DIFENOCONAZOLE (224)

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EXPLANATION

Difenoconazole is a systemic triazole fungicide and acts by inhibition of demethylation during ergosterol synthesis. It is applied by foliar spray or seed treatment and controls a broad-spectrum of foliar, seed and soil-borne diseases caused by *Ascomycetes*, *Basidiomycetes* and *Deuteromycetes* on a variety of crops. Difenoconazole was evaluated for the first time by JMPR 2007. The 2007 Meeting established an acceptable daily intake (ADI) of 0–0.01 mg/kg bw and an acute reference dose (ARfD) of 0.3 mg/kg bw. In 2007, 2010 and 2013, the JMPR evaluated the compound for residues and recommended a number of maximum residue levels.

Difenoconazole was listed by the 46th session of CCPR (2014) for evaluation for additional MRLs. The current Meeting received from the manufacturer additional analytical methods, processing data from soya beans, oilseed rape and rice, GAP information and residue trial data from uses on strawberry, avocado, soya beans, cotton, peanut, rice and oilseed rape (canola).

IDENTITY

The 2007 Meeting noted that the structural formula for difenoconazole contains two chiral carbons resulting in a *cis-trans* pair diastereoisomers. The current Meeting noted that the presented analytical methods not are stereo-selective for the *cis*- and *trans*- isomers

ANALYTICAL METHODS

The current Meeting received new analytical method descriptions and validation data for parent difenoconazole. Methods were validated for all crop matrices; the LOQ were 0.01 mg/kg for determination of difenoconazole with procedural recoveries by matrix in the range of 70–122% at various fortification levels. A summary of the analytical methods for difenoconazole is provided below.

Method, analyte	Matrix	Extraction	Clean-up	Detection, LOQ
Method REM 147.08 Difenoconazole	Plant material 2007 JMPR) Current Meeting: Validation data on oilseed rape seed, meal and refined oil.	Refluxing with methanol-ammonia for 2 hours. Elution with dichloromethane.	Solid-phase extraction (SPE)	LC-MS/MS Difenoconazole LOQ 0.01 mg/kg
Method POPIT MET.032 Difenoconazole	Plant material Validation data on soya beans and peanuts	high-speed homogenisation with a acetone/water mixture (2:1; v/v)	Filtration/centrifugation	HPLC-MS/MS Difenoconazole The ion transition m/z 406→251 is used for quantification LOQ 0.01 mg/kg
Method POPIT MET.033, rev.31 Difenoconazole	Plant material Validation data on avocado, cotton, peanut, rice, soya beans and	high-speed homogenisation with a acetone/water mixture (2:1; v/v)	Filtration/centrifugation	HPLC-MS/MS Difenoconazole The ion transition m/z 406→251 is used for quantification and

Method, analyte	Matrix	Extraction	Clean-up	Detection, LOQ
	strawberry			the ion transition m/z 406→111 for confirmation, LOQ 0.01 mg/kg

Plant materials

Oilseed rape (canola)

The analytical method REM 147.08 was validated for oilseed rape seed and the processed fraction meal and refined oil by Sagan, K (2012 SYN545192) for residues of difenoconazole with an LOQ of 0.01 mg/kg. The method REM 147.08 was reviewed by JMPR in 2007. The recovery (% recovery) and repeatability (RSD) is summarized in Table 1 below.

Table 1 Recovery and repeatability data for the method REM 147.08 for difenoconazole oilseed rape

Commodity	Fortification level (mg/kg)	No of analysis	Recovery (%)	Mean recovery (%)	% RSD
Oilseed Rape (seed)	0.010	4	81,121, 114, 112	107	17
	0.10	4	122, 118, 97, 102	110	11
	0.20	4	91 ,94 ,85	90	5.1
Oilseed Rape (meal)	0.010	3	98,89,100	96	6.1
	0.10	3	89, 103, 115	102	13
	0.20	3	85, 92, 84	87	5.0
Oilseed Rape (oil)	0.010	3	82, 100, 83	88	11
	0.10	3	98, 99,84	94	9.0

Other plant materials

The analytical method POPIT MET.032 was developed to determine and quantify difenoconazole in plant material by Vopi K *et al.* (2010 Syngenta file no. CGA169374_10882).

Residues of difenoconazole are extracted from plant matrices by high-speed homogenisation with an acetone/water mixture (2:1; v/v). The suspension is either filtrated (soya beans) or centrifuged (peanut) and brought to volume with extraction solvent. An aliquot of the extract is evaporated and reconstituted in acetonitrile/water (1:1; v/v). After filtration of the final sample solution, residues of difenoconazole are determined by liquid chromatography with tandem mass spectrometric detection (HPLC-MS/MS). Quantification is performed with calibration curves using 6 standard solutions (from 1×10^{-4} to 3.2×10^{-3} µg/mL, ½ to 16 times the LOQ). The ion transition m/z 406→251 is used for quantification. The method has a validated LOQ of 0.01 mg/kg for soya beans and peanut.

Table 2 Recovery and repeatability data for the method POPIT MET.032 for difenoconazole in soya beans and peanuts

Commodity	Fortification level (mg/kg)	No of analysis	Recovery (%)	Mean recovery (%)	% RSD
Soya (beans)	0.010	8	88; 91; 92; 93; 94; 96; 97; 99	94	3.8
	0.10	6	78; 83; 90; 92; 93; 95	89	7.5
	1.0	5	74; 77; 79; 79; 83	78	4.2
Peanut (kernels)	0.010	7	70; 72; 72; 72; 73; 74; 80	73	4
	0.11	5	70; 82; 83; 85; 90	82	9

The analytical method POPIT MET.033 was developed to determine and quantify difenoconazole in plant material by Maslowski, R *et al*. (2008 Syngenta file no. CGA169374_10881).

Residues of difenoconazole are extracted from plant matrices by high-speed homogenisation with an acetone/water mixture (2:1; v/v). The suspension is filtrated (cotton) and brought to volume by extraction solvent. After filtration of the final sample solution, residues of difenoconazole are determined by liquid chromatography with tandem mass spectrometric detection (HPLC-MS/MS). Quantification is performed with calibration curves using 6 standard solutions. The ion transition m/z 406→251 is used for quantification and the ion transition m/z 406-111 for confirmation. The method has a validated LOQ of 0.01 mg/kg for difenoconazole in avocado, cotton, peanut, rice, soya beans and strawberry.

The recovery (% recovery) and repeatability (RSD) of residues from parent difenoconazole in crop matrices in current evaluation for MRLs is summarized in Table 3 below.

Table 3 Recovery and repeatability data for the method POPIT MET.033 for difenoconazole in various plant matrices

Commodity	Fortification level (mg/kg)	No of analysis	Recovery (%)	Mean recovery (%)	% RSD
Avocado	0.011	7	81; 83; 83; 83; 84; 84; 87	84	2.2
	0.11	5	89; 90; 90; 90; 93	90	1.7
	2.2	5	89; 91; 91; 91; 92	91	1.2
Cotton	0.010	8	75; 79; 80; 81; 85; 88; 91; 92	84	7.3
	0.10	6	81; 82; 82; 82; 87; 91	84	4.7
Peanut	0.010	7	78; 81; 83; 85; 85; 86; 87	84	3.8
	0.10	3	88; 88; 88; 90; 90	89	1.2
Rice	0.01	7	94; 95; 96; 97; 99; 99; 103	98	3.1
	0.1	5	96; 101; 104; 105; 109	103	4.7
	0.51	5	102; 106; 107; 108; 109	106	2.5
Soya beans	0.011	7	90; 92; 92; 93; 94; 95; 96	93	2.2
	0.11	5	88; 88; 88; 89; 89	88	0.6
Strawberry	0.01	5	78; 79; 80; 80; 81; 83; 85	81	3.0
	0.1	5	76; 77; 77; 80; 80	78	2.4
	0.3	5	88; 88; 91; 91; 94	90	2.8
	2	5	87; 87; 91; 92; 95	90	3.8

USE PATTERN

Difenoconazole is a systemic fungicide which belongs to the triazole chemical group of fungicides. Information on registered uses including labels from countries trials had been carried out was provided to the Meeting by one manufacturer. The representative uses relating to crops under consideration for additional MRLs and revising some of the existing CODEX MRLs are summarized in the following table.

Table 4 Registered uses of difenoconazole from labels provided

Crop	Country	Application details								
		type	Met-hod	kg ai/ha	Water L/ha	Crop growth stage	No	Interval (days)	PHI	Comments
Straw-berry	USA	126 g ai/L SC	Foliar spray	0.129	> 94[a]; 140 [b]	Prior to disease onset when conditions conductive for disease	4	7-14	0	Not more than 2 sequential applications before alternating to another fungicide with different mode of action. max 0.21 kg ai/ha per crop and season
Avo-cado	Brazil	250 g ai/L EC	Foliar spray	0.050	500-1000	start at flowering, end when fruit is around 5 cm	4	14	14	ground/ aerial application
Soya bean	USA	126 g ai./L SC	Foliar spray	0.129	> 19[b]	Prior to disease onset when conditions conductive for disease	2	7-10	14	Do not feed soybean hay, forage or silage max 0.25 k g ai/ha per season
Rice	Italy	125 g ai/L SC	Foliar spray	0.125	200-400	BBCH 21-29[d]	2	-	28	ground application
Cotton	Brazil	250 g ai/L EC	Foliar spray	0.075	200-400	when first symptom occur	3	10-15	21	ground/ aerial application
Peanut	Brazil	250 g ai/L EC	Foliar spray	0.0875	100-200	when first symptom occur	3	-	22	ground/ aerial application
Oilseed Rape (canola)	Canada	250 g ai/L EC	Foliar spray	0.125	110-170[c]	BBCH 12-18[e] or BBCH 62-65[f]	1	-	30	ground/ aerial application max 0.125 kg ai/ha per season

[a] For aerial applications

[b] For ground applications

[c] When applying difenoconazole at typical herbicide timing it is recommended to use 50-110 L/ha water

[d] Between beginning and end of tillering.

[e] Apply during rosette stage between 2nd true leaf and bolting. (*Leptosphaeria maculans)* Virulent Black Leg

[f] Apply at 20-50% bloom *(Sclerotinia sclerotiorum)* Sclerotinia Stem Rot

Conditions of the supervised residue trials were generally well reported in detailed field reports. In most trials plots treated plots were not replicated but where results were reported from replicate plots, these are presented as individual values. Most field reports provided data on the sprayers used and their calibration, and reports provided data on plot size, residue sample size and sampling date. Although trials included control plots, no control data are recorded in the tables except where residues in control samples exceeded the LOQ. Residue data are recorded

unadjusted for % recovery. When residues were not detected they are shown as below the LOQ (e.g., < 0.01 mg/kg). Laboratory reports included methods validation with procedural recoveries from spiking at residue levels similar to those occurring in samples from the supervised trials. Data on duration of residue sample under storage were also provided. Residues and application rates have generally been rounded to two significant figures or, for residues near the LOQ, to one significant figure.

RESIDUES RESULTING FROM SUPERVISED TRIALS ON CROPS

Residues values from trials conducted according to a maximum registered GAP with supporting trials have been used for the estimation of maximum residue levels. The results included in the evaluation of the MRL, STMR and HR is underlined.

