Draft Assessment Report

DIFENOCONAZOLE

Volume 3
Annex B.7
Residue data

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B.7 Residue data

Difenoconazole (1-[2-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-4-methyl-[1,3]dioxolan-2-ylmethyl]-1H-[1,2,4] triazole) is a systemic triazole fungicide used for long-lasting preventative and curative broad-spectrum control of cereal, fruit and vegetable diseases including powdery mildew, rust, scab and leaf spots.

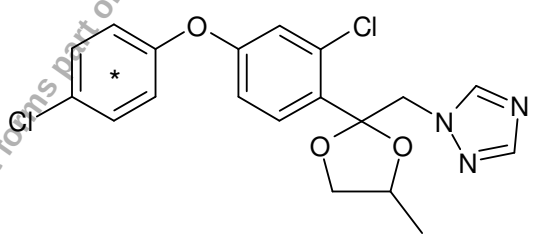
Difenoconazole acts by interference with the ergosterol biosynthesis in target fungi by inhibition of the C-14-demethylation of sterols, which leads to morphological and functional changes of the fungal cell membrane.

The compound is formulated either as a 250 g active ingredient (a.i.)/L emulsifiable concentrate (EC) or as a 30 g a.i./L flowable concentrate (FS). The intended use within EU is in the cultivation of cereals (seed treatment), carrot and pome fruits (foliar application).

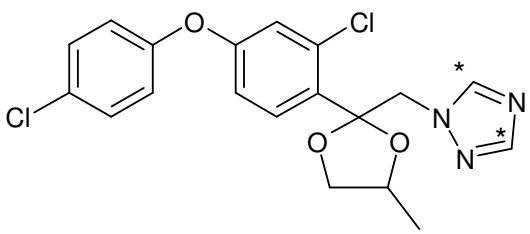
In Southern Europe, difenoconazole will be applied on pome fruit at a maximum of 75 g a.i./ha per application at a maximum of 4 applications per season and with a pre-harvest interval (PHI) of 14 days. In Northern Europe, the application rate will be lower (56.25 g a.i./ha per application) with a maximum of 4 applications per season and with a PHI of 28 days. In Northern and Southern Europe, difenoconazole will be applied on carrots at a maximum of 125 g a.i./ha per application with a maximum of three applications per season and with a PHI of 14 days. In Northern and Southern Europe, difenoconazole will be applied once to seeds of cereals at a maximum of 6 g a.i./100 kg seeds.

B.7.1 Metabolism, distribution and expression of residues in plants (Annex IIA 6.1 and Annex IIIA 8.1)

The metabolism and distribution of difenoconazole in plants has been investigated in four crop types: cereals (spring wheat), root vegetables (potato), fruits (tomato and grape) and pulses/oilseeds (oilseed rape). Difenoconazole contains both aromatic and triazole ring moieties and therefore the metabolism studies were performed using two radiolabelled forms of difenoconazole (Figure B.7.1-1). Difenoconazole is referred to as CGA-169374 in the studies. Metabolites are also referred to by various code numbers when they have been identified in the metabolism studies.



[Phenyl-^{14}C] difenoconazole



[Triazole-^{14}C] difenoconazole

Figure B.7.1-1. Structure of difenoconazole and position of the radiolabel (*).

B.7.1.1 Metabolism of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole in greenhouse grown tomatoes

Reference:	**Madrid S.O. and Huber M. K. (1987a)** The distribution and characterization of Phenyl-^{14}C vs. Triazole-^{14}C-CGA-169374 on spray treated tomatoes - A side by side comparison study in the greenhouse. Biochemistry Department, Agricultural Division, Ciba-Geigy Corporation, Greensboro, North Carolina, United States. Unpublished report ABR-87025, SAM No. 0043.
Test Material:	Phenyl-^{14}C, batch number GAN-IX-5, radiochemical purity 97%, specific activity 48.6 µCi/mg. Triazole-^{14}C, batch number GAN-IX-7, radiochemical purity 98%, specific radioactivity 48.5 µCi/mg.
Guideline:	US EPA Residues Chemistry, Series 171-4 (a) (1) & (2) (1982 and 1986), Washington, DC.
GLP:	No. Conducted prior to the adoption of GLP standards.
Material and methods:	
Test concentration:	123.5 g a.i./ha
Test system:	Tomato plants (variety *Sunny*) were grown in aluminium containers (ca 15L capacity, 1 plant/container) containing Georgia loamy sand soil. The soil characteristics were: pH (6.0), sand (83.2%), silt (12.8%), clay (4%), cation exchange capacity (CEC, 4.72 meq/100 g dry soil) and organic matter (1.4%). [Phenyl-^{14}C] and [triazole-^{14}C] difenoconazole, formulated as a 3.5 EC, were applied using a Devilbiss sprayer assembly with nitrogen as a propellant.
Stage of application:	55, 62, 69, 76, 83, 90 days after planting.
No. of applications:	Six
Sampling time points:	Tomato foliage was sampled after the first application (55 days post planting) and prior to the third application (68 days post planting). Tomato fruit and foliage were sampled prior to the fifth application (82 days post planting) and at a PHI of 7 and 16 days (mature harvest) following the last application.
Method of analysis:	Total radioactive residues (TRR) in the foliage and fruit samples were quantified by combustion/liquid scintillation counting (LSC). Samples of each commodity were subjected to Bligh-Dyer solvent extraction, which yielded organic and aqueous phases and an unextractable fraction. Identification of the residues in the organic soluble phase was carried out by co-chromatography against standard reference compounds using either one-dimensional (1D) or two-dimensional (2D) thin layer chromatography (TLC).
Date of experiment:	29 January 1986 to 5 March 1987

Findings: Residue levels in tomato foliage and fruit are shown in Table B.7.1.1-1.

Table B.7.1.1-1. Distribution of radioactive residues in tomato plant fractions following six applications of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole at a rate of 123.5 g a.i./ha.

Labelled form	Plant Commodity	Sampling Time[a]	TRR (mg/kg)[b]	Organic phase (%)[c]	Aqueous phase (%)[c]	Non-extracted (%)[c]	Total (%)[c]
[phenyl-^{14}C] difenoconazole	Foliage	55 days (following 1st application)	4.044	84.0	3.1	1.4	88.5
	Foliage	68 days (prior to 3rd application)	4.014	78.9	11.7	9.3	99.9
	Foliage	82 days (prior to 5th application)	3.323	80.7	15.0	12.1	107.8
	Fruit (green)	82 days (prior to 5th application)	0.079	68.6	18.4	5.0	92.0
	Foliage (mature)	106 days (16 day PHI)	2.843	56.7	25.5	13.3	95.5
	Fruit (green)	106 days (16 day PHI)	0.016	52.8	31.6	11.5	95.9
	Fruit (ripe)	106 days (16 day PHI)	0.037	48.9	37.2	10.1	96.2
[triazole-^{14}C] difenoconazole	Foliage	55 days (following 1st application)	2.61	91.1	2.8	2.6	96.5
	Foliage	68 days (prior to 3rd application)	2.036	81.7	18.7	9.6	110.0
	Foliage	82 days (prior to 5th application)	2.374	69.3	20.4	8.2	97.9
	Fruit (green)	82 days (prior to 5th application)	0.232	52.11	43.4	6.9	102.4
	Foliage (mature)	106 days (16 day PHI)	2.806	49.4	29.4	12.3	91.1
	Fruit (green)	106 days (16 day PHI)	0.129	1.7	91.0	0.6	93.3
	Fruit (ripe)	106 days (16 day PHI)	0.122	9.1	79.5	1.3	89.9

[a] Days after planting.
[b] mg/kg determined by combustion.
[c] Percent of the TRR determined by combustion.

The total radioactive residues (TRR) in the tomato foliage were 2.843 to 4.044 mg/kg for the [phenyl ^{14}C] difenoconazole treated plants and 2.036 to 2.806 mg/kg for the [triazole-^{14}C] difenoconazole treated plants. At maturity, low levels of TRR were present in green (0.016 mg/kg) and ripe (0.037 mg/kg) tomato fruit harvested at a PHI of 16 days following six applications of [phenyl-^{14}C] difenoconazole. Higher levels of TRR (0.129 and 0.122 mg/kg) were present in the respective [triazole-^{14}C] difenoconazole treated fruit.

In the tomato foliage, organic soluble radioactivity comprised 56.7 to 84.0% and 49.4 to 91.1% of the TRR for the [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole treated plants, respectively. Parent difenoconazole formed the major part of the residue accounting for 35.8 to 58.2% of the TRR (Table B.7.1.1-2). The metabolites CGA-205375 and CGA-205374 accounting for less than 2% of the TRR, although they were not fully resolved by TLC analysis. The metabolite CGA-189138 (2.4 to 5.6% of the TRR) was observed in the [phenyl-^{14}C] difenoconazole treated plants only.

In the tomato foliage, aqueous soluble radioactivity accounted for 3.1 to 25.5% and 2.8 to 29.4% of the TRR for the [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole treatments, respectively, and was composed mainly of polar material that was attributed to conjugates of the metabolites. No 1,2,4-triazole (CGA-71019) was detected in the foliage from plants treated with [triazole-^{14}C] difenoconazole. The unextracted radioactive residues were 0.6 to 13.3% of the TRR for both radiolabelled treatment and were not analysed further.

In the mature tomato fruit (green and ripe), organic soluble radioactivity contained 48.9 to 52.8% of the TRR in the [phenyl-^{14}C] difenoconazole treated plants, but contained only 1.7 to 9.1% of the TRR in the [triazole ^{14}C] difenoconazole treated plants. Aqueous soluble radioactivity was correspondingly higher in the [triazole-^{14}C] difenoconazole treated plants, accounting for 79.5 to 91.0% of the TRR, with the [phenyl-^{14}C] difenoconazole treated aqueous phase containing only 31.6 to 37.2% of the TRR. Unextracted radioactive residues were lower (0.6 to 1.3% TRR) in the [triazole-^{14}C] difenoconazole treated fruit, compared to the [phenyl-^{14}C] difenoconazole treated fruit (10.1 to 11.5% TRR) at the 16 day PHI interval.

Chromatographic analysis of the fruit extracts was not performed. The differences in the distribution of radioactivity between the organic and aqueous soluble fractions were attributed to cleavage of the triazole moiety, with preferential transport of water soluble triazole metabolites to the fruit.

Characterisation of the organic soluble radioactivity extracted from tomato foliage is summarised in Table B.7.1.1-2.

Table B.7.1.1-2. Characterisation of organic soluble radioactive residues in tomato foliage following six applications of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole at a rate of 123.5 g a.i./ha.

