902210

Study Title:
MRID 46902209: Validation of BASF method number DO506 for determination of metconazole (BAS 555 F) and its metabolites M11, M21, M30 and triazol in soil using LC/MS/MS.

MRID 46902210: Independent method validation of BASF analytical method D0506: method for determination of metconazole (BAS 555 F) and its metabolites M11, M21, M30 and triazol in soil using LC/MS/MS.

Guideline Number: 850.6100
PC code: 125619

Reasons for changes:

- These environmental chemistry method (ECM) and independent laboratory validation (ILV) reports are classified as **Acceptable**. For each of the five analytes, the method limit of detection (LOD) is 1 µg/Kg and the limit of quantitation (LOQ) is 10 µg/Kg. **Linearity** is established in the calibration (y=a+bx) using external standards with adequate correlation coefficient ($r^2 > 0.995$) for all analytes. The method in general satisfies the **repeatability** and **reproducibility** criteria with RSDs ≤20% and mean recoveries are in the range of 70-120% except for the triazole (65%-112%) (**Table 1**).

Table 1. Summary of % recovery for ECM (MRID 46902209) and ILV (MRID 46902210)

	ECM (n = 10)		ILV (n = 10)	
Compound	**% Recovery Mean and SD**	**% Recovery Range**	**% Recovery Mean and SD**	**% Recovery Range**
	North Dakota Loam		-[1]	
cis-metconazole	92 ± 5%	80-98%	-	-
trans-metconazole	92 ± 7%	83-103%	-	-
M11	95 ± 14%	75-114%	-	-
M21	102 ± 9%	90-114	-	-
M30	95 ± 12%	81-119%	-	-
triazole	77 ± 6%	66-88%	-	-
	Oklahoma Loamy Sand		Loamy Sand	
cis-metconazole	103 ± 11%	78-122%	98.7 ± 3.46%	93.8-105.8%
trans-metconazole	100 ± 9%	88-115%	100.0 ± 5.33%	93.8-113.8%
M11	102 ± 6%	92-110%	108.8 ± 5.06%	102.0-118.3%
M21	105 ± 11%	86-129%	102.7 ± 4.81%	93.5-110.8%
M30	96 ± 9%	77-109%	112.9 ± 5.69%	100.8-120.8%
triazole	99 ± 11%	78-114%	85.9 ± 11.56%	65.2-112.7%
	Mississippi Silt Loam		-	
cis-metconazole	89 ± 9%	79-102%	-	-
trans-metconazole	96 ± 4%	88-100%	-	-
M11	93 ± 10%	73-107%	-	-
M21	97 ± 9%	85-113%	-	-
M30	88 ± 8%	72-96%	-	-
triazole	89 ± 11%	74-110%	-	-

[1] - = No Data

References

Nejad, H. 2006. Validation of BASF method number D0506 for determination of metconazole (BAS 555 F) and its metabolites M11, M21, M30 and triazol in soil using LC/MS/MS. Unpublished study performed, sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina. BASF Study No.: 138074 and Registration Document No.: 2006/7006766. Experimental start date February 9, 2006, and termination date March 22, 2006 (p. 10). Final report issued June 20, 2006. MRID 46902209

Ibrahim, A. and R. Hauser. 2006. Independent method validation of BASF analytical method D0506: method for determination of metconazole (BAS 555 F) and its metabolites M11, M21, M30 and triazol in soil using LC/MS/MS. Unpublished study performed by ADPEN Laboratories, Inc., Jacksonville, Florida; sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina. ADPEN Study No.: 2K6-238588 (p. 7). BASF Study No.: 238588 and Registration Document No.: 2006/7007031 (pp. 1, 7). Experimental start date March 17, 2006, and termination date March 30, 2006 (p. 7). Final report issued June 21, 2006. MRID 46902210

HE ZHONG

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DN: c=US, o=U.S. Government, ou=USEPA, ou=Staff, cn=HE ZHONG, dnQualifier=0000051219
Date: 2015.11.17 15:22:34 -05'00'

Revised by: He Zhong Date: 11-17-15

Secondarily reviewed by: Greg Orrick Date: 11-17-15

Data Evaluation Record on method validation for metconazole and its transformation products M11, M21, M30 and triazole in soil

PMRA Submission Number {.....} EPA MRID Number 46902210

Data Requirement: PMRA Data Code:
EPA DP Barcode: D331927
OECD Data Point:
EPA Guideline: OPPTS 850.7100.