The Meeting received information on supervised field trials involving difenoconazole for the following crops and commodities:

Group	Crop commodity	Portion of commodity to which MRL apply	Countries	Table No
FB, Berries and other small fruits	Strawberry, fruit	Whole fruit	USA	5
FI, Assorted tropical and sub-tropical fruits – inedible peel	Avocado, fruit	Whole commodity after removal of obviously decomposed or withered leaves	Brazil	6
Pulses,	Soya, seed	Whole commodity	USA	7
GC, Cereal grain	Rice, grain, straw forage	Whole commodity	Europe	8
SO, Oilseed	Cotton, seed	Whole kernel after removal of the seed	Brazil	9
	Peanut, whole plant	Whole kernel after removal of the seed	Brazil	10
	Oilseed rape, seed	Whole kernel after removal of the seed	Canada	11
Animal feeds	Rice forage		Europe	12
	Rice straw		Europe	13

Strawberry

Nine independent supervised residue field trials on strawberries were conducted in USA during growing season 2008-2009. Four foliar applications of difenoconazole (EC formulation) at a target rate of 0.129 kg ai/ha were made with a seven day interval. Duplicate samples (fruits) were taken seven days after the third application and immediately after the last (fourth) application. Samples were stored frozen for a maximum of eight months.

Analysis of difenoconazole was made using LC-MS/MS and method REM 147.08. The limit of quantification was 0.01 mg/kg and the mean recovery was in the range of 70–109% at fortification levels of (n=1–2) 0.01, 0.5, 1.0 and 2.0 mg/kg.

Samples were analysed for triazole metabolites by Analytical Method 160 using LC/MS/MS and Morse Laboratories, Inc. The limit of quantitation (LOQ) for all analytes (as respective parent equivalents) for strawberries was 0.01 mg/kg. The limit of detection (LOD) based on the smallest standard that can be detected is 0.0015 ng/kg. The mean recovery for the metabolites was triazole (87±8%), triazole alanine (92±7%) and triazole acetic (102±7%) at fortification levels (n=16) 0.01, 0.10, 0.02 and 0.5 mg/kg.

Table 5 Residues in strawberry after foliar applications of difenoconazole in field trials from USA

STRAWBERRY Country year (variety)	Application			Residues (mean values in parenthesis) mg/kg mature fruit							Reference
	kg ai/ha	no	(BBCH)	DAT	Difenoconazole	1,2,4,-Triazole	Triazole alanine		Triazole acetic acid		Study: T002101-7 CGA169374_50035
Critical GAP in USA; apply 0.129 kg ai/ha 4 times at a 7-14 day intervals. PHI 0 days											
					treated	treated	treated	control	treated	control	
USA (NY) 2007 Penn Yan (Honeoye)	0.131 0.131 0.128 0.129	1 2 3 4	65 73 81 89	7[c]	0.31, 0.25 (0.28)	< 0.01, < 0.01 (< 0.01)	0.02, 0.02 (0.02)	0.02	< 0.01, < 0.01 (0.01)	< 0.01	Trial: E03NY078481
				0[d]	0.64, 0.66 (0.65)	nd, < 0.01 (< 0.01)	0.03, 0.03 (0.03)	0.02	< 0.01, < 0.01 (0.01)	< 0.01	
USA (NC) 2008 Seven Springs (Camarosa)	0.129 0.129 0.125 0.130	1 2 3 4	81 81 83 85	7[c]	0.20, 0.19 (0.20)	< 0.01,< 0.01	0.02, 0.02 (0.02)	< 0.01	< 0.01,< 0.01 (< 0.01)	< 0.01	Trial: E10NC078482
				0[d]	0.38, 0.43 (0.41)	< 0.01,< 0.01 (< 0.01)	0.02, 0.02 (0.02)	< 0.01	< 0.01,< 0.01 (< 0.01)	< 0.01	
USA (FL) 2008 (Treasures)	0.132 0.127 0.130 0.126	1 2 3 4	81-85[a] 73-81 81-85[a] 81-85[b]	7[c]	0.13, 0.18 (0.16)	< 0.01,< 0.01 (< 0.01)	0.05, 0.05 (0.05)	< 0.01	< 0.01,< 0.01 (< 0.01)	< 0.01	Trial: E16FL078483
				0[d]	0.19, 0.19 (0.19)	< 0.01,< 0.01 (< 0.01)	0.04, 0.05 (0.05)	< 0.01	< 0.01,< 0.01 (< 0.01)	< 0.01	
USA (MN) 2008 Wimauma (Mesabi)	0.132 0.130 0.129 0.137	1 2 3 4	63 65 73 88	7[c]	0.14, 0.20 (0.17)	< 0.01,< 0.01 (< 0.01)	< 0.01 < 0.01	(< 0.01) < 0.01	< 0.01,< 0.01 (< 0.01)	< 0.01	Trial: C12MN078484
				0[d]	0.49, 0.36 (0.43)	< 0.01,< 0.01 (< 0.01)	< 0.01 < 0.01 (< 0.01)	< 0.01	< 0.01,< 0.01 (< 0.01)	< 0.01	
USA (CA) Santa Maria 2008 (Albino)	0.130 0.130 0.129 0.129	1 2 3 4	89 89 89 89	7[c]	0.22, 0.24 (0.23)	< 0.01,< 0.01	0.07, 0.07 (0.07)	0.02	< 0.01,< 0.01	< 0.01	Trial: W30CA078485
				0[d]	0.41, 0.55 (0.48)	< 0.01,< 0.01 (< 0.01)	0.08, 0.07 (0.08)	0.02	< 0.01,< 0.01 (< 0.01)	< 0.01	
				1[d]	0.63	< 0.01	0.07	0.02	0.01	< 0.01	
				3[d]	0.47	< 0.01	0.09	0.03	0.02	< 0.01	
				5[d]	0.42	< 0.01	0.09	0.03	0.02	< 0.01	
USA (CA) 2008 Madera (Seascape)	0.130 0.131 0.129 0.131	1 2 3 4	71 75 79 79	7[c]	0.26, 0.31 (0.29)	< 0.01,< 0.01 (< 0.01)	0.04, 0.03 (0.03)	< 0.01	< 0.01,< 0.01 (< 0.01)	< 0.01	Trial: W29CA078486
				0[d]	0.72, 0.58 (0.65)	< 0.01,< 0.01 (< 0.01)	0.02, 0.04 (0.03)	0.01	< 0.01,< 0.01 (< 0.01)	< 0.01	
USA (CA) 2008 Madera (Chandler)	0.130 0.131 0.130 0.131	1 2 3 4	73 77 79 79	7[c]	0.54, 0.59 (0.57)	< 0.01,< 0.01 (< 0.01)	0.04, 0.05 (0.05)	< 0.01	< 0.01 < 0.0.01 (< 0.01)	< 0.01	Trial: W29CA078487
				0[d]	1.20, 1.22 (1,21)	< 0.01,< 0.01 (< 0.01)	0.06, 0.03 (0.05)	< 0.01	< 0.01,< 0.01 (< 0.01)	< 0.01	
USA (WA) 2008 Mount	0.125 0.123 0.131	1 2 3	73-81 81 85	7[c]	0.13, 0.09 (0.11)	< 0.01,< 0.01 (< 0.01)	< 0.01,< 0.0.01 (< 0.01)	< 0.01	< 0.01,< 0.01 (< 0.01)	< 0.01	Trial: W19WA078488

STRAWBERRY Country year (variety)	Application			Residues (mean values in parenthesis) mg/kg mature fruit							Reference
	kg ai/ha	no	(BBCH)	DAT	Difenoconazole	1,2,4,-Triazole	Triazole alanine		Triazole acetic acid		Study: T002101-7 CGA169374_50035
Critical GAP in USA; apply 0.129 kg ai/ha 4 times at a 7-14 day intervals. PHI 0 days											
					treated	treated	treated	control	treated	control	
Vernon (Puget Reliance)	0.128	4	81-85[a]	0[d]	0.07, 0.07 (0.07)	< 0.01,< 0.0.01 (< 0.01)	< 0.01,< 0.0.0.01 (< 0.01)	< 0.01	< 0.01,< 0.0.01 (< 0.01)	< 0.01	
USA (CA) Guadalupe 2009 (Albino)	0.131 0.131 0.130 0.131	1 2 3 4	75 75 75 75	7[c]	0.23, 0.25 (0.24)	< 0.01,< 0.0.01 (< 0.01)	0.09, 0.08 (0.09)	0.03	0.02, 0.03	< 0.01	Trial: W33CA098489
				0[d]	0.35, 0.39 (0.37)	< 0.01,< 0.0.01 (< 0.01)	0.07, 0.08 (0.08)	0.03	0.02, 0.02	< 0.01	

DAT = days after third or fourth (last) application

[a] Ripe berries

[b] Green, ripe fruit and flowers

[c] samples taken after third treatment

[d] samples taken after fourth treatment

nd = not detected

Avocado

Four independent supervised residue decline field trials were conducted on avocado in Brazil during growing season 2007-2008. Four foliar applications of difenoconazole (SC formulation) at a rate of 0.05 kg ai/ha were made with a fourteen day interval. Single samples (avocado fruits) were collected and stored frozen for a maximum of 8.9 months. This storage period is covered by the storage stability studies (24 months)

Analysis of parent difenoconazole was made using HPLC-MS/MS and method POPIT MET.033. The limit of quantification was 0.01 mg/kg and the mean recovery was between 84±2.2% to 91±1.2% at fortification levels of (n=2) 0.01, 0.11 and 2.2 mg/kg.

Table 6 Residues in avocado after foliar application of difenoconazole from field trials in Brazil

Location	Application			Residues Fruit		Reference
AVOCADO Country, year (variety	Kg ai/ha	no	BBCH	DAT	Difenoconazole (mg/kg)	No A13703G_10284
Brazil (Mogi, Mirimi, SP) 2007/2008 (Giada)	0.050	1	71-73	0	0.13	Study/ Trial M08071-LZF1
	0.050	2	73	3	0.12	
	0.050	3	73-75	7	0.05	
	0.050	4	75-77	14	0.05	
				21	0.03	
Brazil (SP) 2008 (Hass)	0.050	1	76	0	0.29	Study/ Trial M08071-LZF2
	0.050	2	76	3	0.33	
	0.050	3	77	7	0.20	
	0.050	4	77	14	0.26	
				21	0.18	
Brazil (Taquaritnga, SP) 2007/2008	0.050	1	69-71	0	0.12	Study/ Trial: M08071-LZF3
	0.050	2	71	3	0.06	
	0.050	3	71	7	0.07	
	0.050	4	85	14	0.05	

Location	Application			Residues Fruit		Reference
AVOCADO Country, year (variety	Kg ai/ha	no	BBCH	DAT	Difenoconazole (mg/kg)	No A13703G_10284
(Giada)				21	0.01	
Brazil	0.050	1	75	0	0.04	Study/
(MG)	0.050	2	76	3	0.05	Trial
2008	0.050	3	78	7	0.02	M08071-JJB
	0.050	4	79	14	0.02	
(Margarida)				21	0.01	

DAT = days after last treatment

Soya beans (dry)

Eighteen independent supervised residue field trials on soya beans were conducted in USA during growing season 2008. Two foliar applications of difenoconazole (EC formulation) at a target rate of 0.129 kg ai/ha were made at an interval of seven to ten days. Duplicate samples of soya beans were collected except in the residue decline trials when single samples were taken. Samples were stored frozen for a maximum of 4.8 months. This storage period is covered by the storage stability studies (24 months).

Analysis of parent difenoconazole was made using LC-MS/MS and method REM 147.08. The limit of quantification was 0.01 mg/kg and the mean recovery was 100±11% (n=22) at fortification levels of 0.01–10.0 mg/kg.

Samples were also analysed for the triazole metabolites using LC/MS/MS and Morse Laboratories, Inc. (Analytical Method 160). The limit of quantitation (LOQ) for all analytes (as respective parent equivalents) for soybeans was 0.01 mg/kg. The limit of detection (LOD) for all metabolites based on the smallest detectable standard was 0.00003 µg/mL for 1, 2, 3-triazole and triazole alanine in all matrices. The LOD for triazole acetic acid was 0.00005 µg/mL in all matrices. The mean recovery for the metabolites was triazole (91±11%), triazole alanine (90±10%) and triazole acetic (99±7.4%) at fortification levels of 0.01 and 0.1 mg/kg (n=18).