Labelled form	Sampling time[a]	68 days (prior to 3rd application)		82 days (prior to 5th application)		106 days (16 days of PHI)	
	Compound	% TRR	mg/kg[b]	% TRR	mg/kg[b]	% TRR	mg/kg[b]
[phenyl-^{14}C] difenoconazole	Parent difenoconazole	58.2	2.338	54.8	1.689	36.6	1.089
	CGA 205375 / CGA 205374[c]	1.2	0.048	1.3	0.040	0.8	0.024
	CGA 189138	2.4	0.096	3.6	0.111	5.6	0.167
[triazole-^{14}C] difenoconazole	Parent difenoconazole	58.2	1.077	51.4	1.248	35.8	1.103
	CGA 205375 / CGA 205374[c]	1.9	0.035	1.1	0.027	0.9	0.028

[a] Days after planting
[b] Calculated from percentages shown in Table B.7.1.1-1. Analysis performed on the organic phase resulting from Bligh-Dyer extraction of the plant material.
[c] Metabolites not fully separated by TLC. Consists of more than one metabolite.

Conclusions: At maturity, 16 days after the last of six applications of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole at 123.5 g a.i./ha, the TRR in ripe tomato fruit was 0.037 and 0.122 mg/kg and in foliage was 2.843 and 2.806 mg/kg, respectively. Parent difenoconazole was the largest component of the residue in the mature foliage, accounted for 36.6 and 35.8% of the TRR from the [phenyl-^{14}C] and [triazole-^{14}C]-difenoconazole treatments, respectively. Very low levels (< 1% TRR) of the alcohol and ketone metabolites CGA-205375 and CGA-205374 were identified in foliage from both radiolabelled treatments. The acid metabolite CGA-189138 was observed in the [phenyl-^{14}C] difenoconazole treated foliage at a level of 5.6% of the TRR. Aqueous soluble radioactivity extracted from the foliage contained mainly polar compounds that were attributed to conjugates of the free metabolites. 1,2,4-triazole was not detected in the organic extracts from foliage treated with [triazole-^{14}C] difenoconazole. Differences in the distribution of radioactivity between the organic and aqueous soluble fractions were attributed to cleavage of the triazole moiety, with preferential transport of water soluble triazole metabolites (including conjugates) to the fruit. The majority of the residue remained in the foliage for both radiolabels.

Comments by RMS: No data of the greenhouse conditions or discussion of storage stability has been included in the report. However, this is not considered to affect the validity of the study. The application rate in the present study was higher than the critical GAP proposed for pome fruit in Southern and Northern Europe (75 and 56.2 g a.i./ha, respectively) and the interval between applications was 7 days, which was shorter than the minimum of 10 days.

B.7.1.2 Metabolism of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole in field-grown tomatoes

Reference:	**Madrid S.O. and Huber M. K. (1987b)** The distribution and characterization of Phenyl-^{14}C vs. Triazole-^{14}C-CGA-169374 and their metabolites in field-grown tomatoes. Biochemistry Department, Agricultural Division, Ciba-Geigy Corporation, Greensboro, North Carolina, United States. Unpublished Report ABR-87033, SAM No. 0044.
Test Material:	Phenyl-^{14}C, batch number GAN-IX-5, radiochemical purity 97%, specific activity 48.6 µCi/mg. Triazole-^{14}C, batch number GAN-IX-7, radiochemical purity 98%, specific radioactivity 48.5 µCi/mg.
Guideline:	US EPA Residues Chemistry, Series 171-4 (a) (1) & (2) (1982 and 1986), Washington, DC.
GLP:	No. Conducted prior to the adoption of GLP standards.
Material and methods:	
Test concentration:	247 g a.i./ha
Test system:	The study was conducted outside in three trial plots (1m x 4m) containing a sandy loam soil. The soil characteristics were: pH (7.0), sand (49.6%), silt (39.6%), clay (10.8%), CE (6.4 meq/100 g) and organic matter (0.6%). Tomato seedlings (variety *UC-82*) were transplanted into the plots. [Phenyl-^{14}C] difenoconazole and [triazole-^{14}C] difenoconazole, formulated as a 3.5 EC, were applied using two TX-12 nozzles, 38 cm apart on the spray rig with CO_2 as a propellant. Harvested plants were separated into foliage and fruits.
Stage of application:	63 days after planting (flowering stage) , 77 and 91 days after planting.
No. of applications:	Three
Sampling time points:	Tomato foliage was sampled after the first application (63 days post planting) and prior to the second application (77 days post planting). Tomato fruit and foliage were sampled prior to the third application (91 days post planting) and at a PHI of 40 days (mature harvest) following the last application.
Method of analysis:	The TRR in the foliage and fruit samples were quantified by combustion/LSC. Samples of each commodity were subjected to Bligh-Dyer solvent extraction, which yielded organic and aqueous phases and an unextractable fraction. Identification of the residues in the organic soluble phase was carried out by co-

chromatography against standard reference compounds using either one-dimensional (1D) or two-dimensional (2D) thin layer chromatography (TLC).

Date of experiment: 24 April 1986 to 14 April 1987

Findings: Residue levels in tomato foliage and fruit are shown in Table B.7.1.2-1.

Table B.7.1.2-1. Distribution of radioactive residues in tomato plant fractions following three applications of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole at a rate of 247 g a.i./ha.

Labelled form	Plant commodity	Sampling Time[a]	TRR (mg/kg)[b]	Organic phase (%)[c]	Aqueous phase (%)[c]	Non-extracted (%)[c]	Total (%)[c]
[phenyl-^{14}C] difenoconazole	Foliage	63 days (following 1st application)	9.447	88.1	4.5	3.2	95.8
	Foliage	77 days (prior to 2nd application)	1.015	73.4	16.2	9.0	98.6
	Foliage	91 days (prior to 3rd application)	2.127	78.1	12.9	12.7	103.7
	Fruit (green)	91 days (prior to 3rd application)	0.012	--	--	--	--
	Foliage (mature)	131 days (40 day PHI)	3.548	54.5	22.3	15.7	92.5
	Fruit (green)	131 days (40 day PHI)	0.029	--	--	--	--
	Fruit (ripe)	131 days (40 day PHI)	0.026	--	--	--	--
[triazole-^{14}C] difenoconazole	Foliage	63 days (following 1st application)	6.670	98.4	3.4	4.4	106.2
	Foliage	77 days (prior to 2nd application)	0.978	69.1	20.1	8.9	98.1
	Foliage	91 days (prior to 3rd application)	2.943	60.5	14.9	8.0	83.4
	Fruit (green)	91 days (prior to 3rd application)	0.114	10.3	96.9	1.3	108.5
	Foliage (mature)	131 days (40 day PHI)	7.413	49.1	27.8	20.5	97.4
	Fruit (green)	131 days (40 day PHI)	0.241	1.4	98.4	0.6	100.4
	Fruit (ripe)	131 days (40 day PHI)	0.267	5.0	88.4	1.0	94.4

[a] Days after planting.
[b] mg/kg determined by combustion.
[c] Percent of the TRR determined by combustion.

The total radioactive residues (TRR) in the tomato foliage were 1.015 to 9.447 mg/kg for the [phenyl ^{14}C] difenoconazole treated plants and 0.978 to 7.413 mg/kg for the [triazole-^{14}C] difenoconazole treated plants. At maturity, low levels of TRR were present in green (0.029 mg/kg) and ripe (0.026 mg/kg) tomato fruit harvested at a PHI of 40 days following three applications of [phenyl-^{14}C] difenoconazole. Higher levels of TRR (0.241 and 0.267 mg/kg) were present in the respective [triazole-^{14}C] difenoconazole treated fruit.

In the tomato foliage, organic soluble radioactivity comprised 54.5 to 88.1% and 49.1 to 98.4% of the TRR for the [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole treated plants, respectively. Parent difenoconazole formed the major part of the residue accounting for 27.8 to 59.1% of the TRR (Table B.7.1.2-2). The metabolites CGA-205375 and CGA-205374, that were not fully resolved by TLC analysis, accounting for less than 5% of the TRR. The metabolite CGA-189138 (4.3 to 5.2% of the TRR) was observed in the [phenyl-^{14}C] difenoconazole treated plants only.

In the tomato foliage, aqueous soluble radioactivity accounted for 4.5 to 22.3% and 3.4 to 27.8% of the TRR for the [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole treatments, respectively, and was composed mainly of polar material that was attributed to conjugates of the metabolites. No 1,2,4-triazole (CGA-71019) was detected in the foliage from plants treated with [triazole-^{14}C] difenoconazole. Unextracted radioactive residues were 1.3 to 20.5% of the TRR for both radiolabelled treatment and were not analysed further.

In the mature tomato fruit (green and ripe), organic soluble radioactivity contained between 1.4 and 5.0% of the TRR in the [triazole-^{14}C] difenoconazole treated plants. Aqueous soluble radioactivity accounted for 88.4 to 98.4% of the TRR and the unextracted radioactive residues contained 0.6 to 1.0% TRR. Radioactive residues in the [phenyl-^{14}C] difenoconazole treated fruit were not analysed further.

Chromatographic analysis of the fruit extracts was not performed. The differences in the distribution of radioactivity between the organic and aqueous soluble fractions were attributed to cleavage of the triazole moiety, with preferential transport of water soluble triazole metabolites to the fruit.

Characterisation of the organic soluble radioactivity extracted from tomato foliage is summarised in Table B.7.1.2-2.

Table B.7.1.2-2. Characterisation of organic soluble radioactive residues in tomato foliage following three applications of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole at a rate of 247 g a.i./ha.

Labelled form	Sampling time[a]	91 days (prior to 3rd application)		131 days (40 days PHI)	
	Compound	% TRR	mg/kg[b]	% TRR	mg/kg[b]
[phenyl-^{14}C] difenoconazole	Difenoconazole	59.1	1.212	31.3	1.200
	CGA 205375 / CGA 205374[c]	3.8	0.078	3.4	0.130
	CGA 189138	4.3	0.088	5.2	0.199
[triazole-^{14}C] difenoconazole	Difenoconazole	52.1	1.839	27.8	2.116
	CGA 205375 / CGA 205374[c]	3.5	0.109	4.3	0.327

[a] Days after planting
[b] Calculated from percentages shown in Table B.7.1.2-1. Analysis performed on the organic phase resulting from Bligh-Dyer extraction of the plant material.
[c] Metabolites not fully separated by TLC consists of more than one metabolite.