Test material:
Common name: Metconazole.
Chemical name:
IUPAC name: (1RS,5RS,1RS,5SR)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol. (1RS,5RS[cis isomer];1RS,5SR[trans isomer])-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl) cyclopentanol.
CAS name: 5-[(4-Chlorophenyl)methyl]-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol.
CAS No.: 125116-23-6.
Synonyms: BAS 555 F; CL 354,801 (cis); CL 354,802 (trans); AC 900,768; WL 148,271; Caramba; CL900768; KNF-S-474; M0.
Smiles string: c1cc(Cl)ccc1CC2CCC(C)(C)C2(O)CN3N=CN=C3 (EPI Suite, v3.12 SMILES String).

Primary Reviewer: Lynne Binari
Cambridge Environmental
Signature:
Date: 1/16/07

Secondary Reviewer: Kathleen Ferguson
Cambridge Environmental
Signature:
Date: 1/16/07

QC/QA Manager: Joan Gaidos
Cambridge Environmental
Signature:
Date: 1/16/07

Final Reviewer: James Lin
EPA Reviewer
Signature:
Date: 28/01/07

Company Code:
Active Code:
Use Site Category:
EPA PC Code: 125619.

CITATION: Ibrahim, A. and R. Hauser. 2006. Independent method validation of BASF analytical method D0506: method for determination of metconazole (BAS 555 F) and its metabolites M11, M21, M30 and triazol in soil using LC/MS/MS. Unpublished study performed

Data Evaluation Record on method validation for metconazole and its transformation products M11, M21, M30 and triazole in soil

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EPA DP Barcode: D331927
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EPA Guideline: OPPTS 850.7100.

Test material:
Common name: Metconazole.
Chemical name:
IUPAC name: (1RS,5RS,1RS,5SR)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol. (1RS,5RS[cis isomer];1RS,5SR[trans isomer])-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl) cyclopentanol.
CAS name: 5-[(4-Chlorophenyl)methyl]-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol.
CAS No.: 125116-23-6.
Synonyms: BAS 555 F; CL 354,801 (cis); CL 354,802 (trans); AC 900,768; WL 148,271; Caramba; CL900768; KNF-S-474; M0.
Smiles string: c1cc(Cl)ccc1CC2CCC(C)(C)C2(O)CN3N=CN=C3 (EPI Suite, v3.12 SMILES String).

Primary Reviewer: Lynne Binari
Cambridge Environmental
Signature: Lynne Binari
Date: 1/16/07

Secondary Reviewer: Kathleen Ferguson
Cambridge Environmental
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QC/QA Manager: Joan Gaidos
Cambridge Environmental
Signature: JGS
Date: 1/16/07

Final Reviewer: Amer ~~Al-~~Mudallal
EPA Reviewer
Signature:
Date:

Company Code:
Active Code:
Use Site Category:
EPA PC Code: 125619.

CITATION: Ibrahim, A. and R. Hauser. 2006. Independent method validation of BASF analytical method D0506: method for determination of metconazole (BAS 555 F) and its metabolites M11, M21, M30 and triazol in soil using LC/MS/MS. Unpublished study performed

by ADPEN Laboratories, Inc., Jacksonville, Florida; sponsored and submitted by BASF Corporation, Research Triangle Park, North Carolina. ADPEN Study No.: 2K6-238588 (p. 7). BASF Study No.: 238588 and Registration Document No.: 2006/7007031 (pp. 1, 7). Experimental start date March 17, 2006, and termination date March 30, 2006 (p. 7). Final report issued June 21, 2006.

EXECUTIVE SUMMARY

An independent laboratory validation of a method used to detect and quantify (1RS,5RS[cis];1RS,5SR[trans])-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl) cyclopentanol (metconazole, BAS 555 F) and its transformation products

- (1RS,5SR-5-[R-(4-chlorobenzyl)(hydroxy) methyl]-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl) cyclopentanol (R benzylic alcohol, M11, KNF-474-M-11, CL 382390),
- (1RS,5RS-5-[R-(4-chlorobenzyl)(hydroxy) methyl]-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl) cyclopentanol (R benzyl alcohol, M21, KNF-474-M-21, CL 382391),
- methanone,4-chlorophenyl-[2-hydroxy-3,3-dimethyl-2-(1H-1,2,4-triazol-1-ylmethyl) cyclopentyl]-,cis-(±)-(9Cl) (R benzyl ketone, M30, KNF-474-M-30, CL 382389), and
- 1,2,4-(1H)-triazole (triazole, BF 480-16, CL 196719)