Table 7 Residues in soya beans after foliar application of difenoconazole from field trials in USA

SOYA BEAN Country, year (variety)	Application			Residues (mean value in parenthesis) mg/kg beans							Reference
	kg ai/ ha	no	(BBCH)	DAT	Difenoconazole	1,2,4,-Triazole	Triazole alanine		Triazole acetic acid		Study: T002400-07 and ML 08-1488-SYN No A7402T_10144
Critical GAP USA; apply 0.129 kg ai/ha maximum 2 times at 7-10 days intervals. PHI 14 days											
					treated	treated	treated	control	treated	control	Trial
USA (NC)	0.127	12	88	0	0.44	nd	0.20	0.1	< 0.01	< 0.01	Trial:
Seven Spring[a]	0.124		88	7	0.02	nd	0.15	0.1	< 0.01	< 0.01	E10NC081261
				14	< 0.01, < 0.01 (0.01)	nd, nd	0.12, 0.13 (0.13)	0.09	< 0.01, < 0.01	< 0.01	
2008				20	< 0.01	nd	0.162	0.11	< 0.01	< 0.01	
(DKB 64-51 (SE 74480))				28	< 0.01	< 0.01	0.12	0.16	< 0.01	< 0.01	
USA (IA)	0.121	1	93	0	0.08	nd,	0.069	0.098	< 0.01,	< 0.01	Trial:
2008	0.123	2	95						< 0.01		C30IA081274
Bagely				7	< 0.01, < 0.01	nd, 0.01	0.082	0.12	< 0.01	< 0.01	
(93M11)				14	0.019, 0.018	nd, nd	0.088,	0.08	< 0.01	< 0.01	

SOYA BEAN Country, year (variety)	Application			Residues (mean value in parenthesis) mg/kg beans							Reference
	kg ai/ ha	no	(BB CH)	DAT	Difenoconazole	1,2,4,-Triazole	Triazole alanine		Triazole acetic acid		Study: T002400-07 and ML 08-1488-SYN No A7402T_10144
Critical GAP USA; apply 0.129 kg ai/ha maximum 2 times at 7-10 days intervals. PHI 14 days											
					treated	treated	treated	control	treated	control	Trial
					(0.019)		0.092 (0.09)				
				21	< 0.01	nd	0.08	0.12	< 0.01	< 0.01	
				33	0.016	nd	0.042	0.09	< 0.01	< 0.01	
USA (NC) 2008 Seven Spring[a] (95M50	0.123 0.124	1 2	93 88	14	< 0.01, < 0.01 (0.01)	nd, nd	0.043, 0.046 (0.05)	0.085	< 0.01, < 0.01	< 0.01	Trial: E10NC081262
USA (MO) 2008 Fisks (Armor 47G7)	0.124 0.123	1 2	81 87	14	< 0.01, 0.014 (0.012)	nd, nd	0.114, 0.09 (0.10)	0.134	< 0.01, < 0.01	< 0.01	Trial: C23MO081263
USA 2008 (Washington, LA) (AG5605)	0.129 0.124	1 2	82 85	14	0.012, 0.019 (0.016)	nd, nd	0.068, 0.037 (0.05)	0.066	< 0.01, < 0.01	< 0.01	Trial: E18LA081264
USA 2008 (Washington, LA) (AG5605)	0.124 0.123	1 2	82 85	14	0.042, 0.038 (0.04)	< 0.01, < 0.01	0.089, 0.073 (0.08)	0.062	< 0.01, < 0.01	< 0.01	Trial: E18LA081265
USA (MO) 2008 Oregon (Pioneer 93M11)	0.123 0.127	1 2	R6 R6-R7	14	0.012, 0.013 (0.013)	< 0.01, nd	0.084, 0.085 (0.09)	0.053	< 0.01, < 0.01	< 0.01	Trial: C19MO081266
USA (MO) 2008 St Joseph (Pioneer) 93M96)	0.126 0.124	1 2	R5 R6	14	< 0.01, < 0.01, (0.01)	nd, nd	0.154, 0.191 (0.17)	0.09	< 0.01, < 0.01	< 0.01	Trial:, C19MO081267
USA (WI) 2008 Dunn (S17-A1)	0.122 0.119	1 2	75 81	14	< 0.01, < 0.01, (0.01)	nd, nd	0.07, 0.078 (0.074)	0.11	< 0.01, < 0.01	< 0.01	Trial: C08WI1081268
USA (WI) 2008 Fitchburg (S17-V2)	0.125 0.125	1 2	74 80	14	0.026, 0.015 (0.021)	nd, nd	0.086, 0.086 (0.09)	0.07	< 0.01, < 0.01	< 0.01	Trial: C08WI1081269
USA (ND) 2008 Asgrow AG0202	0.123 0.125	1 2	82 88	15	< 0.01, < 0.01 (0.01)	nd, nd	0.118, 0.198 (0.16)	0.110	< 0.01, < 0.01	< 0.01	Trial: C13ND081270
USA (NE) York, (NC+2A46RR)	0.123 0.125	1 2	93 95-97	11	< 0.01, < 0.01 (0.01)	nd, nd	0.129, 0.126 (0.13)	0.146	< 0.01, < 0.01	< 0.01	Trial: E13NE081271
USA (Osceola, NE) 2008 (NC+2A46RR)	0.122 0.123	1 2	93-95 97	13	< 0.01, < 0.01 (0.01)	nd, nd	0.156, 0.178 (0.17)	0.162	< 0.01, < 0.01	< 0.01	Trial: E13NE081272

SOYA BEAN Country, year (variety)	Application			Residues (mean value in parenthesis) mg/kg beans							Reference
	kg ai/ ha	no	(BB CH)	DAT	Difenoconazole	1,2,4,-Triazole	Triazole alanine		Triazole acetic acid		Study: T002400-07 and ML 08-1488-SYN No A7402T_10144
Critical GAP USA; apply 0.129 kg ai/ha maximum 2 times at 7-10 days intervals. PHI 14 days											
					treated	treated	treated	control	treated	control	Trial
USA (IA) Berkely 2008 (93M11)	0.121 0.126	1 2	79 95	14	< 0.01, < 0.01 (0.01)	nd, nd	0.079, 0.077 (0.08)	0.059	< 0.01, < 0.01	< 0.01	Trial: C301A081273
USA 2008 (Lime Springs, IA) (52726085)	0.125 0.125	1 2	86 88	14	0.022, 0.15 (0.087)	nd, nd	0.0590, 0.0635 (0.06)	0.052	< 0.01, < 0.01	< 0.01	Trial E19A081275
USA 2008 (Lime Springs, IA) (52726085)	0.123 0.123	1 2	86 88	14	0.067, 0.092 (0.079)	< 0.01, < 0.01	0.034, 0.0295 (0.03)	0.037	< 0.01, < 0.01	< 0.01	Trial E19A081276
USA (IA) 2008 Richland (Pioneer 93M11)	0.124 0.125	1 2	79 83	15	< 0.01, < 0.01 (0.01)	nd, nd	0.0575, 0.0462, (0.05)	0.036	< 0.01, < 0.01	< 0.01	Trial C18A081277
USA (IA) Hedrick 2008 (Pioneer 93M11)	0.123 0.124	1 2	79 95	14	< 0.01, < 0.01 (0.01)	nd, < 0.01	0.0555 0.0418 (0.05)	0.036	< 0.01, < 0.01	< 0.01	Trial C18A081278
USA (ND) Gardner 2008 5B077RR	0.127 0.124	2	85 87	14	< 0.01, < 0.01 (0.01)	nd, < 0.01	0.310 0.324 (0.32)	0.299	0.023,0.026 (0.03)	0.016	Trial C12MN081279
USA (MN) Perley 2008 (5A009RR)	0.130 0.121	2	85 89	14	< 0.01, < 0.01 (0.01)	nd, nd	0.264, 0.282 (0.28)	0.332	0.013 0.013 (0.01)	0.013	Trial C12MN081280

DAT = days after last treatment

nd = not detected

[a] Different due to different of 2.5 weeks difference in application times and different cultvars

R5 = BBCH 50-59Beginning Seed: Seed in one of the four uppermost nodes with fully developed leaves is 1/8 in. long.

R6 = BBCH60-69 Full Seed: Pod containing a green seed filling the pod cavity is present at one of the top four nodes.

R7 =BBCH 70-79 Beginning Maturity: One normal pod on the main stem has reached its mature pod colour. At this stage, the crop is safe from a killing frost.

Rice

Eight independent supervised residue field trials on rice were conducted in Italy in 2009 and 2010. Two foliar applications of difenoconazole (EC or SC) were made with a fifteen days interval at a target rate of 0.125 kg ai/ha. Duplicate samples of whole plant, grain and straw were collected and

maintained in frozen storage for periods up to 14 months for whole plants and 13 months for grain and straw. This storage period is covered by the storage stability studies (24 months)

Analysis of parent difenoconazole (on one of the duplicate sample) was made using LC-MS/MS and method REM 147.08. The limit of quantification was 0.01 and the mean recovery was 102±14% (whole plant), 108±12% (grain) and 105±10% for straw at fortification levels of (n=1–2) 0.01, 0.1 and 8 mg/kg.

Analysis of the metabolites was made using Syngenta method GRM053.01A for triazole metabolites T, TA, TAA and triazole lactic acid (TLA). The method is validated for cereals (including rice) whole plant, grain and straw with a LOQ of 0.01 mg/kg for each metabolite.

Table 8 Residues in rice grain after foliar application of difenoconazole from field trials in Europe

RICE Country, year (variety) Trial no	Application			Residues* mg/kg								Reference
	g ai/ ha	**no**	(BBCH)	DAT	Matrix	Difenoconazole	Triazole alanine	Triazole acetic acid		Triazole lactic acid		No A7402T_ 10138, 10139, No A13703G _10496
Critical GAP EU; apply 0.125 kg ai/ha maximum 2 times at BBCH 21-29 with a 15 days interval. PHI 28 days.												
						treated	treated	control	treated	control	treated	control	
Europe Italy 2009 (Ercole S-09-01473-01	133[a]	1	71-74 83	21	Grain	0.85	0.07	-	0.05	-	< 0.01	-	Study: S09-01473
	132[a]	2	83	28	Grain	0.76	0.06	0.03	0.06	0.02	< 0.01	< 0.01	
Europe Italy 2009 (ValoneNano) S-09-01473-02	118[a]	1	71-75	21	Grain	0.9	0.03	-	0.04	-	< 0.01	-	Study: S09-01473
	122[a]	2	73-77	28	Grain	0.85	0.03	0.02	0.04	0.01	< 0.01	< 0.01	
Europe Italy 2010 (Ercole) S10-00370-01[g]	133[a]	1	69-73	21	Grain	0.75	0.12	-	0.07	-	< 0.01	-	Study: S10-00370
	133[a]	2	77-83	28	Grain	0.68	0.12	0.06	0.09	0.07	< 0.01	< 0.01	
Europe Italy 2010 (Scudo) S10-00370-02[f]	127[a]	1	69-73	21	Grain	1.2	0.40	-	0.33	-	0.01	-	Study: S10-00370
	113[a]	2	77-83	28	Grain	1.1	0.33	0.24	0.26	0.25	< 0.01	< 0.01	
Europe	144[b]	1	69	21	Grain	0.84	0.09	0.03	0.05	0.03	< 0.01	< 0.01	Study:

RICE Country, year (variety) Trial no	Application			Residues* mg/kg									Reference
	g ai/ ha	**no**	(BBCH)	DAT	Matrix	Difenoconazole	Triazole alanine		Triazole acetic acid		Triazole lactic acid		No A7402T_10138, 10139, No A13703G_10496
Critical GAP EU; apply 0.125 kg ai/ha maximum 2 times at BBCH 21-29 with a 15 days interval. PHI 28 days.													
						treated	treated	control	treated	control	treated	control	
Italy 2010 (Volano) S10-00370-01	144 [b]	2	72-73										S10-00372
				28	Grain	0.86	0.08	-	0.04	-	< 0.01	-	
Europe Italy 2010 (Scudo) S10-00370-02 [e]	147 [b]	1	69-73	21	Grain	1.8, 1.3 (1.6)	0.27 [c]	0.36 [c]	0.14 [c]	0.18 [c]	< 0.01 [c]	< 0.01 [c]	Study: S10-00372
	145 [b]	2	76										
				28	Grain	1.4	0.39	-	0.17	-	< 0.01	-	
Europe Italy 2010 (Ercole) S10-00370-03 [d]	146 [b]	1	69-73	21	Grain	0.95	0.10	0.06	0.05	0.03	< 0.01	< 0.01	Study: S10-00372
	146 [b]	2	77-83										
				28	Grain	0.78	0.13	-	0.04	-	< 0.01	-	
Europe Italy 2010 (SIS R215) S10-00370-03	143 [b]	1	83-85	21	Grain	1.2	0.20	0.08	0.07	0.04	< 0.01	< 0.01	Study: S10-00372
	139 [b]	2	85-87										
				28	Grain	1.1	0.17		0.07		< 0.01		

*1,2,4-triazole was measured but was not detected in any trial, therefore not reported here.