Conclusions: At maturity, 40 days after the last of three applications of [phenyl-^{14}C] difenoconazole and [triazole-^{14}C] difenoconazole at 247 g a.i./ha, the TRR in ripe tomato fruit was 0.026 and 0.267 mg/kg and in foliage was 3.548 and 7.413 mg/kg, respectively. Parent difenoconazole was the largest component of the residue in the mature foliage, accounted for 31.3 and 27.8% of the TRR from the [phenyl-^{14}C] and [triazole-^{14}C]-difenoconazole treatments, respectively. Very low levels (3.4 to 4.3% TRR) of the alcohol and ketone metabolites CGA-205375 and CGA-205374 were identified in foliage from both radiolabelled treatments. The acid metabolite CGA-189138 was observed in the [phenyl-^{14}C] difenoconazole treated foliage at a level of 5.2% of the TRR. Aqueous soluble radioactivity extracted from the foliage contained mainly polar compounds that were attributed to conjugates of the free metabolites. 1,2,4-triazole was not detected in the organic extracts from foliage treated with [triazole-^{14}C] difenoconazole.
Differences in the distribution of radioactivity between the organic and aqueous soluble fractions were attributed to cleavage of the triazole moiety, with preferential transport of water soluble triazole metabolites (including conjugates) to the fruit.

Comments by RMS: No weather data and no discussion of storage stability has been included in the report. However, the lack of these data is not considered to affect the validity of the study. The application rate in the present study was 3 to 4 times higher than the critical GAP proposed for pome fruit in Southern and Northern Europe (75 and 56.2 g a.i./ha, respectively). The pre-harvest intervals were longer (40 days) than the minimum recommended in Southern and Northern Europe (14 and 28 days, respectively). Thus, higher residues would be expected with shorter PHI. The interval between applications was 14 days, which was longer than the minimum of 10 days.

B.7.1.3 Metabolism of [triazole-^{14}C] difenoconazole in greenhouse grown tomatoes

Reference:	**Velagaleti P.R. (1990a)** Metabolism of Triazole-^{14}C-CGA-169374 in spray-treated tomatoes. Battelle, Columbus, Ohio, United States. Unpublished Report N-0964-0600, SAM No. 0355.
Test Material:	Triazole-^{14}C, batch number RAF-VIII-93, radiochemical purity 98.1 to 97.2%(over the treatment period), specific radioactivity 44.4 µCi/mg.
Guideline:	US EPA Residues Chemistry, Series 171-4 (a) (1) & (2) (1982 and 1986), Washington, DC.
GLP:	Yes (In part). The biological phase and some of the analytical phase of this study were conducted prior to the adoption of GLP standards. The remaining analytical work and final report were conducted and prepared under the GLP standards.
Material and methods:	
Test concentration:	123 g a.i./ha
Test system:	Tomato plants (variety *Sunny*) were grown in plastic containers (ca 35 cm diameter x ca 38 cm deep) containing Ohio sandy loam soil. The soil characteristics were: pH (6.9), sand (60.8%), silt (29.9%), clay (9.2%), CEC (11.2 meq/100 g) and organic matter (0.71%). [Triazole-^{14}C] difenoconazole, formulated as a 3.5 EC, were applied using a Devilbiss sprayer assembly with nitrogen as a propellant. The potted plant was rotated during spraying to facilitate uniform application. The greenhouse conditions were 8-10 hours daylight, the relative humidity ranged from 32-100% and temperature from 13-37°C.
Stage of application:	62, 69, 76, 83, 90, 97 days after planting.
No. of applications:	Six
Sampling time points:	Tomato foliage was sampled after the first application, on the day prior to the 3rd , 5th and 6th applications and at a final harvest PHI interval of 34 days. Tomato fruit was sampled on the day prior to the fifth application and 6th applications and at intervals commencing at a PHI 7 days and ending at the final

harvest PHI of 34 days.

Method of analysis: The TRR in the foliage and fruit samples were quantified by combustion/LSC. Samples of each commodity were subjected solvent extraction according to Bligh-Dyer and Ting-Dugger, yielding organic and aqueous phases and an unextractable fraction. Identification of the residues in the organic and aqueous soluble phases was carried out by co-chromatography against standard reference compounds using either one-dimensional (1D) or two-dimensional (2D) thin layer chromatography (TLC). Aqueous extracts were further analysed by fractionation on Sephadex A-25 anion exchange resin. Selected fractions were purified with XAD-4 resin and preparative TLC, prior to cellulase de-conjugation and analysis for the resulting aglycones by 1D-TLC.

Date of experiment: 20 March 1989 to 5 July 1990

Findings: Residue levels in tomato foliage and fruit are shown in Table B.7.1.3-1.

Table B.7.1.3-1. Distribution of radioactive residues in tomato plant fractions following six applications of [triazole-^{14}C] difenoconazole at a rate of 123 g a.i./ha.

Plant commodity	Sampling Time[a]	TRR (mg/kg)[b]	Organic phase (%)[c]	Aqueous phase (%)[c]	Non-extracted (%)[c]	Total (%)[c]
Foliage	62 days (post 1st application)	3.802	89.2	1.3	1.9	92.4
Foliage	76 days (prior to 3rd application)	3.451	99.0	10.7	3.5	113.2
Foliage	90 days (prior to 5th application)	6.416	80.0	13.2	4.3	97.5
Fruit (green)	90 days (prior to 5th application)	0.174	66.0	28.9	3.0	97.9
Foliage	97 days (prior to 6th application)	9.734	70.8	15.3	13.6	99.6
Fruit (green)	97 days (prior to 6th application)	0.151	52.4	40.6	1.7	94.8
Fruit (green)	104 days (7 day PHI)	0.158	50.2	42.7	1.9	94.7
Foliage (mature)	131 days (34 day PHI)	7.719	76.7	24.5	6.8	107.9
Fruit (green)	131 days (34 day PHI)	0.139	14.5	77.0	2.4	94.0
Fruit (intermediate ripe)	131 days (34 day PHI)	0.128	15.6	71.9	1.6	89.1
Fruit (ripe)	131 days (34 day PHI)	0.203	54.9	34.4	2.9	92.2

[a] Days after planting.
[b] mg/kg difenoconazole equivalents.
[c] Percent of the TRR determined by combustion.

Levels of TRR present in green, intermediate and ripe tomato fruit harvested at a PHI of 34 days following six applications of [triazole-^{14}C] difenoconazole were 0.139, 0.128 and 0.203 mg/kg, respectively (Table B.7.1.3-1). Corresponding levels of TRR in fruit harvested at earlier intervals up to 7 days PHI ranged from 0.151 to 0.174 mg/kg. Levels of TRR in the foliage were much higher in the range 3.451 to 9.734 mg/kg.

In tomato fruit sampled at a PHI of 7 and 34 days, 91.5 to 92.9 % of the TRR was solvent extractable, with 14.5 to 50.2% present as organic soluble radioactivity and 42.7 to 77% present as aqueous soluble radioactivity. Parent difenoconazole and the metabolite CGA-131013 were the largest components of the TRR (12.5 to 50.9% and 19.3 to 42.4%, respectively; Table B.7.1.3-2). Low levels of the metabolites CGA-205374 and CGA-205375 were also detected (< 1% of the TRR), in addition to at least 7 unidentified metabolite regions that occurred mainly in aqueous extracts, but none individually exceeded 13.6% of the TRR (0.019 mg/kg). The unextracted residue was low (<3% of the TRR) and was not analysed further.

The extractable organic soluble radioactivity declined from 89.2 % of the TRR in the foliage harvested immediately after the first application to 76.7% harvested at maturity. Aqueous extracts at the same intervals increased from 1.3 to 24.5% TRR. Unextracted residues were in the range 1.9 to 13.6% of the TRR. Parent difenoconazole formed the major part of the residue in the mature tomato foliage, accounting for 68.8% of the TRR (Table B.7.1.3-1). Very low levels of the alcohol and ketone metabolites CGA-205375 and CGA-205374 were also detected (1.2 and 1.6% of the TRR, respectively; Table B.7.1.3-2).

Unidentified metabolites collectively accounted for 28.2% of the TRR and following separation by anion exchange chromatography and TLC analysis were shown to contain at least 13 individual regions. Treatment of selected regions with cellulase enzyme and analysis by TLC showed that a substantial proportion of the residue (12.8% of the TRR) was present as sugar conjugates of either CGA-205375, hydroxy-CGA-205375 or hydroxy-difenoconazole.

Characterisation of the radioactive residues detected in tomato fruit and foliage is summarised in Table B.7.1.3-2.

Table B.7.1.3-2. Characterisation of radioactive residues in tomato foliage and fruit following six applications of [triazole-^{14}C] difenoconazole at a rate of 123 g a.i./ha.

Plant Part	Fruit (green)		Fruit (green)		Fruit (intermediate)		Fruit (ripe)		Foliage	
PHI	7 days		34 days		34 days		34 days		34 days	
TRR (mg/kg)	0.158		0.139		0.128		0.203		7.719	
Compound	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Difenoconazole	46.9	0.074	12.5	0.017	12.6	0.016	50.9	0.103	68.0	5.249
CGA 205374	0.73	0.001	0.4	0.001	0.2	<0.001	0.5	0.001	1.6	0.126
CGA 205375	0.63	0.001	0.3	0.001	0.5	0.001	0.7	0.002	1.2	0.096
CGA 131013	21.7	0.03	42.4	0.059	39.1	0.050	19.3	0.039	--	--
Unidentified	22.8[a]	0.036	35.9[b]	0.050	35.3[c]	0.045	17.8[d]	0.036	28.2[e]	2.177
Non-extracted	1.9	0.003	2.4	0.003	1.6	0.002	2.9	0.006	6.8	0.525
Total	94.6	0.149	93.9	0.131	89.3	0.114	92.1	0.187	105.8	7.719

[a] Consists of at least 7 components that individually did not exceed 6.2% of the TRR (0.010 mg/kg).
[b] Consists of at least 7 components that individually did not exceed 13.6% of the TRR (0.019 mg/kg).
[c] Consists of at least 7 components that individually did not exceed 8.9% of the TRR (0.011 mg/kg).
[d] Consists of at least 7 components that individually did not exceed 7.0% of the TRR (0.014 mg/kg).
[e] Consists of at least 13 components that individually did not exceed 4.5% of the TRR (0.347 mg/kg) and includes up to 12.8% of the TRR (0.988 mg/kg) as conjugated CGA 205375, hydroxy CGA 205375 and hydroxy difenoconazole.

Conclusions: The TRR in green fruit harvested 7 days after the last of six applications of [triazole-^{14}C] difenoconazole at 123 g a.i./ha was 0.158 mg/kg, being parent difenoconazole the largest component of the residue (46.9% of the TRR).