in soil was performed to support registration of metconazole (p. 7; Figure 1, pp. 22-24; Appendix 3, pp. 97-98). This method validation was conducted in accordance with USEPA OPPTS 850.7100 "Public Draft" - Data Reporting Guideline for Environmental Chemistry Methods and in compliance with 40 CFR Part 160 GLP Standards (p. 3; Appendix 3, pp. 87-88). Aliquots (10 ± 0.1 g) of loamy sand soil (0-15 cm, pH 7.0, organic matter 0.7%, clay 10%, bulk density 1.23 g/cm^3, CEC 6.7 meq/100 g, moisture at 1/3 bar 6.9%; Table VII, p. 36 of MRID 46902206) from Oklahoma were weighed into 250-mL centrifuge bottles, then cis-metconazole (purity ≥98.8%, Lot/Batch No.: AC8879-136A; trans BAS 555 F, purity ≥98.6%, Lot/Batch No.: AC9339-122A), M11 (purity 98.5%, Lot/Batch No.: AS 2106a), M21 (purity 98.3%, Lot/Batch No.; AS 2110a), M30 (purity 98.4%, Lot/Batch No.: AS 2111a) and triazole (purity 99.0%, Lot/Batch No.: AC10194-134) were each applied at 10 and 100 µg a.i./kg (five treated soil samples per application rate; pp. 10-11; Appendix 2, pp. 72-79; Appendix 3, pp. 102-103). Metconazole (cis, trans), M11, M21 and M30 in acetone were applied as a mixed fortification solution, while triazole in 0.05% aqueous formic acid was applied separately; all application solution volumes were 0.1 mL (Appendix 3, pp. 102-103). Fortified soil samples were extracted once with methanol:0.2N HCl (90:10, v:v) followed by once with methanol:0.2N HCl (50:50, v:v); extraction solvent volumes were 100 mL (p. 11; Appendix 3, p. 103). Each extraction was done using a reciprocal shaker (moderate speed, rpm not specified) for 60 minutes for the initial extraction and 20 minutes for the second extraction, then extract and soil were separated via centrifugation (*ca.* 5,000 rpm, 10 minutes). Aliquots (10 mL) of each extract were combined and centrifuged (*ca.* 2,000 rpm, 10 minutes).

For determination of cis/trans-metconazole, M11, M21 and M30, an aliquot (2 mL) of the combined extract sample was diluted with aqueous 0.05% formic acid:methanol (60:40, v:v, 3 mL), vortexed and analyzed by LC/MS/MS under the following conditions: Phenomenex Luna C18 LC column [4.6 x 150 mm, 5 µm (reported 6 mm diameter typographical error)], gradient mobile phase combining (A) 0.1% aqueous formic acid and (B) 0.1% formic acid in acetonitrile [percent A:B at 0.0 min. 90:10 (v:v), 6.5 min. 30:70, 10.5 min. 10:90, 10.6-13.0 min. 90:10], injection volume 20 µL, flow rate 1.0 mL/minute, PE Sciex API 4000 MS, turbo ion spray

interface, ion mode positive, multiple reaction monitoring (Table 2, p. 20; Appendix 3, pp. 103-104).

For determination of triazole an aliquot (2 mL) of the extract was diluted with water (1 mL) and partitioned once (2 mL) with methylene chloride (p. 11, Appendix 3, p. 104). The resulting aqueous phase was taken to dryness (N-Evaporator, 45°C), then the resulting residues were reconstituted in water (2 mL) via vortex, centrifuged (*ca.* 2,000 rpm, 10 minutes) and analyzed by LC/MS/MS under the following conditions: Thermo Hypercarb column (4.6 x 50 mm, 3 µm), gradient mobile phase combining (A) 1% aqueous formic acid and (B) 1% formic acid in methanol [percent A:B at 0.0-2.0 min. 95:5 (v:v), 2.5-5.0 min. 10:90, 5.1-7.0 min. 95:5], injection volume 30 µL, flow rate 0.8 mL/minute, PE Sciex API 4000 MS, turbo ion spray interface, ion mode positive, multiple reaction monitoring (Table 2, p. 21; Appendix 3, pp. 105, 110).

Metconazole (cis/trans), M11, M21, M30 and triazole extracted from the fortified samples were identified by comparison to the retention times of reference standards (Figure 4, pp. 29-52; Figure 5, pp. 55-56, 58, 60, 62, 64). The limits of quantitation (LOQ) and detection (LOD) were set at 10 µg a.i./kg and 0.1 ng/mL, respectively for all test compounds (p. 11).