- Data not available

[a] EC formulation

[b] SC formulation in mixture with azoxystrobin

DAT = days after last treatment

nd = not detected

Cotton

Eight independent supervised residue field trials on cotton were conducted in Brazil during growing season 2006 and 2007/08. Four trials were made with four foliar applications of difenoconazole (SC formulation) at a target rate of 0.075 kg ai/ha, an interval of 14 days and sampling after 7, 4 and 21 days. An additional four trials were made with five foliar applications of difenoconazole (SC formulation) at a target rate of 0.075 kg ai/ha, an interval of 21 days (after the last two applications)

and sampling after 30 days. Single samples (cotton bolls) were taken and stored frozen maximum 8.1 months. This storage period is covered by the storage stability studies (24 months).

Analysis of parent difenoconazole in seeds was made using HPLC-MS/MS and method POPIT MET.033. The limit of quantification was 0.01 mg/kg for and the mean recovery was 84±5% at fortification levels of (n=8) 0.01 and 0.1 mg/kg.

Table 9 Residues in cotton after foliar application of difenoconazole from field trials in Brazil

COTTON Country, year (variety	Application				Residues			Reference
	g ai/ha	no	interval days	BBCH	DAT	matrix	Difenoconazole (mg/kg)	No A13703G_10323, No A15265A_10006
Critical GAP Brazil; apply 0.075 kg ai/ha maximum 3 times at 10-15 days intervals. PHI 21 days								
Brazil (Holambra)	75	4	0	71	7	seed	0.02	Study: M05022 Trial: M505022-LZF1
			14	75	14	seed	0.02	
			14	81	21	seed	0.02	
2006			14	87				
(IAC24,)								
Brazil (Bandeiantes)	75	4	0	73	7	seed	0.02	Study: M05022 Trial: M505022-LZF2
			14	79	14	seed	0.02	
			14	79-80	21	seed	0.02	
2006			14	80				
(IPR 96)								
Brazil (Uberlandia)	75	4	0	73	7	seed	0.04	Study: M05022 Trial: M05022-JJB1
			14	79	14	seed	0.01	
			14	81	21	seed	< 0.01	
2006			14	83				
(IPR 96)								
Brazil (Guaira)	75	4	0	75	7	seed	0.01	Study: M05022 Trial: M05022-JJB2
			14	77	14	seed	0.02	
			14	79	21	seed	0.01	
2006 (Delta Penta)			14	83				
Brazil (Coelho)	75	5	0	13-19				Study: M08065 Trial: M08065 -LZF1
			21	29				
			21	40				
2007/08			14	51				
(Delta Oppal,)			77	70	30	seed	< 0.01	
Brazil (Bandeirantes)	75	5	0	12				Study: M08065 Trial: M08065 LZF2
			20	21-22				
			22	39				
2007/08			45	71				
(Copetec 401)			52	71-73	30	seed	< 0.01	
Brazil (Uberlandia)	75	5	0	14				Study: M08065 Trial: M08065 JJB1
			21	18-19				
			21	57				
2007/08			14	60				
(Nu Opal,)			99	81	30	seed	< 0.01	
Brazil (Goiania)	75	5	0	14				Study: M08065 Trial: M08065 -JJB2
			21	22				
			21	60				
2007/08			14	63				
(Nu Opal,)			66	80	30	seed	< 0.01	

DAT = days after last treatment

Peanut

Eight supervised residue field trials on peanuts were conducted in Brazil during growing seasons 2008 and 2009/10. Four trials were made with six foliar applications (SC formulation) at a rate of 0.125 kg ai/ha. Single samples (peanut plants) were collected 14, 22 and 38 days after last application and after the plants were dried, the pods were removed from the plants and threshed using a small machine. The seeds were stored frozen at maximum 7.6 months. This storage period is covered by the storage stability studies (24 months).

Analysis of parent difenoconazole from seeds in these trials was made using method HPLC-MS/MS and method POPIT MET.033. The limit of quantification was 0.01 mg/kg and the mean recovery was between 84±3% to 89±2% at fortification levels of (n=5–7) 0.01 and 0.1 mg/kg.

An additional four field trials were conducted in Brazil during growing season 2007/08 with three foliar applications at rate of 0.0875 kg difenoconazole (EC formulation). Single samples (peanut plants) were sampled and peanut kernel stored frozen for a maximum of 4.5 months. The storage period is covered by the storage stability studies (24 months).

Analysis of parent difenoconazole from seeds in these trials was made using HPLC-MS/MS and method POPIT MET.032. The limit of quantification was 0.01 mg/kg and the mean recovery was between 73±3% to 82±9% at fortification levels of (n=5–7) 0.01 and 0.1 mg/kg.

Table 10 Residues in peanut kernel after foliar application of difenoconazole in field trials from Brazil

PEANUT Country, year (variety	Application				Residues			Reference
	kg ai/ha	no	interval days	BBCH	DAT	matrix*	Difenoconazole (mg/kg)	No A16976A_10030, No A7402N_10001
Critical GAP Brazil; apply 0.0875 kg ai/ha maximum 3 times, interval not defined. PHI 22 days.								
Brazil (Sao Palo,) 2009/10 (Runner)	0.125	6	0	59-60	7	peanuts	< 0.01	Study: M10070 Trial: M10070-LZF
			13	61-63	14	peanuts	< 0.01	
			14	63-65	22	peanuts	< 0.01	
			14	65-67	28	peanuts	< 0.01	
			14	73-75				
			14	78-79				
Brazil (Parana) 2009/10 (Super Tatu)	0.125	6	0	60	7	peanuts	< 0.01	Study: M10070 Trial: M10070-JJB
			14	67	14	peanuts	< 0.01	
			14	71	22	peanuts	< 0.01	
			14	75	28	peanuts	< 0.01	
			14	79			< 0.01	
			14	81			< 0.01	
Brazil (Jacoboticabal, Sao Palo) 2009/10 (Alto oleico)	0.125	6	0	13-14	7	peanuts	< 0.01	Study: M10070 Trial: M10070-AMA1
			14	23-29	14	peanuts	< 0.01	
			14	51-61	22	peanuts	< 0.01	
			14	63-67	28	peanuts	< 0.01	
			14	69-71				
			14	75				

PEANUT Country, year (variety	Application				Residues			Reference
	kg ai/ha	no	interval days	BBCH	DAT	matrix*	Difenoconazole (mg/kg)	No A16976A_10030, No A7402N_10001
Critical GAP Brazil; apply 0.0875 kg ai/ha maximum 3 times, interval not defined. PHI 22 days.								
Brazil (Vista Alegro do Alto, Sao Paulo) 2009/10 (Alto oleico)	0.125	6	0	13-15	7	peanuts	< 0.01	Study: M10070 Trial: M10070-AMA1
			14	55	14	peanuts	< 0.01	
			14	61	22	peanuts	< 0.01	
			14	65	28	peanuts	< 0.01	
			14	69			< 0.01	
			14	69			< 0.01	
Brazil (Sao Paulo) 2008 (Super Tatu Vermelho)	0.088	3	0	73	14	peanuts	< 0.01	Study: M08013 Trial: M08013-LZF1
			7	75	22	peanuts	< 0.01	
			7	76-77	28	peanuts	< 0.01	
Brazil (Parana) 2008 (Tatu Vermelho)	0.088	3	0	77	14	peanuts	< 0.01	Study: M08013 Trial: M08013-LZF2
			7	77-79	22	peanuts	< 0.01	
			7	70-80	28	peanuts	< 0.01	
Brazil (Goias) 2008 (Tatu)	0.088	3	0	77	14	peanuts	< 0.01	Study: M08013 Trial: M08013-JJB1
			7	79	22	peanuts	< 0.01	
			7	82	28	peanuts	< 0.01	
Brazil (Minas Gerais) 2008 (Tatu)	0.088	3	0	79-81	14	peanuts	< 0.01	Study: M08013 Trial: M08013-JJB2
			7	81-83	22	peanuts	< 0.01	
			7	83-85	28	peanuts	< 0.01	

DAT = days after last treatment

*Peanut plants were sampled. After the plants were dried, the pods were removed from the pods. Threshing was done on a small machine

Rape seed (Canola)

Thirteen independent supervised field trials on oilseed rape were conducted in Canada during growing season 2011. One foliar application (EC formulation) was made at the target rate of 0.125 kg ai/ha. Duplicate samples of were collected 30 days after the application. Rape seed samples were stored frozen for periods up to 4.7 months. This storage period is covered by the storage stability studies (24 months).

Analysis of parent difenoconazole from seeds in these trials was made using LC-MS/MS and method REM 147.08. The limit of quantification was 0.01 mg/kg for and the mean recovery was between 88±11% to 107±17% at fortification levels of (n=3–4) 0.01, 0.1 and 0.2 mg/kg.