The TRR in green, intermediate ripe and ripe tomato fruit harvested 34 days after the last application was 0.139, 0.128 and 0.203 mg/kg, respectively. Parent difenoconazole accounted for 12.5 to 50.9% of the TRR. Substantial amounts (19.3 to 42.4% TRR) of the triazole alanine metabolite CGA-131013 were detected in the fruit, in addition to low levels (< 1% TRR) of the alcohol and ketone metabolites CGA-205375 and CGA-205374. At least 7 unidentified regions of radioactivity were present predominantly in the aqueous extracts, but none individually exceeded 13.6% of the TRR (0.019 mg/kg). Levels of unextracted radioactivity did not exceed 3% of the TRR.

The TRR in mature foliage harvested at the 34 day PHI after the last application was 7.719 mg/g, being parent difenoconazole the largest component of the residue (68% of the TRR). Small amounts (<2% TRR) of the alcohol and ketone metabolites CGA-205375 and CGA-205374 were detected. The metabolite CGA-131013 was not observed. Unidentified metabolites collectively accounted for 28.2% of the TRR and following treatment of selected regions with cellulase enzyme showed that a substantial proportion of the residue (12.8% of the TRR) was present as sugar conjugates of either CGA-205375, hydroxy-CGA-205375 or hydroxy-difenoconazole.

Comments by RMS: No discussion of storage stability has been included in the report. The application rate in the present study was higher than the critical GAP proposed for pome fruit in Southern and Northern Europe (75 and 56.2 g a.i./ha, respectively). The pre-harvest intervals were longer (34 days) than the minimum recommended in Southern and Northern Europe (14 and 28 days, respectively). Thus, higher residues would be expected with shorter PHI. The interval between applications was 7 days, which was shorter than the minimum of 10 days.

B.7.1.4 Metabolism of [phenyl-^{14}C] difenoconazole in greenhouse grown tomatoes

Reference:	**Schweitzer M.G. (1990a)** Metabolism of Phenyl-^{14}C CGA-169374 in spray-treated tomatoes. Battelle, Columbus, Ohio, United States. Unpublished Report N-0964-0700, SAM No. 0356.
Test Material:	Phenyl-^{14}C, batch number RAF-VIII-96, radiochemical purity 98.8 to 96.2% (over the treatment period), specific radioactivity 42.6 µCi/mg.
Guideline:	US EPA Residues Chemistry, Series 171-4 (a) (1) & (2) (1982 and 1986), Washington, DC.
GLP:	Yes (In part). The biological phase and some of the analytical phase of this study were conducted prior to the adoption of GLP standards. The remaining analytical work and final report were conducted and prepared under the GLP standards.
Material and methods:	
Test concentration:	123 g a.i./ha
Test system:	Tomato plants (variety *SunnyHybrid*) were grown in plastic containers (ca 34 cm diameter x ca 28 cm deep) containing Ohio sandy loam soil. The soil characteristics were: pH (6.9), sand (60.8%), silt (29.9%), clay (9.2%), CEC (11.2 meq/100 g) and organic matter (0.71%). [Phenyl-^{14}C] difenoconazole, formulated as a 3.5 EC, were applied using a Devilbiss sprayer assembly with nitrogen as a propellant. The potted plant was rotated during spraying to facilitate uniform application. The greenhouse conditions were 14 hour daylight, the relative humidity ranged from 32-100% and temperature from 13-

	41°C.
Stage of application:	62, 69, 76, 83, 90, 97 days after planting.
No. of applications:	Six
Sampling time points:	Tomato foliage was sampled after the first application (62 days post planting), on the day prior to the 3rd , 5th and 6th applications and at a final harvest PHI interval of 34 days. Tomato fruit was sampled on the day prior to the fifth application and 6th applications and at intervals commencing at a PHI 7 days and ending at the final harvest PHI of 34 days.
Method of analysis:	Total radioactive residues (TRR) in the foliage and fruit samples were quantified by combustion/LSC. Samples of each commodity were extracted with acetonitrile and extracts concentrated to remove the solvent prior to partition with ethyl acetate. Identification of the residues in the organic and aqueous soluble phases was carried out by co-chromatography against standard reference compounds using either one-dimensional (1D) or two-dimensional (2D) TLC. Aqueous extracts were further analysed by fractionation on Sephadex A-25 anion exchange resin and cellulase enzyme de-conjugation.
Date of experiment:	20 March 1989 to 15 August 1990

Findings: Residue levels in tomato foliage and fruit are shown in Table B.7.1.4-1.

Table B.7.1.4-1. Distribution of radioactive residues in tomato plant fractions following six applications of [phenyl-^{14}C] difenoconazole at a rate of 123 g a.i./ha.

Plant commodity	Sampling Time[a]	TRR (mg/kg)[b]	CH_3CN extract (%)[c]	Organic phase (%)[c]	Aqueous phase (%)[c]	Non-extracted (%)[c]	Total (%)[c]
Foliage	62 days (post 1st application)	2.61	100.3	n.a.	n.a.	1.1	101.4
Foliage	76 days (prior to 3rd application)	4.0	91.2	n.a.	n.a.	4.1	95.3
Foliage	90 days (prior to 5th application)	5.33	84.4	n.a.	n.a.	10.3	94.7
Fruit (green)	90 days (prior to 5th application)	0.20	85.2	n.a.	n.a.	11.8	97.0
Foliage	97 days (prior to 6th application)	6.84	84.2	n.a.	n.a.	12.1	96.3
Fruit (green)	97 days (prior to 6th application)	0.19	102.1	n.a.	n.a.	5.0	107.1
Fruit (green)	104 days (7 day PHI)	0.22	82.3	n.a.	n.a.	13.6	95.9
Foliage (mature)	131 days (34 day PHI)	8.29	88.6	92.1	7.9	5.4	94.0
Fruit (green)	131 days (34 day PHI)	0.04	94.0	n.a.	n.a.	12.3	106.3
Fruit (ripe)	131 days (16 day PHI)	0.17	98.9	86.2	13.8	5.3	104.2

[a] Days after planting.
[b] mg/kg difenoconazole equivalents.
[c] Percent of the TRR determined by combustion.
n.a. = Not applicable.

In mature tomato foliage, 88.6% of the TRR was extractable into solvent. Parent difenoconazole was the predominant residue accounting for 64.7% of the TRR (Table B.7.1.4-2). Low levels of the alcohol, ketone and acid metabolites CGA-205374, CGA-205375 and CGA 189138 were also detected (3.9, 1.3 and 0.9% of the TRR, respectively). Unidentified metabolites collectively accounted for 10.8% of the TRR and following separation by anion exchange chromatography and TLC analysis were shown to contain at least 10 individual regions. Treatment of selected regions with cellulase enzyme and analysis by TLC showed that a proportion of the residue (5.0% of the TRR) was present as sugar conjugates of either CGA-205375, hydroxy-CGA-205375 or hydroxy-difenoconazole.

Levels of TRR present in green and ripe tomato fruit harvested at a PHI of 34 days following six applications of [phenyl-^{14}C] difenoconazole were 0.04 to 0.17mg/kg, respectively. Corresponding levels of TRR in green fruit harvested at earlier intervals up to 7 days PHI were 0.22 mg/kg while levels of TRR in the mature foliage were much higher (up to 8.29 mg/kg).

In mature ripe tomato fruit, 98.9% of the TRR was extractable, being parent difenoconazole the largest compound (66.3% of TRR). Less than 2% of the TRR consisted of the metabolites CGA 205374 and CGA 205375. Unidentified metabolites collectively accounted for 15.8% of the TRR and following separation by anion exchange chromatography and TLC analysis were shown to contain at least 6 individual regions with cellulase enzyme and analysis by TLC showed that a proportion of the unidentified radioactivity (3.7% of the TRR) was present as sugar conjugates of either CGA-205375, hydroxy-CGA-205375 or hydroxy-difenoconazole. Unextracted radioactive residues in the fruit comprised 5.3% of the TRR (0.009 mg/kg) and were not analysed further.

Characterisation of the radioactive residues detected in tomato fruit and foliage is summarised in Table B.7.1.4-2.

Table B.7.1.4-2. Characterisation of radioactive residues in mature tomato foliage and fruit following six applications of [phenyl-^{14}C] difenoconazole at a rate of 123 g a.i./ha.

Plant Part	Final harvest ripe fruit		Final harvested foliage	
PHI	34 days		34 days	
TRR (mg/kg)	0.17		8.29	
Compound	% TRR	mg/kg	% TRR	mg/kg
Difenoconazole	66.3	0.110	64.7	5.36
CGA 189138	n.d.	n.d.	0.9	0.08
CGA 205374	1.4	0.002	3.9	0.32
CGA 205375	1.7	0.003	1.3	0.11
Unidentified	15.8[b]	0.027	10.8[c]	0.88
Polar compounds[a]	13.6	0.023	10.9	0.91
Non-extracted	5.3	0.009	5.4	1.23
Total	107.2	0.179	97.9	8.91

[a] Radioactivity remaining at the origin of the TLC plate following development.
[b] Consists of at least 6 components that individually did not exceed 8.4% of the TRR (0.014 mg/kg) and includes up to 3.7% of the TRR (0.006 mg/kg) as conjugated CGA 205375, hydroxy CGA 205375 and hydroxy difenoconazole.
[c] Consists of at least 10 components that individually did not exceed 2.1% of the TRR (0.17 mg/kg) and includes up to 5.0% of the TRR (0.988 mg/kg) as conjugated CGA 205375, hydroxy CGA 205375 and hydroxy difenoconazole.
n.d. = Not detected.

Conclusions: The TRR in the mature ripe tomato fruit, harvested 34 days after the final of six applications of 123 g a.i../ha, was 0.17 mg/kg, being parent difenoconazole the largest component of the residue (66.3% of the TRR). Small amounts of the alcohol and ketone metabolites CGA-205375 and CGA-205374 were also detected (<2% of the TRR). At least 6 unidentified regions of radioactivity were present in fruit, predominantly in aqueous extracts, however, these unidentified regions individually did not exceed 8.4% of the TRR (0.014 mg/kg). Levels of unextracted radioactivity did not exceed 5.3% of the TRR.