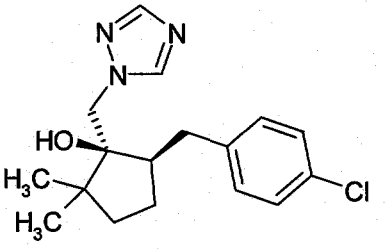
Overall recoveries from soil fortified at 10 and 100 µg a.i./kg averaged (n = 10) 98.7 ± 3.46% (range 93.8-105.8%) of the applied for cis-metconazole, 100.0 ± 5.33% (93.8-113.8%) for trans-metconazole, 108.8 ± 5.06% (102.0-118.3%) for M11, 102.7 ± 4.81% (93.5-110.8%) for M21, 112.9 ± 5.69% (100.8-120.8%) for M30 and 85.9 ± 11.56% (65.2-112.7%) for triazole (DER Attachment 1). Recoveries at the 10 µg a.i./kg and 100 µg a.i./kg fortification levels averaged (n = 5) 100.1 ± 3.68% (95.8-105.8%) and 97.3 ± 2.55% (93.8-100.8%), respectively, for cis-metconazole, 99.2 ± 3.25% (93.8-103.0%) and 100.9 ± 6.68% (95.5-113.8%), respectively, for trans-metconazole, 108.5 ± 6.69% (102.0-118.3%) and 109.1 ± 2.53% (105.3-112.3%), respectively, for M11, 99.3 ± 4.08% (93.5-104.0%) and 106.1 ± 2.54% (103.0-110.8%), respectively, for M21, 112.1 ± 7.01% (100.8-120.8%) and 113.7 ± 3.79% (107.8-118.3%), respectively, for M30, and 92.5 ± 10.94% (80.0-112.7%) and 79.3 ± 7.74% (65.2-87.3%), respectively, for triazole. Detector responses were linear for cis-metconazole (r^2 = 0.9999), trans-metconazole (r^2 = 0.9984), M11 (r^2 = 0.9995), M21 (r^2 = 0.9997) and M30 (r^2 = 1.000) over a range of 2-1,000 pg (0.1-50 ng/mL, based on 20 µL injection volume) and also for triazole (r^2 = 0.9983) over a range of 7.5-300 pg (0.25-10 ng/mL, based on 30 µL injection volume; Appendix 1, pp. 66-71). Chromatograms of reagent blank and unfortified control soil samples indicated there were no significant interfering peaks at the retention times for cis/trans-metconazole and its products M11, M21, M30 and triazole (Figure 5, pp. 53-54, 57, 59, 61, 63).

Attachment 1: Structure of Parent Compound and Transformation Products

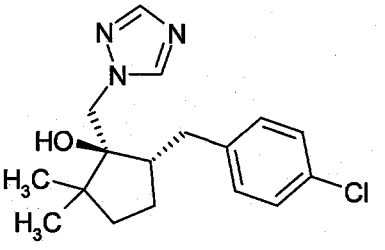
Metconazole [BAS 555 F; CL 354,801 (cis); CL 354,802 (trans); AC 900,768; WL 148,271; Caramba; CL 900768; KNF-S-474; M0]

IUPAC Name:	(1RS,5RS,1RS,5SR)-5-(4-Chlorobenzyl)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentanol.
CAS Name:	5-[(4-Chlorophenyl)methyl]-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol.
CAS Number:	125116-23-6
SMILES String:	c1cc(Cl)ccc1CC2CCC(C)(C)C2(O)CN3N=CN=C3 (EPI Suite, v3.12 SMILES String).

Cis-metconazole

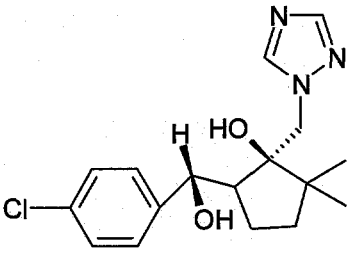


Trans-metconazole



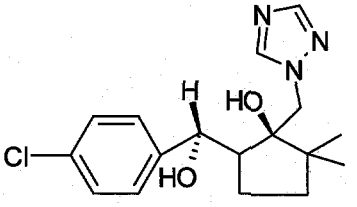
(1RS,5SR-5-[R-(4-Chlorobenzyl)(hydroxy) methyl]-2,2-dimethyl-1-(1H-1,2,4-triazol-1-yl-methyl)cyclopentanol [CL382390; M11; KNF-474-M-11; R Benzylic alcohol]

IUPAC Name: Not reported.
CAS Name: Not reported.
CAS Number: Not reported.