Table 11 Residues of parent difenoconazole in oilseed rape from field trials in Canada

OILSEED RAPE, (CANOLA) Country, year (variety	Application					Residues (mean value in parenthesis)			Reference
	g ai/hl	water L/ha	kg ai/ha	no	BBCH	DAT	matrix	Difenoconazole (mg/kg)	No A15457B_50038, Study: CER 05903/11
GAP Canada; apply 0.125 kg ai/ha one time at BBCH 12-18[a]or at BBCH 62-65[b]. PHI 30 days.									
Canada (Elm Creek, MB) 2011 (1841 RR)	302	45	0.136		69-73	29	seed	0.017, 0.013 (0.015)	Trial: T938
Canada (Morden, MB) 2011 (1841 RR)	305	45	0.137		67-69	30	seed	0.81, 0.043 (0.062)	Trial: T938C
Canada (Kinley, SK) 2011 (1841 RR)	282	45	0.127	1	67-71	30	seed	0.056, 0.070 (0.063)	Trial: T939
Canada (Kinley, SK) 2011 (72-55) RR)	63	200	0.126	1	69-73	30	seed	0.023, 0.023 (0.023)	Trial: T940
Canada (Elgin, MB) 2011 (72-55) RR)	277	45	0.125	1	68	30	seed	0.042, 0.024 (0.033)	Trial: T941
Canada (Elgin, MB) 2011 (72-55) RR)	63	200	0.125	1	78-79	30	seed	0.036, 0.021 (0.029)	Trial: T942
Canada (Rosthern, SK) 2011 (1841 RR)	65	200	0.130	1	73-76	31	seed	0.031, 0.044 (0.038)	Trial: T943
Canada (Minto, MB) 2011 (72-55) RR)	62	200	0.123	1	67	35	seed	< 0.01, < 0.01 (< 0.01)	Trial: T944
Canada (Alvena, SK) 2011 (72-55) RR)	58	200	0.116	1	65-66	31	seed	0.010, 0.019, (0.015)	Trial: T945
Canada (Fort Sask.AB) 2011 (72-55) RR)	65	200	0.129	1	67-71	32	seed	0.040; 0.026 (0.033)	Trial: T946
Canada (Minto, MB) 2011 (1841 RR)	62	200	0.124	1	67	25	seed	0.025	Trial: T947
						30		< 0.01, 0.012 (< 0.01)	
						35		< 0.01	
						40		< 0.01	

OILSEED RAPE, (CANOLA) Country, year (variety	Application					Residues (mean value in parenthesis)			Reference
	g ai/hl	water L/ha	kg ai/ha	no	BBCH	DAT	matrix	Difenoconazole (mg/kg)	No A15457B_50038, Study: CER 05903/11
GAP Canada; apply 0.125 kg ai/ha one time at BBCH 12-18[a]or at BBCH 62-65[b]. PHI 30 days.									
Canada (Elgin, MB) 2011 (1841 RR)	62	200	0.124	1	68	31	seed	0.011, < 0.01 (0.011)	Trial: T948
Canada (Rosthern, SK) 2011 (72-55 RR)	65	200	0.130	1	73-76	31	seed	0.037, 0.035 (0.036)	Trial: T949

DAT = days after last treatment

[a] Virulent Black Leg

[b] Sclerotinia Stem Rot

Animal feeds

Rice straw and whole crops silage,

For information on the trials see, Table 8.

Table 12 Residues of difenoconazole in rice whole crop silage following foliar application in field trials from Europe

RICE Country, year (variety)	Application			Residues* mg/kg									Reference
	g ai/ha	no	(BBCH)	DAT	Matrix	Difenoconazole	Triazole alanine		Triazole acetic acid		Triazole lactic acid		No A7402T_10138, 10139, No A13703G_10496
Critical GAP EU; apply 0.125 kg ai/ha maximum 2 times at 15 days interval. PHI 28 days.													
						treated	treated	control	treated	control	treated	control	
Europe Italy 2009 (Ercole	133[a]	1	71-74 83	0	Whole plant	3.5	0.01	0.01	0.03	0.02	0.01	0.02	Study: S09-01473 Trial no: S-09-01473-01
	132 [a]	2	83	7	Whole plant	1.8	0.04		0.05		0.02		
				14	Whole plant	1.4	0.04		0.04		0.02		
Europe Italy 2009 (ValoneNano)	118 [a]	1	71-75	0	Whole plant	6.3	< 0.01	< 0.01	0.03	0.03	0.02	0.02	Study: S09-01473 Trial no: S-09-01473-02
				7	Whole plant	2.6	< 0.01	-	0.04		0.02		
				14	Whole plant	1.6	< 0.02	-	0.05		0.02		
Europe Italy 2010 (Ercole)	133 [a]	1	69-73	0	Whole plant	6.1	0.07	0.05	0.07	0.07	0.02	0.03	Study: S10-00370 Trial no: S10-00370-01[g]
	133 [a]	2	77-83	7	Whole plant	2.1	0.04		0.07		0.02		
				14	Whole plant	1.4	0.06		0.10		0.01		
Europe Italy	127 [a]	1	69-73	0	Whole plant	5.2	0.17	0.16	0.19	0.24	0.09	0.09	Study: S10-00370
	113 [a]	2	77-	7	Whole	2.6	0.15		0.20		0.06		Trial no:

RICE Country, year (variety)	Application			Residues* mg/kg									Reference
	g ai/ ha	no	(BBCH)	DAT	Matrix	Difenoconazole	Triazole alanine		Triazole acetic acid		Triazole lactic acid		No A7402T_10138, 10139, No A13703G_10496
Critical GAP EU; apply 0.125 kg ai/ha maximum 2 times at 15 days interval. PHI 28 days.													
						treated	treated	control	treated	control	treated	control	
2010			83		plant								S10-00370-02[f]
(Scudo)				14	Whole plant	1.4	0.20		0.22		0.06		
Europe Italy	144[b]	1	69	0	Whole plant	3.7	0.02	0.02	0.06	0.04	0.04	0.03	Study: S10-00372
2010	144 [b]	2	72-73	7	Whole plant	3.3	0.04		0.07		0.05		Trial no: S10-00370-01
(Volano)				14	Whole plant	2.5	0.02		0.07		0.03		
Europe Italy	147 [b]	1	69-73	0	Whole plant	4.6	0.19	0.13	0.20	0.13	0.06	0.06	Study: S10-00372
2010	145 [b]	2	76	7	Whole plant	2.8	0.20		0.24		0.06		Trial no: S10-00370-02[e]
(Scudo)				14	Whole plant	2.5	0.17		0.23		0.06		
Europe Italy	146 [b]	1	69-73	0	Whole plant	5.6	0.06	0.04	0.08	0.05	0.02	0.02	Study: S10-00372
2010	146 [b]	2	77-83	7	Whole plant	2.4	0.06		0.08		0.01		Trial no: S10-00370-03 [d]
(Ercole)				14	Whole plant	1.8	0.07		0.06		0.02		
Europe Italy	143 [b]	1	83-85	0	Whole plant	4.6	0.13	0.06	0.11	0.06	0.04	0.04	Study: S10-00372
2010	139 [b]	2	85-87	7	Whole plant	2.9	0.12		0.10		0.04		Trial no: S10-00370-03
(SIS R215)				14	Whole plant	2.5	0.09		0.06		0.04		

*1,2,4-triazole was measured but was not detected in any trial.

- Data not available

[a] EC formulation

[b] SC formulation in mixture with azoxystrobin

[c] Treated and untreated grain samples 21 DAT have been mixed up

DAT = days after last treatment

nd = not detected

Table 13 Residues of difenoconazole in rice straw following foliar application in field trials from Europe

RICE Country, year (variety)	Application			Residues* mg/kg									Reference
	g ai/ ha	no	(BBCH)	PHI	Matrix	Difenoconazole	Triazole alanine		Triazole acetic acid		Triazole lactic acid		No A7402T_10138, 10139, No A13703G_10496
Critical GAP EU; apply 0.125 kg ai/ha maximum 2 times at 15 days interval. PHI 28 days.													
						treated	treated	control	treated	control	treated	control	
Europe Italy	133[a]	1	71-74 83	0									Study: S09-01473 Trial no:

RICE Country, year (variety)	Application			Residues* mg/kg									Reference
	g ai/ ha	no	(BBCH)	PHI	Matrix	Difenoconazole	Triazole alanine		Triazole acetic acid		Triazole lactic acid		No A7402T_10138, 10139, No A13703G_10496
Critical GAP EU; apply 0.125 kg ai/ha maximum 2 times at 15 days interval. PHI 28 days.													
						treated	treated	control	treated	control	treated	control	
2009 (Ercole	132 [a]	2	83	21	Straw	1.1	< 0.01		0.06		0.03		S-09-01473-01
				28	Straw	1.0	< 0.01	< 0.01	0.07	0.02	0.03	0.02	
Europe Italy	118 [a]	1	71-75	0									Study: S09-01473
	122 [a]	2	73-77	21	Straw	1.4	< 0.01		0.08		0.03		Trial no:
2009				28	Straw	1.4	< 0.01	< 0.01	0.09	0.03	0.02	0.02	S-09-01473-02
(ValoneNano)													
Europe Italy	133 [a]	1	69-73	0									Study: S10-00370
	133 [a]	2	77-83										Trial no:
2010													S10-00370-01[g]
(Ercole)				28	Straw	2.6	0.01	0.02	0.12	0.10	0.05	0.03	
Europe Italy	127 [a]	1	69-73	0									Study: S10-00370
	113 [a]	2	77-83	21	Straw	1.9	0.05		0.28		0.12		Trial no:
2010													S10-00370-02[f]
(Scudo)				28	Straw	1.6	0.03	0.04	0.30	0.21	0.12	0.15	
Europe Italy	144 [b]	1	69	0									Study: S10-00372
	144 [b]	2	72-73										Trial no:
2010				21	Straw	2.3	< 0.01	< 0.01	0.09	0.08	0.07	0.04	S10-00370-01
(Volano)				28	Straw	1.8	0.01		0.09		0.07		
Europe Italy	147 [b]	1	69-73	0									Study: S10-00372
	145 [b]	2	76										Trial no:
2010				21	Straw	3.0	0.03	0.04	0.38	0.23	0.16	0.11	S10-00370-02[e]
(Scudo)				28	Straw	2.2	0.04		0.29		0.11		
Europe Italy	146 [b]	1	69-73	0									Study: S10-00372
	146 [b]	2	77-83										Trial no:
2010													S10-00370-03 [d]
				21	Straw	4.3	0.02	0.01	0.14	0.08	0.05	0.04	
(Ercole)				28	Straw	2.2	0.02		0.11		0.05		
Europe Italy	143 [b]	1	83-85	0									Study: S10-00372
	139 [b]	2	85-87										Trial no:
2010													S10-00370-03
				21	Straw	2.2	0.03	0.02	0.16	0.10	0.13	0.08	
(SIS R215)				28	Straw	2.0	0.03		0.17		0.11		

*1,2,4-triazole was measured but was not detected in any trial.

- Data not available

[a] EC formulation

[b] SC formulation in mixture with azoxystrobin

[c] Treated and untreated grain samples 21 DAT have been mixed up

DAT = days after last treatment

nd = not detected

FATE OF RESIDUES IN STORAGE AND PROCESSING

Residues after processing

As a measure of the transfer of residues into processed products, a processing factor was used, which is defined as:

Processing factor (Pf) parent difenoconazole =

$$\frac{\text{Residues in processed product (mg/kg)}}{\text{Residues in raw agricultural commodity (mg/kg)}}$$

Processing factor (PF) for each triazole metabolite =

$$\frac{\text{Residues in treated processed product – residues in untreated processed product (mg/kg)}}{\text{Residues in treated raw agricultural commodity (RAC) – residues in untreated RAC (mg/kg)}}$$

If residues in the RAC were below LOQ, no processing factor could be derived. In case of residues below the LOQ, but above the LOD in the processed product, the numeric value of the LOQ was used in the calculation. If residues in the processed product were below the LOD, the numeric value of the LOQ was used for the calculation but the PF was expressed as "less than" (e.g. < 0.5). If residues in the processed commodity were below what was found in untreated processed commodity no processing factor was calculated.

Soya beans

Two studies on the conduct of difenoconazole during processing of soya bean into meal, hulls and refined oil and one study for the processing into aspired grains was conducted by Willard, TR and Mäyer JT (2008, T002400-07). Field trials of soya bean was treated with two applications at a target rate of 0.65 kg ai/ha. Samples of soya beans were collected 14 days after the last application. Duplicate field samples and processed fractions were analysed for parent difenoconazole using method REM 147.08. LOQ for parent difenoconazole was 0.01 mg/kg and the mean recovery was in meal 108% at fortification level of (n=2) 0.01–5.0, in hulls 106% at fortification level of (n=2) 0.01–5.0, in refined oil 88% at fortification level of (n=2) 0.01–0.05 and in aspirated grain fraction (AEG) 112±6.6% at fortification level (n=4) 0.01–250 mg/kg- Each triazole metabolite was analysed using method No 160 rev.2 Morse Laboratories. LOQ for all triazole analytes were 0.01 mg/kg.

Samples of RAC (soya beans) were stored frozen for a maximum of 4.8 months and the duration of the storage for the processed fractions meals, hulls, refined oil and aspired grain fractions were 3.2, 5.5, 3.2 and 10.4 months, respectively.