The TRR in mature foliage harvested at the 34 day PHI after the last application was 8.29 mg/kg. Parent difenoconazole was the largest component of the radioactive residue (64.7% of the TRR). Small amounts (< 4% TRR) of the alcohol (CGA-205375), ketone (CGA-205374) and acid (CGA189138) were also

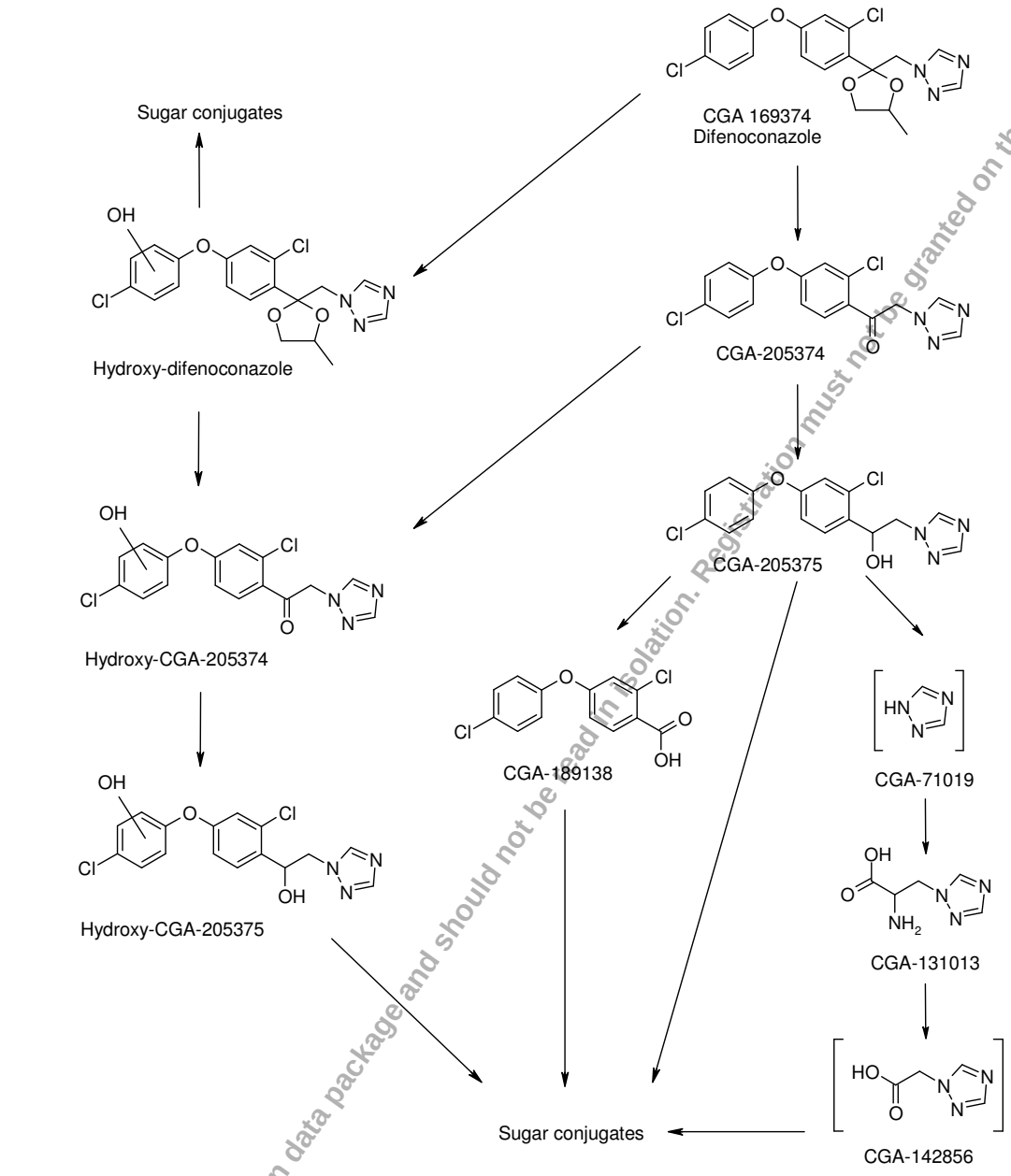
detected in the foliage. Unidentified metabolites collectively accounted for 10.8% of the TRR and following treatment of selected regions with cellulase enzyme showed that a proportion (5% of the TRR) was present as conjugates of either CGA-205375, hydroxy-CGA-205375 or hydroxy-difenoconazole.

Comments by RMS: No discussion of storage stability has been included in the report. The application rate in the present study was higher than the critical GAP proposed for pome fruit in Southern and Northern Europe (75 and 56.2 g a.i./ha, respectively). The pre-harvest intervals were longer (34 days) than the minimum recommended in Southern and Northern Europe (14 and 28 days, respectively). Thus, higher residues would be expected with shorter PHI. The interval between applications was 7 days, which was shorter than the minimum of 10 days.

According to the document 7028/VI/95 rev. 3, 22/7/1997, tomato belongs to the category fruits. However, tomato and pome fruit belong to different flowering plant families, i.e. the *Solanaceae* and *Rosaceae* family, respectively.

Based on the metabolites detected in foliage and fruit samples, from the four available studies, the pathway of metabolism of difenoconazole in tomato plants occurred primarily via phenyl ring hydroxylation, formation of the difenoconazole ketone (CGA-205374) via hydrolysis of the dioxolane ring, reduction of the ketone to the alcohol (CGA-205375) and triazole ring cleavage. Secondary metabolism occurs via sugar conjugation of hydroxyl metabolites and amino acid (serine) conjugation of the cleaved triazole ring to form triazole alanine (CGA-131013). Metabolites formed as a result of triazole ring cleavage were transported preferentially to the fruit.

The proposed pathway of metabolism in tomato plants is given in Figure B 7.1.3-1.



[ ] Proposed intermediate not identified in the studies.

Figure B.7.1.3-1. Proposed metabolic pathway of difenoconazole in tomato plants.

B.7.1.5 Metabolism of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole in outdoor grown spring wheat following seed treatment

Reference:	**Hubbard L. (1991a)** Uptake and metabolism of ^{14}C-CGA-169374 by wheat resulting from seed treatment application under field conditions. Metabolism Department, Agricultural Division, Ciba-Geigy Corporation, Greensboro, North Carolina, United States. Unpublished report ABR-90009, SAM No. 0415.
Test Material:	Phenyl-^{14}C, batch number RAF-VIII-96, radiochemical purity 98.5%, specific activity 42.6 µCi/mg. Triazole-^{14}C, batch number RAF-VIII-93, radiochemical purity 98.3%, specific radioactivity 44.4 µCi/mg.
Guideline:	Pesticide Assessment Guidelines, -Subdivision O, Residue Chemistry, Series 171-4(a) (1) & (2), US Environmental Protection Agency, Washington, DC.
GLP:	Yes (In part). The biological and analytical phases of this study were conducted prior to the adoption of GLP standards. The final report and metabolite identification were written and conducted under the GLP standards.
Material and methods:	
Test concentration:	24 g a.i./100 kg seed
Test system:	The study was conducted outdoors in field plots (1.2 x 1.2 m) at two locations in the US, New York (loam soil) and Illinois (silty clay loam soil). [Phenyl-^{14}C] and [triazole-^{14}C] difenoconazole, formulated as a 3 FS, were applied to spring wheat seeds (variety *Marshall FL-890836*) at a nominal rate of 24 g a.i./100 kg seed. Actual treatment rates were 23 g a.i./100 kg seed for the [triazole-^{14}C] difenoconazole and 32 g a.i./100 kg seed for the [phenyl-^{14}C] difenoconazole. The seed was sown at a rate of 78 kg seed/ha, which resulted in treatment rates of 17.9 g and 25 g a.i./ha for the [triazole-^{14}C] and [phenyl-^{14}C] label, respectively. At harvest, wheat was separated into grains, hulls and straw.
Stage of application:	Seed treatment
No. of applications:	One
Sampling time points:	Wheat foliage was sampled at growth stages equivalent to 25 and 50% maturity at intervals of 31 to 34 days and 48 to 62 days, respectively post planting. Wheat straw, hulls and grain were harvested at full maturity between 59 and 83 days post planting. At the Illinois location both [phenyl-^{14}C] and [triazole^{14}C] difenoconazole treated plants were harvested, but only [triazole-^{14}C] difenoconazole treated plants were harvested at the New York location due to crop failure.
Method of analysis:	The TRR in each harvested sample was quantified by combustion/LSC. Samples of each commodity containing residues > 0.05 mg/kg were subjected to Bligh-Dyer and Ting-Dugger solvent extraction, which yielded organic and

aqueous phases and an unextractable fraction. Identification of the residues in the organic and aqueous soluble phases was carried out by co-chromatography against standard reference compounds using either 1D or 2D-TLC. Aqueous extracts were further analysed by fractionation on Sephadex A25 anion exchange resin and de-conjugation using cellulase enzyme.

Date of experiment: 4 May 1989 to 21 June 1990

Findings: Residue levels in foliage, hulls, straw and grain are shown in Table B.7.1.5-1.

Table B.7.1.5-1. Distribution of radioactive residues in wheat plant fractions following seed treatment with [phenyl-^{14}C] and [triazole- ^{14}C] difenoconazole.

Labelled form Location Soil type	Plant commodity	Sampling Time[a]	TRR (mg/kg)[b]	Organic phase (%)[c]	Aqueous phase (%)[c]	Non-extracted (%)[c]	Total (%)[c]
[phenyl-^{14}C] difenoconazole Illinois Silty clay loam soil[d]	Foliage	31 days (25% mature)	0.095	n.d.	41.6	23.4	65.0
	Foliage	48 days (50% mature)	0.008	n.a.	n.a.	n.a.	n.a.
	Straw	59 days (mature)	0.013	n.a.	n.a.	n.a.	n.a.
	Hulls	59 days (mature)	0.004	n.a.	n.a.	n.a.	n.a.
	Grain	59 days (mature)	0.004	n.a.	n.a.	n.a.	n.a.
[triazole-^{14}C] difenoconazole Illinois Silty clay loam soil[d]	Foliage	34 days (25% mature)	0.007	n.a.	n.a.	n.a.	n.a.
	Foliage	52 days (50% mature)	0.010	n.a.	n.a.	n.a.	n.a.
	Straw	72 days (mature)	0.011	n.a.	n.a.	n.a.	n.a.
	Hulls	72 days (mature)	0.016	n.a.	n.a.	n.a.	n.a.
	Grain	72 days (mature)	0.024	n.a.	n.a.	n.a.	n.a.
[triazole-^{14}C] difenoconazole New York Loam soil[e]	Foliage	33 days (25% mature)	0.049	n.a.	n.a.	n.a.	n.a.
	Foliage	62 days (50% mature)	0.053	n.d.	89.4	6.45	95.9
	Straw	83 days (mature)	0.059	9.4	87.1	16.1	112.6
	Hulls	83 days (mature)	0.075	n.a.	n.a.	n.a.	n.a.
	Grain	83 days (mature)	0.135	n.d.	89.9	9.5	99.3

[a] Days after planting.
[b] mg/kg determined by combustion.
[c] Percent of the TRR determined by combustion.
[d] pH (5.8), sand (18%), silt (47%), clay (35%), CEC (17.4 meq/100 g) and organic matter (2.3%).
[e] pH (6.7), sand (40%), silt (39%), clay (21%), CEC (7.2 meq/100 g) and organic matter (3.3%).
n.d. = Not detected.
n.a. = Not applicable.