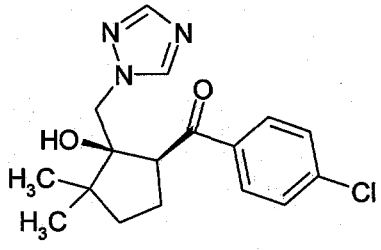
(1RS,5RS-5-[R-(4-Chlorobenzyl)(hydroxy) methyl]-2,2-dimethyl-1-(1H-1,2,4-triazol-1-yl-methyl)cyclopentanol [CL382391; M21; KNF-474-M-21; R Benzyl alcohol]

IUPAC Name: Not reported.
CAS Name: Not reported.
CAS Number: Not reported.



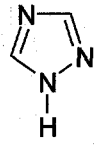
2-Hydroxy-3,3-dimethyl-2-[1,2,4]triazol-1-ylmethyl-cyclopentyl-(4-chlorophenyl)-methanone [CL382389; M30; KNF-474-M-30; R Benzyl Ketone]

IUPAC Name: Not reported.
CAS Name: Not reported.
CAS Number: Not reported.



1,2,4-(1H)-Triazole [Triazol; BF 480-16; CL 196719]

IUPAC Name: Not reported.
CAS Name: Not reported.
CAS Number: Not reported.



Attachment 2: Excel Spreadsheets

Chemical: Metconazole (BAS 555 F)
PC: 125619
MRID: 46902210
Guideline: OPPTS 850.7100

Method validation for metconazole (cis/trans-BAS 555 F) and its products M11, M21, M30 and triazole in Oklahoma loamy sand soil.
Confirmation of means/standard deviations for applied metconazole and its products.

	cis-Metconazole				trans-Metconazole				M11			
Fortified (ppb)	Measured (ppb)	Recovery (%)	Mean (%)	Std. dev.[1] (%)	Measured (ppb)	Recovery (%)	Mean (%)	Std. dev.[1] (%)	Measured (ppb)	Recovery (%)	Mean (%)	Std. dev.[1] (%)
10	9.725	97.3			9.375	93.8			10.475	104.8		
	9.875	98.8			9.850	98.5			11.825	118.3		
	10.275	102.8			10.300	103.0			10.200	102.0		
	10.575	105.8			10.200	102.0			11.475	114.8		
	9.575	95.8	100.1	3.68	9.850	98.5	99.2	3.25	10.275	102.8	108.5	6.69
100	95.250	95.3			113.750	113.8			105.250	105.3		
	100.750	100.8			101.000	101.0			112.250	112.3		
	99.250	99.3			96.750	96.8			109.500	109.5		
	93.750	93.8			95.500	95.5			107.250	107.3		
	97.250	97.3	97.3	2.55	97.500	97.5	100.9	6.68	111.000	111.0	109.1	2.53
Overall mean		98.7				100.0				108.8		
std.dev.		3.46				5.33				5.06		
maximum		105.8				113.8				118.3		
minimum		93.8				93.8				102.0		
n =		10				10				10		
	M21				M30				Triazole			
Fortified (ppb)	Measured (ppb)	Recovery (%)	Mean (%)	Std. dev.[1] (%)	Measured (ppb)	Recovery (%)	Mean (%)	Std. dev.[1] (%)	Measured (ppb)	Recovery (%)	Mean (%)	Std. dev.[1] (%)
10	10.250	102.5			11.150	111.5			11.267	112.7		
	10.100	101.0			10.075	100.8			8.000	80.0		
	10.400	104.0			11.800	118.0			8.867	88.7		
	9.350	93.5			12.075	120.8			8.800	88.0		
	9.550	95.5	99.3	4.08	10.950	109.5	112.1	7.01	9.333	93.3	92.5	10.94
100	105.750	105.8			107.750	107.8			87.333	87.3		
	105.250	105.3			118.250	118.3			83.333	83.3		
	105.750	105.8			114.000	114.0			83.333	83.3		
	103.000	103.0			111.500	111.5			65.200	65.2		
	110.750	110.8	106.1	2.54	117.000	117.0	113.7	3.79	77.333	77.3	79.3	7.74
Overall mean		102.7				112.9				85.9		
std.dev.		4.81				5.69				11.56		
maximum		110.8				120.8				112.7		
minimum		93.5				100.8				65.2		
n =		10				10				10		

[1]Standard deviations determined using "biased" or "n" method for entire sample population; study authors used "nonbiased" or "n-1" method.
Results from Appendix 1, pp. 66-71 of the study report.
Means and standard deviations calculated using Microsoft programs functions @average (A1:A2) and @stdevp(A1:A2).