Processing of meal, hulls and refined oil

Cleaned whole soybeans were fed into a roller mill to crack the hull and liberate the kernel. After hulling, the material was passed through an aspirator to separate hull and kernel material. The moisture content of the kernel material was determined and adjusted to 13.5%. Kernel material was heated to 71–79 °C and flaked in a flaking roll with a gap setting of 0.2–0.33 mm. Flakes were extruded in a continuous processor, where they were turned into collets by direct steam injection and compression. After extrusion, the collets were oven dried, placed in stainless steel batch extractors and submerged in hexane at 49–60 °C. After 30 minutes, the hexane was drained and fresh hexane was added to repeat the cycle twice.

The solvent was evaporated from the extracted flakes and the oil fraction to give meal and crude oil. The crude oil was treated with sodium hydroxide to remove free fatty acids. The neutralized oil was centrifuged and the supernatant, refined oil decanted.

Processing of aspirated grain fraction

To generate aspirated grain fractions (AGF), the samples were placed in a dust generation room containing a holding bin, two bucket conveyors, and a screw conveyor. As the samples were moved for 120 minutes in the system, aspiration was used to remove light impurities (grain dust). Light impurities were classified by sieving using 2.36, 2.0, 1.18, 0.85 and 0.425 mm sieves. After classification of each sample, the material collected through the 2.36 mm sieve was recombined to produce one aspirated grain fraction.

Residues determined in soya bean and processed fractions meal, hulls, refined oil and aspirated grain fraction are shown in table 14 and 15.

Table 14 Residues from parent difenoconazole in soya beans (RAC and processed fractions)

Trial Location, year , (variety), dose rate, interval DALT	Processed fraction	Difenoconazole parent, mg/kg (mean value in parenthesis)	Processing factor parent
C13ND081270 USA, (ND) 2008 (Asgrow) 0.614+0.608 kg ai/ha interval 7days, DALT=14	Soya bean, seeds (RAC)	< 0.01, 0.0128 < 0.0247 (0.016)	-
	Meal	< 0.01, < 0.01 (< 0.01)	0.63
	Hulls	0.0540, 0.0536 (0.054)	3.38
	Refined Oil	0.0158, 0.0180 (0.017)	1.06
C12MN081279 USA, (ND) 2008 (5B077RR) 0.618+0.634 interval 7 days, DALT=14	Soya bean (RAC)	0.049, 0.074, 0.107 (0.077)	
	Meal	< 0.01, < 0.01 (< 0.01)	0.13
	Hulls	0.045, 0.048 (0.047)	0.61
	Refined Oil	0.028, 0.036 (0.032)	0.42
C12MN081281 USA,(ND) 2008 (5A009RR) 0.618+0.621 interval 7 days, DALT=12	Soya bean , seed (RAC)	0.363, 0.31, 0.368 (0.347)	
	AGF	190, 214, 244 (216)	622

Table 15 Levels of triazole metabolites from difenoconazole in soya bean (RAC and processed fractions) *In parenthesis average of the three replicates*

Trial Location, year , (variety), dose rate, interval DALT	Matrix	Treatment 1=control 2=treated	1,2,4 Triazole mg/kg	Pf	Triazole alanine mg/kg	Pf	Triazole acetic acid mg/kg	Pf
C13ND081270 USA, (ND) 2008 (Asgrow) 0.614+0.608 kg ai/ha interval 7 days, DALT=14	Soya bean (RAC)	1	nd		0.068		< 0.01	
	Soya bean (RAC)	2	nd		0.113		< 0.01	
	Soya bean (RAC)	2	nd		0.164		< 0.01	
	Soya bean (RAC)	2	nd (nd)		0.160 (0.146)		< 0.01 (< 0.01)	
	Meal	1	nd		0.113		< 0.01	
	Meal	2	nd	< 0.01	0.143,	0.45	< 0.01	0.01
	Meal	2	nd (nd)		0.152 (0.148)		< 0.01 (nd)	
	Hulls	1	nd		0.026		< 0.01	
	Hulls	2	nd	< 0.01	0.052	0.31	< 0.01	0.01
	Hulls	2	nd		0.049 (0.05)		< 0.01	

Trial Location, year , (variety), dose rate, interval DALT	Matrix	Treatment 1=control 2=treated	1,2,4 Triazole mg/kg	Pf	Triazole alanine mg/kg	Pf	Triazole acetic acid mg/kg	Pf
			(nd)				(< 0.01)	
	Refined Oil	1	nd		nd		< 0.01	
	Refined Oil	2	nd	< 0.01	nd	< 0.01	< 0.01	0.01
	Refined Oil	2	nd (nd)		nd (nd)		< 0.01 (< 0.01)	
C12MN081279 USA, (ND) 2008 (5B077RR) 0.618+0.634 interval 7 days, DALT=14	Soya bean (RAC)	1	nd		0.396		0.017	
	Soya bean (RAC)	2	nd		0.555		0.019	
	Soya bean (RAC)	2	nd		0.585		0.02	
	Soya bean (RAC)	2	nd (nd)		0.605 (0.582)		0.022 (0.02)	
	Meal	1	< 0.01		0.388		0.028	
	Meal	2	< 0.01	0.01	0.545	0.91	0.034	1.5
	Meal	2	< 0.01 (< 0.01)		0.570 (0.558)		0.032 (0.033)	
	Hulls	1	< 0.01		0.182		0.014	
	Hulls	2	< 0.01	0.01	0.221	0.22	0.013	-
	Hulls	2	< 0.01 (< 0.01)		0.226 (0.224)		0.01 (0.012)*	
	Refined Oil	1	nd		nd		nd	
	Refined Oil	2	nd	< 0.01	nd	< 0.01	nd	< 0.01
	Refined Oil	2	nd (nd)		nd (nd)		nd (nd)	
C12MN081281 USA, (ND) 2008 (5A009RR) 0.618+0.621 interval 7 days, DALT=12	Soya bean (RAC)	1	< 0.01		0.600		0.027	
	Soya bean (RAC)	2	nd		0.615		0.032	
	Soya bean (RAC)	2	< 0.01		0.605		0.035	
	Soya bean (RAC)	2	nd (< 0.01)		0.590 (0.60)		0.033 (0.033)	
	AGF	1	< 0.01		0.342		0.030	
	AGF	2	0.026	2.4	0.132	-	0.214	33.84
	AGF	2	0.021		0.106		0.205	
	AGF	2	0.024 (0.024)*		0.113 (0.117)		0.224 (0.214)	

Pf: Processing factor

Treatment 1 Untreated control, one sample per trial

Treatment 2 Treated twice with 0.65 kg ai/ha at ca 7 day interval starting 28 days prior to harvest of mature seed

- not calculated due to a reduced amount in treated processed soya bean than in untreated processed soya bean, or not detected in treated or untreated processed soya beans.

AGF: Aspirated Grain Fraction

Rice

A study on the behaviour of difenoconazole during processing of rice was conducted by Yozgatli HP, and Breyer N (2010, S10-02953, No. A7402T_10217). Two field trials of rice were treated with two applications of difenoconazole with a target rate of 0.25 kg ai/ha. Samples of rice grain were collected at 21 and 28 days after the final application. Rice (grain) was processed into polished rice, parboiled rice, cooked rice and rice flour. Two mass-balance studies to determine the accountability of the residue and two follow-up studies were conducted to determine residue transfer on each process.

Field samples and processed fractions were analysed for parent difenoconazole using method REM 147.08 and LOQ was 0.01 mg/kg. The RAC (rice grain) and processed fractions were stored in the freezer ≤ 18 °C for a maximum of 17 months.

Cleaning and husking

Grain samples from the field were dried if required to achieve a moisture content of 12.1–14.2%. The rice was then cleaned using a sample cleaner. Shriveled (undeveloped and broken) grain was sorted out (< 1.9 mm). Samples of cleaned grain, shriveled grain and impurities were taken.

A portion of the cleaned grain was husked with a rubber husker. Samples of husks, brown rice and abrasion / broken grain were taken.

Polishing

Brown rice was processed into bran and polished rice. If the period between husking and polishing was more than 12 hours, an additional sample of brown rice was taken before polishing. The brown rice was then polished using a vertical shelling machine (abrasive decortication). Samples of bran / rub-off and polished rice were taken.

Parboiling

Samples of cleaned grain were taken before the parboiling process. The cleaned rice was steeped in water and heated to 76–85 °C. The steeped grain was stored in its closed container at room temperature and had a moisture content of 37.1–47.3 % at the end of the procedure (duration 3–4.4 h). Excess steeping water (which was not absorbed) was removed. A sample of the steeping water was taken.

The steeped grain was transferred to an autoclave and steamed at 104–115 °C for about 15 min. Samples of steamed grain and steaming water were taken before the steamed grain was transferred to the drying oven. The grain was dried for 16 h at temperatures between 36 °C and 88 °C until a final moisture content of 7.6–14.9 % was achieved. A sample of parboiled rice was taken.

The parboiled rice was husked using a rubber husker and samples of husks, parboiled brown rice and abrasion / broken grain were taken.

The husked parboiled brown rice was then polished using a vertical shelling machine. Samples of bran / rub-off and polished parboiled rice were taken.

Cooking

Samples of each type of rice were taken just before cooking.

Brown rice was cooked for 50–75 min in boiling water (97–100 °C) and a sample of cooked brown rice was taken. Brown parboiled rice was cooked for 65–85 min in boiling water (99–102 °C) and a sample of cooked parboiled brown rice was taken. Polished rice was cooked for 31–62 min in boiling water (98–100 °C) and a sample of cooked rice was taken. Polished parboiled rice was cooked for 48–68 min in boiling water (98–104 °C) and a sample of cooked parboiled rice was taken.

Milling flour

Samples of polished rice and polished parboiled rice were taken just before milling. Polished rice was milled using a cross beater mill and a sample of flour (polished rice) was taken. Similarly, polished parboiled rice was milled using a cross beater mill and a sample of flour (parboiled rice) was taken.

A summary flow chart of the overall processing scheme is given in Figure 1.

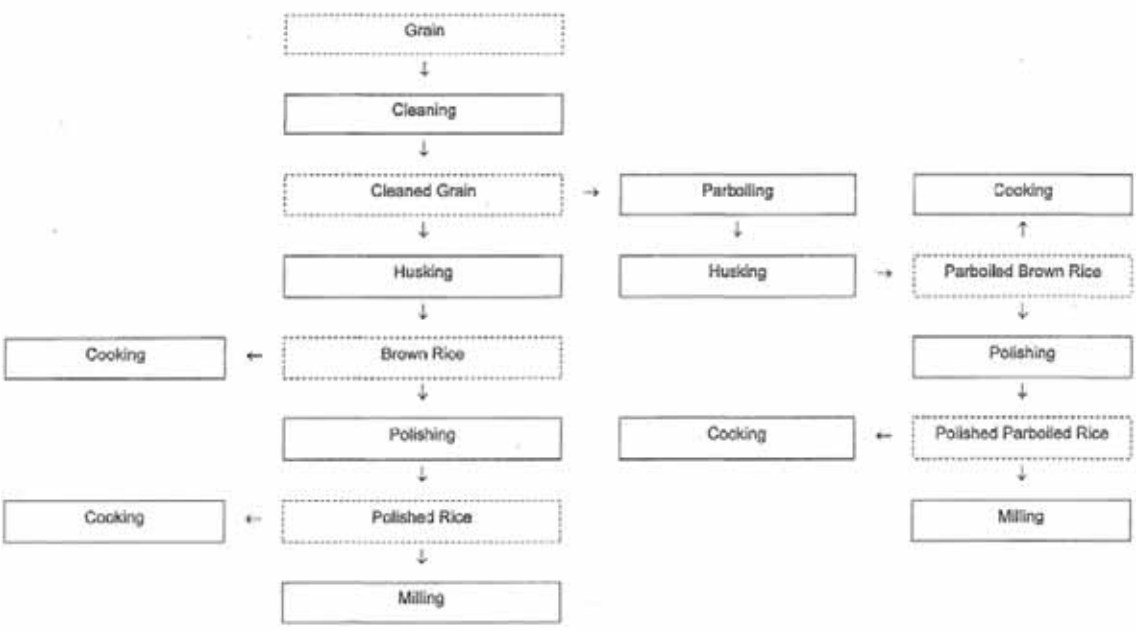

Figure 1 Processing Scheme for Rice grain

Study 1

Table 16a Residues from parent difenoconazole in rice grain (RAC and processed fractions)