The total radioactive residues (TRR) after seed treatment in the immature foliage and mature hulls, straw and grain range from 0.007 to 0.095, 0.004 to 0.075, 0.011 to 0.059 and 0.004 to 0.135 mg/kg, respectively. The TRR for triazole-^{14}C treated plants grown in New York were higher than the corresponding plants grown in Illinois. This may be the result of differences in soil types.

In immature foliage, levels of TRR were higher in the phenyl-^{14}C treated plants compared to the triazole-^{14}C labelled foliage samples while the residues in grain and hulls were higher in the triazole-^{14}C treated plants compared to the phenyl-^{14}C labelled hulls and grain samples. This may indicate cleavage of alkyl bridge between the phenyl and triazole moieties and preferential movement of triazole metabolites throughout the plant, particularly to grain.

In the mature [triazole-^{14}C] difenoconazole treated straw and grain the majority of the radioactivity was aqueous soluble and was identified as either 1,2,4-triazole (CGA-71019) or triazole acetic acid (CGA-142856) following separation using Sephadex A-25 anion exchange resin and detection by 2D-TLC.

These compounds were not analysed quantitatively, although triazole was the major component. The aqueous soluble radioactivity from the 25% mature phenyl -^{14}C labelled foliage contained sugar conjugates of the metabolite CGA-205375, whilst there was evidence that the 50% mature triazole-^{14}C labelled foliage contained the triazole specific metabolites CGA-71019 and CGA-142856, although there was insufficient radioactivity to conclusively identify these compounds.

Conclusions: At harvest, after application of phenyl-^{14}C labelled difenoconazole to wheat seeds, low levels of TRR were found in grain and straw (0.004 and 0.013 mg/kg, respectively). In immature foliage the TRR was 0.095 mg/kg . 41.6% of the radioactivity was aqueous soluble and consisted of sugar conjugates of the metabolite CGA-205375.

Residues in mature straw from the triazole-^{14}C labelled difenoconazole treated seed for two different locations were 0.011 and 0.059 mg/kg, respectively. The corresponding grain residues were 0.024 and 0.135 mg/kg. The majority of the radioactivity was aqueous soluble and was identified as either 1,2,4-triazole (CGA-71019) or triazole acetic acid (CGA-142856). In the immature foliage the TRR was 0.053 mg/kg. 89.4% of the radioactivity was aqueous soluble and consisted of the triazole specific metabolites CGA-71019 and CGA-142856. Metabolites formed as a result of triazole ring cleavage were transported preferentially to the grain.

Based on the compounds detected, difenoconazole is metabolised primarily via formation of the difenoconazole alcohol (CGA-205375) and subsequent triazole ring cleavage. Secondary metabolism occurs via sugar conjugation of the metabolite CGA-205375 and amino acid (serine) conjugation of 1,2,4-triazole (CGA-71019) to form triazole alanine (CGA-131013) and triazole acetic acid (CGA-142856).

Comments by RMS: No discussion of storage stability or weather data have been included in the report. However, the lack of these data is not considered to affect the validity of the study. The application rate in the present study was 2-4 times higher than the proposed critical GAP (6 g a.i./100 kg seed or 12 g a.i./ha). The study was well performed and reported.

B.7.1.6 Metabolism of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole in greenhouse grown spring wheat following seed treatment

Reference:	**Hubbard L. (1991b)** Uptake and metabolism of ^{14}C-CGA-169374 by wheat resulting from seed treatment application under greenhouse conditions. Metabolism Department, Agricultural Division, Ciba-Geigy Corporation, Greensboro, North Carolina, United States. Unpublished Report ABR-90010, SAM No. 0416.
Test Material:	Phenyl-^{14}C, batch number CL-XVI-89, radiochemical purity 97.9%, specific activity 30.7 µCi/mg. Triazole-^{14}C, batch number DPS-II-23-I, radiochemical purity 97.8%, specific radioactivity 29.0 µCi/mg.
Guideline:	Pesticide Assessment Guidelines, -Subdivision O, Residue Chemistry, Series 171-4(a) (1) & (2), US Environmental Protection Agency, Washington, DC.
GLP:	Yes (In part). The biological and analytical phases of this study were conducted prior to the adoption of GLP standards. The final report and metabolite identification were written and conducted under the GLP standards.
Material and methods:	
Test concentration:	24 g a.i./100 kg seed
Test system:	The metabolism of difenoconazole following use as a seed treatment was studied in spring wheat (variety *Hill 81 FL-8820170*). The study was conducted indoors under greenhouse conditions. Plants were grown in 16 L containers containing a sandy loam soil at a rate of 30 seeds per container. The soil characteristics were: pH (5.0), sand (82.4%), silt (12%), clay (5.6%), CEC (5.3 meq/100 g) and organic matter (1.8%). [Phenyl-^{14}C] and [triazole-^{14}C] difenoconazole, were each formulated as a 3 FS flowable concentrate and applied as a seed coating at a target rate of 24 g a.i./100 kg seed. Actual treatment rates were 25 g a.i./100 kg seed for the phenyl-^{14}C label and 30 g a.i./100 kg seed for the triazole-^{14}C label. At harvest, wheat was separated into grains, hulls and straw.
Stage of application:	Seed treatment
No. of applications:	One
Sampling time points:	Immature wheat foliage was sampled at growth stages equivalent to 25 and 50% maturity at intervals of 40 and 72 days, respectively post planting. Wheat straw, hulls and grain were harvested at full maturity at 236 days post planting.
Method of analysis:	The TRR in each harvested sample was quantified by combustion/LSC. Samples of each commodity containing residues > 0.05 mg/kg were subjected to Bligh-Dyer and Ting-Dugger solvent extraction, which yielded organic and aqueous phases and an unextractable fraction. Immature wheat tops were also

extracted using methanol/water and partitioned with chloroform and ethyl acetate after removal of methanol by rotary evaporation. Identification of the residues in the organic and aqueous soluble phases was carried out by co-chromatography against standard reference compounds using either 1D-TLC or 2D-TLC. Aqueous extracts were further analysed by fractionation on Sephadex A-25 anion exchange resin and cellulase enzyme de-conjugation.

Date of experiment: 25 January 1989 to 8 June 1990

Findings: Residue levels in foliage, hulls, straw and grain are shown in Table B.7.1.6-1.

Table B.7.1.6-1. Distribution of radioactive residues in wheat plant fractions following seed treatment with [phenyl-^{14}C] and [triazole- ^{14}C] difenoconazole.

Labelled form	Plant commodity	Sampling Time[a]	TRR (mg/kg)[b]	Organic phase (%)[c]	Aqueous phase (%)[c]	Non-extracted (%)[c]	Total (%)[c]
[phenyl-^{14}C] difenoconazole	Foliage	40 days (25% mature)	0.075	70.7	20.2	15.3	106.2
	Foliage	72 days (50% mature)	0.016	n.a.	n.a.	n.a.	n.a.
	Straw	236 days (mature)	0.016	n.a.	n.a.	n.a.	n.a.
	Hulls	236 days (mature)	0.005	n.a.	n.a.	n.a.	n.a.
	Grain	236 days (mature)	0.003	n.a.	n.a.	n.a.	n.a.
[triazole-^{14}C] difenoconazole	Foliage	40 days (25% mature)	0.148	33.3	43.3	15.1	91.7
	Foliage	72 days (50% mature)	0.010	n.a.	n.a.	n.a.	n.a.
	Straw	236 days (mature)	0.069	16.8	71.6	18.3	106.7
	Hulls	236 days (mature)	0.141	n.d.	96.1	9.8	105.9
	Grain	236 days (mature)	0.183	n.d.	80.4	25.3	105.7

[a] Days after planting.
[b] mg/kg determined by combustion.
[c] Percent of the TRR determined by combustion.
n.d. = Not detected.
n.a. = Not applicable.

The TRR after seed dressing in the immature foliage and mature hulls, straw and grain range from 0.010 to 0.148, 0.005 to 0.141, 0.016 to 0.069 and 0.003 to 0.183 mg/kg, respectively. The levels of TRR were higher in the triazole-^{14}C labelled difenoconazole treated plants compared to the phenyl-labelled samples. This may indicate cleavage of alkyl bridge between the phenyl and triazole moieties and preferential movement of triazole metabolites throughout the plant.

Residues in the immature foliage from the phenyl-^{14}C labelled difenoconazole treated seed were 0.075 mg/kg. 70.7% of the radioactivity was organic soluble and consisted of parent difenoconazole (8% of the TRR) and the metabolite CGA-205375 (23% of the TRR). Radioactivity in the aqueous phase (20.2% of the TRR) contained three regions accounting for between 2 and 10% of the TRR and treatment with cellulase enzyme indicated the presence of sugar conjugates, with hydroxylated species postulated as the aglycones.

In the mature [triazole-^{14}C] difenoconazole treated straw and grain the majority of the radioactivity was aqueous soluble (71.6 to 80.4%) and was tentatively identified as either 1,2,4-triazole (CGA-71019) or triazole acetic acid (CGA-142856) following separation using Sephadex A-25 anion exchange resin and detection by 2D-TLC.

Residues in the immature foliage was 0.148 mg/kg. 33.3% of the radioactivity was organic soluble and consisted of a small amount of parent difenoconazole (7% of the TRR), with the remainder present as polar

material which was attributed to carry over of aqueous soluble compounds during the partition procedure. Radioactivity in the corresponding aqueous phase was 43.3% of the TRR and was tentatively identified as either triazole alanine (CGA-131013) or triazole acetic acid (CGA-142856). Conclusive identification of the metabolites was not possible due to interference from co-extracted plant material.
1,2,4-triazole was not detected in any of the samples.

Conclusions: At harvest, after application of phenyl-^{14}C labelled difenoconazole to wheat seeds, low levels of TRR were detected in grain and straw (0.003 and 0.016 mg/kg, respectively). In the immature foliage the TRR was 0.075 mg/kg and the majority of the radioactivity was organic soluble (70.7%) which consisted in parent difenoconazole (8% of the TRR) and in the alcohol metabolite CGA-205375 (23% of the TRR). Radioactivity in the corresponding aqueous phase (20.2% of the TRR) contained three regions accounting for between 2 and 10% of the TRR and treatment with cellulase enzyme indicated the presence of sugar conjugates, with hydroxylated species postulated as the aglycones.