Trial Location, year, (variety), dose rate, interval , DALT	Processed fraction	Difenoconazole parent mg/kg (mean in parenthesis)	processing factor
S10-02953-01 Italy, 2010 (Scudo) 258+256 g ai/ha interval 15 days, DALT = 24 Sandy clay loam	Rice grain, field (RAC)	3.0	-
	Mass balance trial (S10-02953-01-006)		
	Cleaning and husking		
	Grain, not cleaned	2.4	
	Cleaned grain	1.9, 2.5 (2.2)	1.09
	Impurities	7.6	3.45
	Shriveled grain	1.4	0.64
	Husks	8.4	3.50
	Abrasion/broken grain	4.0	1.82
	Brown rice	0.15	0.07
	Polishing		
	Brown rice	0.28	0.13
	Bran/rub rice	0.28	0.13
	Polished rice	0.041	0.02
		Parboiling	
	Cleaned grain	2.4	
	Steeping water	0.04	0.02
	Steamed grain	1.3	0.54
	Steaming water	< 0.01	0.004
	Parboiled rice	2.0	0.83
	Husks	5.4	2.25
	Abrasion/broken grain	2.2	0.92
	Parboiled brown rice	0.84	0.35

Trial Location, year, (variety), dose rate, interval , DALT	Processed fraction	Difenoconazole parent mg/kg (mean in parenthesis)	processing factor
	Bran/rub-off	3.3	1.38
	Polished parboiled rice	0.56	0.23
		Cooking	
	Cooked brown rice	0.12	0.05
	Cooked parboiled rice	0.51	0.21
	Cooked rice	0.023	0.01
	Cooked parboiled brown rice	0.22	0.09
		Milling	
	Flour (polished rice)	0.054	0.02
	Flour (parboiled rice)	0.44	0.18
	Follow-up-trial (S10-02953-01-007)		
	Cleaning and husking		
	Grain, not cleaned	2.9	-
	Cleaned grain	2.1, 1.9 (2.0)	0.69
	Husks	8.6	4.30
	Brown rice	0.14	0.07
	Polishing		
	Brown rice	0.10	0.05
	Bran/rub rice	0.25	0.13
	Polished rice	0.027	0.01
	Parboiling		
	Cleaned grain	1.9	0.66
	Parboiled rice	1.7	0.59
	Husks	5.1	1.76
	Parboiled brown rice	0.88	0.30
	Bran/rub-off	3.0	1.03
	Polished parboiled rice	0.49	0.17
	Cooking		
	Cooked brown rice	0.14	0.05
	Cooked parboiled rice	0.35	0.12
	Cooked rice	0.011	0.004
	Cooked parboiled brown rice	0.25	0.12
	Milling		
	Flour (polished rice)	0.039	0.01
	Flour (parboiled rice)	0.42	0.14

Study 2

Table 16b Residues from parent difenoconazole in rice grain (RAC and processed fractions)

Trial Location, year, (variety), dose rate, interval, DALT	Processed fraction	Difenoconazole parent (mg/kg)	processing factor
	Rice grain, field (RA	1.7	-
S10-02953-02 Italy, 2010 (Ercole)	Mass balance trial ((S10-02953-01-006)		
	Cleaning and husking		
	Grain, not cleaned	2.0	
	Cleaned grain	1.8, 1.2 (2.0)	1.0
251+252 g ai/ha interval 15 days, DALT = 21	Impurities	5.6	2.8
	Shriveled grain	1.8	0.9
	Husks	8.4	4.2
	Abrasion/broken grain	1.8	0.9
	Brown rice	0.077	0.04
Sandy clay loam	Polishing		
	Brown rice	0.09	0.05
	Bran/rub rice	0.28	0.14
	Polished rice	0.017	0.009
		Parboiling	
	Cleaned grain	1.4	0.7
	Steeping water	0.019	0.01

Trial Location, year, (variety), dose rate, interval, DALT	Processed fraction	Difenoconazole parent (mg/kg)	processing factor
	Steamed grain	0.88	0.63
	Steaming water	0.001	00007
	Parboiled rice	1.5	1.07
	Husks	5.2	3.71
	Abrasion/broken grain	2.1	1.5
	Parboiled brown rice	0.76	0.54
	Bran/rub-off	2.1	1.5
	Polished parboiled rice	0.35	0.25
	Cooking		
	Cooked brown rice	0.062	0.04
	Cooked parboiled rice	0.37	0.26
	Cooked rice	< 0.01	0.007
	Cooked parboiled brown rice	0.18	0.13
	Milling		
	Flour (polished rice)	0.016	0.01
	Flour (parboiled rice)	0.37	0.26
	Follow-up-trial (S10-02953-01-007)		
	Cleaning and husking		
	Grain, not cleaned	1.9	
	Cleaned grain	1.3, 1.3, (1.3)	0.68
	Husks	8.0	4.21
	Brown rice	0.074	0.04
	Polishing		
	Brown rice	0.099	0.05
	Bran/rub rice	0.38	0.2
	Polished rice	0.013	0.007
	Parboiling		
	Cleaned grain	1.5	0.79
	Parboiled rice	1.7	1.13
	Husks	4.7	3.13
	Parboiled brown rice	0.68	0.45
	Bran/rub-off	1.9	1.27
	Polished parboiled rice	0.37	0.25
	Cooking		
	Cooked brown rice	0.045	0.03
	Cooked parboiled rice	0.37	0.25
	Cooked rice	< 0.01	0.003
	Cooked parboiled brown rice	0.17	0.11
	Milling		
	Flour (polished rice)	0.013	0.009
	Flour (parboiled rice)	0.35	0.23

Table 17 Summary of parent difenoconazole residues in rice grain processed commodities from trials made in Italy

Processed fraction	Processing factors	Processing factors (mean)
Cleaned grain	1.09, 0.69, 1.0, 0.68	0.85
Husks	3.5, 4.3, 4.2, 4.21	4.05
Bran/rub-off	0.13, 0.13, 0.14, 0.2	0.15
Brown rice	0.07, 0.07, 0.04, 0.04	0.06
Parboiled rice	0.83, 0.59, 1.07, 1.13	0.91
Parboiled brown rice	0.35, 0.30, 0.54, 0.45	0.41
Polished rice	0.02, 0.01, 0.009, 0.007	0.01
Polished parboiled rice	0.23, 0.17, 0.25, 0.25	0.23

Processed fraction	Processing factors	Processing factors (mean)
Cooked brown rice	0.05, 0.05, 0.04, 0.03	0.04
Cooked parboiled rice	0.21, 0.12, 0.26, 0.25	0.21
Cooked rice	0.01, 0.004, 0.007, 0.003	0.006
Cooked parboiled brown rice	0.09, 0.12, 0.13, 0.11	0.11
Flour (polished rice)	0.02, 0.01, 0.01, 0.009	0.01
Flour (parboiled rice)	0.18, 0.14, 0.26, 0.23	0.20

Rape seed

Two studies on the behaviour of difenoconazole during processing of rape seed into meal and refined oil was conducted by Sagen K (2011, CER 05903/11). Field trials of oilseed were treated with one application of the target rate 0.375 kg ai/ha. Samples were harvested 30 days after the application. Rape seed was used for the production of meal and refined oil. Field samples and processed fractions (single samples) were analysed for parent difenoconazole using method REM 147.08. The LOQ was 0.01 mg/kg. The duration of storage for the processed fractions press-cake meal and refined oil were 3.2 months and 1.6 months, respectively.

- Whole oilseed rape seeds were flaked
- Flakes were pressed to separate the oil
- The extracted meal was air dried
- A sample of air dried meal was heat treated to duplicate toasting of rape seed meal
- The pressed and extracted oils were combined
- The crude solvent oil and the centrifuged press oil were blended, acid degummed, refined, washed with water and bleached
- The bleached oil was deodorized.

Figure 2 Processing scheme for rape seed

Table 18 Residues from parent difenoconazole in rape seed (RAC and processed fractions)

Trial Location, year, (variety), dose rate, interval , DALT	Processed Fraction	Difenoconazole parent, mg/kg	processing factors
Trial T948 Canada, (Elgin MB) 2011 (1841 RR) 0.367 kg ai/ha, DALT = 31	rape seed	0.033	
	meal	0.014	0.42
	oil	< 0.01	0.3
Trial T949 Canada, (Rosthern SK) 2011 (72-55 RR) 0.390 kg ai/ha, DALT = 31	rape seed	0.18	
	meal	0.12	0.67
	oil	< 0.01	0.06

Table 19 Summary of calculated processing factors in soya bean, rice and oilseed rape from difenoconazole treated raw commodities

RAC	Processed fraction	Calculated processing factors				PF best estimate
		Difenoconazole	1,2,4 Triazole*	Triazole alanine**	Triazole lactic acid***	
soya bean	Meal	0.63, 0.01	< 0.01, 0.01,	0.45, 0.91	0.01, 1.5	
	Hulls	3.38, 0.61	< 0.01, 0.01,	0.31, 0.22	0.01, -	
	Oil (refined)	1.06, 0.42	< 0.01, < 0.01,	< 0.01, < 0.01,	0.01, < 0.01	
	AGF	622	2.4	-	33.8	
rice	Husks	3.5, 4.3, 4.2, 4.21	nm	nm	nm	
	Bran/rub-off	0.13, 0.13, 0.14, 0.2	nm	nm	nm	
	Brown rice	0.07, 0.07, 0.04, 0.04	nm	nm	nm	
	Parboiled rice	0.83, 0.59, 1.07, 1.13	nm	nm	nm	
	Parboiled brown rice	0.35, 0.30, 0.54, 0.45	nm	nm	nm	
	Polished rice	0.02, 0.01, 0.009, 0.007	nm	nm	nm	
	Polished parboiled rice	0.23, 0.17, 0.25, 0.25	nm	nm	nm	
	Cooked brown rice	0.05, 0.05, 0.04, 0.03	nm	nm	nm	
	Cooked parboiled rice	0.21, 0.12, 0.26, 0.25	nm	nm	nm	
	Cooked rice	0.01, 0.004, 0.007, 0.003	nm	nm	nm	
	Cooked parboiled brown rice	0.09, 0.12, 0.13, 0.11	nm	nm	nm	
	Flour (polished rice)	0.02, 0.01, 0.01, 0.009	nm	nm	nm	
	Flour (parboiled rice)	0.18, 0.14, 0.26, 0.23	nm	nm	nm	
Oilseed rape	meal	0.42, 0.67	nm	nm	nm	
	refined oil	0.3, 0.06	nm	nm	nm	

- not calculated due to less occurrence in treated processed soya bean than in untreated processed soya beans.

nm: not measured

APPRAISAL

Difenoconazole is a systemic triazole fungicide and acts by inhibition of demethylation during ergosterol synthesis. It is applied by foliar spray or seed treatment and controls a broad spectrum of foliar, seed and soil-borne diseases caused by Ascomycetes, Basidiomycetes and Deuteromycetes, on a variety of crops. Difenoconazole was evaluated for the first time by JMPR 2007. The 2007 Meeting established an acceptable daily intake (ADI) of 0–0.01 mg/kg bw and an acute reference dose (ARfD) of 0.3 mg/kg bw. Maximum residue levels for a number of commodities were recommended by JMPR in 2007, 2010 and 2013.