Residues in mature straw and grain from the [triazole-^{14}C] difenoconazole treated seed were 0.069 and 0.183 mg/kg. The majority of the radioactivity was aqueous soluble (71.6 to 80.4% of the TRR) and was tentatively identified as either triazole alanine (CGA-131013) or triazole acetic acid (CGA-142856). Conclusive identification of the metabolites was not possible due to interference from co-extracted plant material.

Based on the compounds detected, difenoconazole is metabolised primarily via formation of the difenoconazole alcohol (CGA-205375) and subsequent triazole ring cleavage. Secondary metabolism via sugar conjugation of hydroxylated species was proposed. Amino acid (serine) conjugation of 1,2,4-triazole (CGA-71019) to form triazole alanine (CGA-131013) and triazole acetic acid (CGA-142856) was also proposed as the main source of metabolites in the grain.

Comments by RMS: No data of the greenhouse conditions or discussion of storage stability has been included in the report. However, this is not considered to affect the validity of the study. The application rate in the present study was 2 – 4 times higher than the proposed critical GAP (6 g a.i./100 kg seed or 12 g a.i./ha). The study was well performed and reported.

B.7.1.7 Metabolism of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole in greenhouse grown spring wheat following foliar application

Reference:	**Hubbard L. (1991c)** Uptake and metabolism of ^{14}C-CGA-169374 by wheat resulting from foliar spray application in a greenhouse environment (nature of residue - plant metabolism). Metabolism Department, Agricultural Division, Ciba-Geigy Corporation, Greensboro, North Carolina, United States. Unpublished Report ABR-90011, (Amendment 1: May 28, 1993, Amendment 2: June 01, 1993), SAM No. 0417.
Test Material:	Phenyl-^{14}C, batch number RAF-VIII-95, radiochemical purity 98.6%, specific activity 19.0 µCi/mg. Triazole-^{14}C, batch number RAF-VIII-92, radiochemical purity 98.4%, specific radioactivity 19.2 µCi/mg.
Guideline:	Pesticide Assessment Guidelines, -Subdivision O, Residue Chemistry, Series 171-4(a) (1) & (2), US Environmental Protection Agency, Washington, DC.
GLP:	Yes (In part). The biological and analytical phases of this study were conducted prior to the adoption of GLP standards. The final report and metabolite identification were written and conducted under the GLP standards.
Material and methods:	
Test concentration:	247 g a.i./ ha
Test system:	The study was conducted indoors under greenhouse conditions, with spring wheat (variety *James*) grown in 16 L containers containing a loamy sand soil. The soil characteristics were: pH (5.0), sand (82.4%), silt (12%), clay (5.6%), CEC (5.3 meq/100 g) and organic matter (1.8%). [Phenyl-^{14}C] difenoconazole and [triazole-^{14}C] difenoconazole, formulated as a 250 EC, were applied as a spray.
Stage of application:	43, 50, 58, 65 days post planting
No. of applications:	Four
Sampling time points:	Immature wheat tops were sampled at growth stages equivalent to 25 and 50% maturity at intervals of 43 (post first application) and 58 days post planting (8 days following the second application), respectively. Wheat straw, hulls and grain were harvested at full maturity at 94 days post planting (29 day PHI).
Method of analysis:	The TRR in each harvested sample was quantified by combustion/LSC. Samples of each commodity containing residues > 0.05 mg/kg were subjected to Bligh-Dyer and Ting-Dugger solvent extraction, which yielded organic and aqueous phases and an unextractable fraction. Identification of the residues in the organic and aqueous soluble phases was carried out by co-chromatography against standard reference compounds using either 1D-TLC or 2D-TLC. Aqueous extracts were further analysed by fractionation on Sephadex A-10 gel

filtration and Sephadex A-25 anion exchange resins and and by enzyme de-conjugation using cellulase.

Non-extracted radioactivity from [phenyl-^{14}C] difenoconazole treated grain was also treated with cellulase enzyme to further characterise the residue. Straw samples from plants treated with [triazole-^{14}C] difenoconazole were further analysed to more fully characterise the residue. Corresponding straw and grain samples from the studies B.7.1.5 and B.7.1.6 were also re-analysed for comparative purposes (the samples were stored frozen 3 ½ years). Samples were extracted with methanol/water and the Bligh-Dyer/Ting-Dugger methods and extractable radioactivity was separated using XAD-4 and DEAE Sephadex A-25 column chromatography. Individual components were characterised using TLC and HPLC, with structural confirmation obtained using mass spectrometry (MS). Aqueous soluble components and unextracted residues were subjected to enzyme digestion using cellulase, amylase and protease. Purified components were also derivatised (butylation and acetylation) to assist identification.

Date of experiment: 17 April 1989 to 11 January 1991

Findings: Residue levels in foliage, hulls, straw and grain are shown in Table B.7.1.7-1.

Table B.7.1.7-1. Distribution of radioactive residues in wheat plant fractions following four applications of [phenyl-^{14}C] and [triazole- ^{14}C] difenoconazole at a rate of 247 g a.i./ha.

Labelled form	Plant commodity	Sampling Time[a]	TRR (mg/kg)[b]	Organic phase (%)[c]	Aqueous phase (%)[c]	Non-extracted (%)[c]	Total (%)[c]
[phenyl-^{14}C] difenoconazole	Foliage	43 days (25% mature)	6.88	89.5	1.9	5.8	92.1
	Foliage	58 days (50% mature)	8.32	68.5	22.4	10.3	101
	Straw	94 days (29 day PHI)	46.7	51.8	29.8	13.9	95.6
	Hulls	94 days (29 day PHI)	5.20	26.4	26.1	40.6	93.1
	Grain	94 days (29 day PHI)	0.064	n.d.	n.d.	81.5	81.5
[triazole-^{14}C] difenoconazole	Foliage	43 days (25% mature)	6.27	84.5	1.7	7.0	93.2
	Foliage	58 days (50% mature)	8.70	65.5	22.1	10.3	97.9
	Straw	94 days (29 day PHI)	53.8	50.1	27.4	13.2	90.7
	Hulls	94 days (29 day PHI)	4.13	23.3	34.8	31.1	89.3
	Grain	94 days (29 day PHI)	1.40	n.d.	69.5	22.7	92.3

[a] Days after planting.
[b] mg/kg determined by combustion.
[c] Percent of the TRR determined by combustion.
n.d. = Not detected.

The TRR in immature foliage and mature hulls, straw and grain range from 6.27 to 8.70, 4.13 to 5.20, 46.7 to 53.8 and 0.064 to 1.40 mg/kg, respectively.

The distribution of radioactivity between organic and aqueous soluble fractions was similar between the [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole labelled samples of immature foliage, mature straw and hulls, but not in grain. The TRR in the phenyl-^{14}C labelled difenoconazole treated grain was present exclusively as an unextracted residue (81.5% of the TRR), whilst in the corresponding triazole-^{14}C difenoconazole labelled treated grain, 69.5% of the TRR was present as aqueous soluble radioactivity and 22.7% of the TRR was unextracted residue.

The majority of the radioactivity in immature foliage from both radiolabelled forms of difenoconazole was parent difenoconazole (85 to 90% of the TRR (5.6 to 5.8 mg/kg), Table B.7.1.7-2). In the corresponding straw, difenoconazole accounted for 50% of the TRR in plants from each treatment group (23 to 27 mg/kg), with small amounts (less than 10% of the TRR) present as sugar conjugates of the metabolites CGA-205375, hydroxy-CGA-205375 and hydroxy-difenoconazole.

In triazole-^{14}C labelled difenoconazole treated grain, 69.5% of the radioactivity was aqueous soluble and consisted of 1,2,4-triazole (CGA-71019, 10% of the TRR)) and triazole acetic acid (CGA-142856, 20% of the TRR), while in the phenyl-^{14}C labelled difenoconazole treated grain, the presence of conjugated CGA-189138 or CGA-189138 metabolites was proposed following treatment of the unextracted residue with cellulase enzyme.

Table B.7.1.7-2. Characterisation of radioactive residues in wheat plant fractions following four foliar applications of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole at a rate of 247 g a.i./ha.

Labelled form	Plant part	Immature foliage		Straw				Grain	
	Sampling time[a]	43 days (25% mature)		94 days (29 day PHI)				94 days (29 day PHI)	
[phenyl-^{14}C] difenoconazole	TRR (mg/kg)[b]	6.88		46.7				0.064	
	Extract	Organic phase		Organic phase		Aqueous phase		Aqueous phase	
	Compound	% TRR	mg/kg[b]	% TRR	mg/kg[b]	% TRR	mg/kg[b]	% TRR	mg/kg[b]
	Difenoconazole	85	5.8	50	23	--	--	--	--
	CGA-205375[c]	--	--	--	--	--[d]	--[d]	--	--
	OH-CGA 205375[c]	--	--	--	--	--[d]	--[d]	--	--
	OH-difenoconazole[c]	--	--	--	--	--[d]	--[d]	--	--
	Unidentified compounds[e]	--	--	--	--	--	--	35	0.02
[triazole-^{14}C] difenoconazole	TRR (mg/kg)[b]	6.27		53.8				1.40	
	Difenoconazole	90	5.6	50	27	--	--	--	--
	CGA-205375[c]	--	--	--	--	5	2.7	--	--
	OH-CGA 205375[c]	--	--	--	--	1	0.54	--	--
	OH-difenoconazole[c]	--	--	--	--	1	0.54	--	--
	CGA-142856[c]	--	--	--	--	--	--	20	0.28
	CGA-71019[f]	--	--	--	--	--	--	10	0.14

[a] Days after planting
[b] mg/kg difenoconazole equivalents.
[c] Metabolites present as sugar conjugates, characterised by cellulase enzyme de-conjugation.
[d] Chromatographic determinations not quantitated (approximately 10% of the total residues (ca 4.7 mg/kg) were found in this aqueous phase).
[e] Contains possible conjugates of CGA-189138 or CGA-189138 metabolites.
[f] After gel filtration

Comments by RMS: No data of the greenhouse conditions or discussion of storage stability has been included in the report. However, this is not considered to affect the validity of the study. The GAP in the present study is not in accordance with the current GAP that prevails in several EU member states (GAP: 75 – 125 g a.i./ha, one foliar application, PHI: 35 – 42). However, the intended application in the EU regions is to wheat seeds. Three metabolites in wheat straw treated with [triazole-^{14}C] difenoconazole were found at levels that exceed 0.1 mg/kg, but they were less than 10% of the TRR and were not characterised further. However, an additional study was carried out. Straw samples from wheat plants treated with [triazole-^{14}C] difenoconazole were further analysed to more fully characterise the residue (see Table B.7.1.7-4).

Corresponding straw and grain samples from the studies B.7.1.5 and B.7.1.6 were also re-analysed for comparative purposes (3 ½ years of storage).