Definition of residues for plant products (compliance with MRLs and dietary intake assessment): *difenoconazole*.

Definition of residues for animal products: sum of difenoconazole and CGA 205375 (1-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-2-(1, 2, 4-triazol)-1-yl-ethanol), expressed as difenoconazole.

Difenoconazole was listed by the Forty-sixth Session of CCPR (2014) for the review of additional maximum residue levels. GAP information with supporting residue studies in strawberries, avocadoes, soya beans, cotton, peanuts, rice and oilseed rape (canola) was evaluated by the present Meeting.

Methods of analysis

The analytical method used for determination of difenoconazole residues in samples derived from supervised field trials and processing studies in strawberries, soya beans, rice and oilseed was evaluated by previous Meetings.

Two new pre-registration methods for plant matrices were presented to the 2015 Meeting. In these methods difenoconazole is extracted by high-speed homogenisation with an acetone/water mixture (2:1). After clean-up the residues were determined by (HPLC-MS/MS).The method has a validated LOQ of 0.01 mg/kg for difenoconazole in avocadoes, cotton, oilseed rape including processed commodities, peanuts, rice, soya beans and strawberries. The methods were used for determination of difenoconazole residues in samples from supervised field trials on cotton and peanuts presented to the current Meeting.

Stability of pesticide residues in stored analytical samples

The stability of residues from difenoconazole in stored samples was evaluated by the 2007 Meeting. The periods of demonstrated stability cover the frozen storage intervals used in the residue trials for which maximum residue levels were estimated.

Results of supervised residue trials on crops

The Meeting received new supervised trial data for foliar application of difenoconazole (EC or SC formulations) on strawberries, avocadoes, soya beans, rice, cotton, peanuts and oilseed rape, and noted that residue data from rice, soya beans and oilseed rape also were provided to the 2007 JMPR.

The results from new trials and those previously reported by the 2007 JMPR which either matched the critical GAP, or when results could be proportionally adjusted to reflect GAP application rates, were considered in estimating maximum residue levels, STMRs and HRs for the commodities for which GAP information was available. The proportionality approach was considered to scale the results from trials where the application rates range from 0.3× GAP to 4× GAP and where all other parameters matched the critical GAP.

Strawberry

Data from supervised trials on strawberries from USA conducted in 2008 and 2009 were presented to the Meeting. The critical GAP in USA is maximum foliar applications up to 0.129 kg/ha, an application interval of 7–14 days and a PHI of 0 days. The maximum application rate for difenoconazole is 0.515 kg ai/ha per crop and season.

Strawberries belong to the high acid category and storage data covering this category was not evaluated by 2007 JMPR and not included in the residue trials. As difenoconazole has a pKa of 1.1 an estimation of maximum residue levels was not made.

Avocado

Four independent supervised trials from Brazil conducted in 2007 and 2008 were presented to the Meeting. The critical GAP in Brazil is four foliar applications of 0.05 kg ai/ha at BBCH 62–79 (starting at flowering until fruit is around 5 cm) and with intervals of 14 days. The PHI is 14 days.

The trials from Brazil (4× 0.05 kg ai/ha at BBCH 71–79, interval 14 days, PHI 14 days) matched the critical GAP. Residues of difenoconazole in avocado fruits 14 days after the last application were (n=4) 0.02, 0.05 (2) and 0.26 mg/kg. The highest residue of 0.26 mg/kg was measured in an individual fruit sample.

The Meeting estimated a maximum residue level, an STMR value and an HR value for difenoconazole in avocado of 0.6 mg/kg, 0.05 mg/kg and 0.26 mg/kg, respectively.

Soya bean (dry)

Twenty one supervised trials from USA conducted in 2008 were presented to the Meeting. The critical GAP in USA is two foliar applications of 0.129 kg ai/ha, with an interval of seven days and a PHI of 14 days.

Six trials from Brazil (2× 0.075 kg ai/ha and a PHI of 30 days) presented to the 2007 JMPR did not match the critical GAP.

Eighteen independent trials from USA (2× 0.129 kg ai/ha, interval 7–10 days, PHI 14 days) matched the critical GAP. Residues of difenoconazole in soya beans were (n=18) < 0.01(12), 0.012, 0.013, 0.019, 0.021, 0.04 and 0.087 mg/kg. The highest residue of 0.15 mg/kg was measured in individual seed samples.

The Meeting estimated a maximum residue level and STMR value for difenoconazole in soya bean seeds of 0.1 mg/kg and 0.01 mg/kg, respectively. The Meeting withdraws its previous recommendation of 0.02* mg/kg for maximum residue level for soya beans (dry).

Rice

Eight supervised trials from Europe (Italy) conducted in 2009 and 2010 were presented to the current Meeting. A registered label was not available to the Meeting and an estimation of a maximum residue level was not made.

Cotton

Eight independent supervised trials from Brazil conducted in 2006–2008 were presented to the Meeting. The critical GAP in Brazil is three foliar applications of 0.075 kg ai/ha, an interval of 10–15 days and a PHI of 21 days.

Four trials (5× 0.075 kg ai/ha, BBCH 13–81, interval 21 days, PHI 30 days) were not according to GAP. Samples were only taken 30 days after last application, and the applications were two more than specified in the critical GAP.

Four trials were made with four applications of 0.075 kg ai/ha starting from BBCH 71 up to BBCH 83 and a PHI of 21 days. These trials matched the critical GAP from Brazil. Residues

of difenoconazole in cotton were (n=4) < 0.01, 0.01 and 0.02 (2) mg/kg. An estimation of maximum residue levels was not made as four trials were considered insufficient.

Oilseeds

Peanut

Eight independent supervised trials from Brazil conducted in 2008–2010 were presented to the Meeting. The critical GAP in Brazil is three applications of 0.0875 kg ai/ha and a PHI of 22 day.

Four of the trials (3× 0.088 kg ai/ha, PHI 22 days) were according to the critical GAP and residues of parent difenoconazole were not detected. Another four trials (6× 0.125 kg ai/ha) were conducted as residue decline trials and residues of parent difenoconazole was not found.

As residues of difenoconazole not was detected at an exaggerated number of applications and application rates, the Meeting concluded a zero residue situation occurs after application of difenoconazole to peanuts in accordance with the Brazilian critical GAP.

Residues of difenoconazole in peanuts from eight independent trials matching GAP were (n=8) < 0.01 mg/kg.

The Meeting estimated a maximum residue level and STMR values for difenoconazole in peanut kernels of 0.01* mg/kg and 0 mg/kg, respectively.

Rape seed (canola)

Data from supervised trials on rape seed (canola) from Canada conducted in 2011 were presented to the Meeting. The critical GAP in Canada is one foliar application of 0.125 kg ai/ha and a PHI of 30 days.

Nine independent trials from Canada matching the critical GAP were available to the Meeting. Residues from difenoconazole in rape seed were (n=9) < 0.01, 0.011 (1), 0.015 (2), 0.033 (2), 0.038, 0.062 and 0.063 mg/kg.

The Meeting estimates a maximum residue level, and STMR value for difenoconazole in oilseed rape (rape seed) of 0.15 mg/kg and 0.03 mg/kg, respectively. The Meeting replaces its previous recommendation of 0.05 mg/kg for the maximum residue level for rape seed.

Animal feeds

Rape seed (canola), forage, fodder

Residue data for rape seed forage was not presented to the Meeting.

Soya bean

The Meeting noted that the GAP for difenoconazole in USA does not permit soya bean hay, forage or silage as animal feeds.

Rice whole crop (silage), and straw

Eight supervised trials from Europe (Italy) conducted in 2009 and 2010 were presented to the Meeting. Forage and straw samples were collected. A registered GAP was not available for rice. An estimation of maximum residues levels was not made.

Fate of residues during processing

The 2007 JMPR reported that difenoconazole was essentially stable during the hydrolysis conditions simulating food processing conditions and also estimated processing factors for a range of commodities. Relevant processing factors for difenoconazole and STMR-Ps for the commodities

considered at this Meeting and used for dietary intake and risk assessment or for estimating livestock animal burden are summarized below.

Raw agricultural commodity	Processed commodity	Processing factors [a] (mean)	RAC (mg/kg)	STMR-P
			STMR	mg/kg
Soya bean	RAC		0.01	
	Meal	0.38		0.004
	Hulls	2		0.02
	Oil (refined)	0.8		0.08
	AGF [b]	622		6.22
Rape seed (canola)	RAC		0.03	
	Meal	0.55		0.016
	Refined oil	0.05		0.002

[a] The processing factor is the ratio of the total residue in the processed item divided by the total residue in the RAC

[b] Aspirated grain fraction

The Meeting noted that in the studies available difenoconazole residues did not concentrate in food commodities during processing. In feed commodities however residues increased in soya bean hulls and soya bean aspirated grain fractions (AGF).

Residues in animal commodities

Estimated dietary burdens of farm animals

The dietary burdens for beef cattle and dairy cattle were calculated using the OECD diets listed in Appendix IX of the 2009 edition of the FAO Manual. Potential feed items included: almond hulls, cabbage heads and leaves, bean vines, carrot hulls, canola meal, grape pomace, pea vines, potato culls, potato process waste, soya beans, soya bean aspirated grain fraction, sunflower meal, and wheat grain and hay.

The estimated the dietary burden for cattle and poultry and were not significantly different from the dietary burdens estimated by the 2013 JMPR. The only additional feed item included was soya bean.

The Meeting confirmed the previous recommendations for animal commodities.

RECOMMENDATIONS

On the basis of the data from supervised trials the Meeting concluded that the residue levels listed are suitable for establishing maximum residue limits and for IEDI and IESTI assessment.

Definition of residue for plant products (compliance with MRLs and dietary intake assessment): *difenoconazole.*

Definition of residue for animal products (compliance with MRLs and dietary intake assessment): sum of difenoconazole and CGA 205375 (1-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-2-(1, 2,4-triazol)-1-yl-ethanol), expressed as difenoconazole.

The residue is fat soluble (2007 JMPR Meeting).

CCN	**Commodity**	**Recommended Maximum residue level (mg/kg)**		**STMR or STMR-P mg/kg**	**HR or HR-P mg/kg**
		New	**Previous**		
FI 0326	Avocado	0.6		0.05	0.26
SO 0697	Peanut	0.1 *		0	
SO 0495	Rape seed	0.15	0.05	0.03	
VD 0541	Soya bean (dry)	0.1	0.02 *	0.01	

CCN	Commodity	Recommended Maximum residue level (mg/kg)		STMR or STMR-P mg/kg	HR or HR-P mg/kg
		New	Previous		
OR 0541	Soya bean oil, refined			0.08	
OR 0495	Rape seed oil, edible			0.002	
AB 0541	Soya bean hulls			0.02	
AB 1265	Soya bean meal			0.004	
	Soya bean asp gr fn[a]			6.22	

[a] aspirated grain fraction

DIETARY RISK ASSESSMENT

Long-term intake

The IEDI of difenoconazole based on the STMRs estimated by this and previous Meetings for the 17 GEMS/Food regional diets were 7–70% of the maximum ADI of 0.01 mg/kg bw (see Annex 3 of the 2015 Report). The Meeting concluded that the long-term dietary intake of residues of difenoconazole is unlikely to present a public health concern.

Short-term intake

The ARfD for difenoconazole is 0.3 mg/kg bw. The International Estimated Short-Term (IESTI) of difenoconazole for the commodities for which STMR, HR and maximum residue levels were estimated by the current Meeting are shown in Annex 4 to the 2015 Report. The IESTI represented a maximum of 3% of the ARfD. The Meeting concluded that the short-term intake of difenoconazole residues from uses considered by the current Meeting was unlikely to present a public health concern.

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