Findings: No significant change was observed in the distribution of the radioactivity over time in the straw and grain extracts (Table B.7.1.7-3). As shown in Table B.7.1.7-4, the extracted radioactivity consisted of parent difenoconazole (45.4% of the TRR) together with the metabolite CGA-205375 (17.3% of the TRR) and four metabolites that individually did not exceed 5.2% of the TRR.

Table B.7.1.7-3. Comparison of radioactive residues in mature grain and straw from [triazole-^{14}C] difenoconazole seed and foliar treated wheat plants.

Treatment	Plant commodity	Sampling Time[a]	TRR (mg/kg)[b]	Organic phase (%)[c]	Aqueous phase (%)[c]	Non-extracted (%)[c]	Total (%)[d]
Field seed treatment	Straw	83 days	0.061 (0.059)[e]	8.1 (9.4)[e]	86.7 (87.1)[e]	13.7 (16.1)[e]	108.5 (112.6)[e]
Greenhouse seed treatment	Straw	236 days	0.081 (0.069)[f]	12.9 (16.8)[f]	57.5 (71.6)[f]	33.6 (18.3)[f]	104 (106.7)[f]
Greenhouse foliar treatment	Straw	94 days	53.8[g]	54.4 (50.1)[g]	23.0 (27.4)[g]	16.1 (13.2)[g]	93.5 (90.7)[g]
Greenhouse seed treatment	Grain	236 days	0.583[*] (0.183)[f]	n.d.	79.0 (80.4)[f]	36.0 (25.3)[f]	115 (105.7)[f]
Greenhouse foliar treatment	Grain	94 days	1.19 (1.40)[g]	n.d.	90.0 (69.5)[g]	30.0 (22.7)[g]	120 (92.3)[g]

[*] the difference (0.183 vs. 0.583) may have resulted from loss of moisture during storage or from a non-homogenous sample.
[a] Days after planting.
[b] mg/kg determined by combustion.
[c] Percent of the TRR determined by combustion.
[d] Sum of extractable and non-extractable.
[e] Previous result (study B.7.1.5).
[f] Previous result (study B.7.1.6).
[g] Previous result (see Table B.7.1.7-1).
n.d.=Not detected.

Table B.7.1.7-4. Characterisation of components in [triazole-^{14}C] difenoconazole foliar treated, greenhouse grown mature wheat straw.

Extractable				
Extract	Organic phase	Aqueous phase	Cellulase released	Total
Compound	% TRR	% TRR	% TRR	% TRR
Difenoconazole	43.3	1.2	0.8	45.3
CGA-205374	0.5	--	0.5	1.0
CGA-205375	2.2	0.3	13.4	15.9
CGA-71019	3.4	--	--	3.4
CGA-131013	--	--	--	--
CGA-142856	--	--	--	--
Non-extractable				
Extract	Organic phase	Aqueous phase	Total in bound	
Compound	% TRR	% TRR	% TRR	
Difenoconazole	0.1	--	0.1	
CGA-205374	< 0.1	0.6	0.6	
CGA-205375	0.4	1.0	1.4	
CGA-71019	0.1	1.7	1.8	
CGA-131013	--	1.4	1.4	
CGA-142856	--	1.1	1.1	
			Overall total: 72 % of TRR	

Conclusions: At harvest, following four applications of phenyl-^{14}C labelled difenoconazole at a rate of 247 g a.i/ha per application, the TRR in straw and grain was 46.7 and 0.064 mg/kg, respectively. The majority of the radioactivity in straw samples was organic soluble (51.8%) and consisted mainly of parent difenoconazole (50% of the TRR). Radioactivity in the corresponding aqueous phase was 29.8% of the TRR. Cellulase treatment in grain samples released ca 35% of the TRR and it was proposed that this water soluble radioactivity contained conjugates of CGA-189138 or CGA-189138 metabolites. In immature foliage the TRR was 6.88 mg/kg and the majority of the radioactivity was organic soluble (89.5%) and consisted mainly of parent difenoconazole (85% of the TRR).

Residues in mature straw and grain from the [triazole-^{14}C] difenoconazole foliar treated wheat plant at a rate of 247 g a.i/ha per application were 53.8 and 1.40 mg/kg, respectively. In grain, the majority of the radioactivity was aqueous soluble (69.5% of the TRR) and after gel filtration, 1,2,4-triazole (CGA-71019; 10% of the TRR) and triazole acetic acid (CGA-142856; 20% of the TRR) were identified. Radioactive residues in the straw were mainly composed of parent difenoconazole found in organic soluble fractions, and water-soluble metabolite conjugates. After cellulase treatment, the metabolites CGA-205375 (5% of the TRR), hydroxy-CGA-205375 (1% of the TRR) and hydroxy-difenoconazole (1% of the TRR) were identified.

In both radiolabelled experiments harvested grain contained no organic soluble radioactivity.

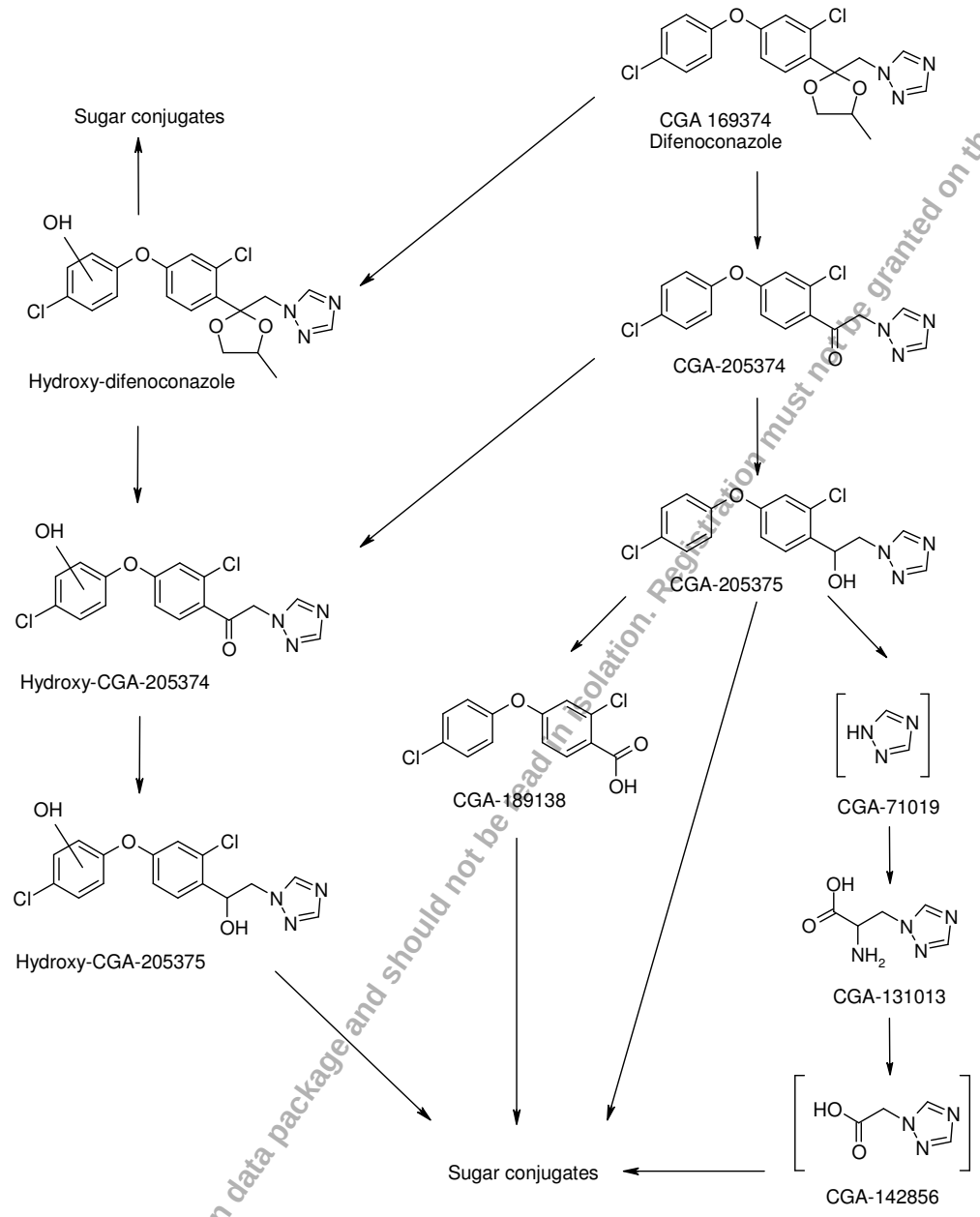
Comparison of foliar and seed treated grain residues showed they had similar extraction and partitioning characteristics. The majority of the residues were aqueous soluble (up to 80.4% of the TRR) with the remainder present as non-extractable radioactivity.

Further analysis of the [triazole-^{14}C] difenoconazole treated straw confirmed the presence of difenoconazole (45.4% of the TRR) and sugar conjugated CGA-205375 (17.3% of the TRR) as the major components of

the residue, in addition to smaller amounts of the metabolites CGA-71019 (5.2% of the TRR), CGA-205374 (1.6% of the TRR), CGA-131013 (1.4% of the TRR) and CGA-142856 (1.1% of the TRR).

Based on the compounds detected in the available studies, difenoconazole is metabolised in wheat plants primarily via phenyl ring hydroxylation, formation of the difenoconazole ketone (CGA-205374) via hydrolysis of the dioxolane ring, reduction of the ketone to the alcohol (CGA-205375) and triazole ring cleavage. Secondary metabolism occurs via sugar conjugation of hydroxylated parent compound and metabolites, and amino acid (serine) conjugation of 1,2,4-triazole (CGA-71019) to form triazole alanine (CGA-131013) which was further degraded to triazole acetic acid (CGA-142856). Metabolites formed as a result of triazole ring cleavage were transported preferentially to the grain.

The proposed pathway of metabolism in wheat plants is shown in Figure B.7.1.7-1.



[ ] Proposed intermediate not identified in the studies.

Figure B.7.1.7-1. Proposed metabolic pathway of difenoconazole in greenhouse grown wheat plants following seed or foliar treatment.

B.7.1.8 Metabolism of [phenyl-^{14}C] difenoconazole in greenhouse grown potatoes

Reference:	**Schweitzer M.G. (1990b)** Metabolism of phenyl-^{14}C CGA-169374 in spray treated potatoes. Battelle, Columbus, Ohio, United States. Unpublished Report N-0964-0400, SAM No. 0357.
Test Material:	Phenyl-^{14}C, batch number RAF-VIII-96, radiochemical purity 98.5%, specific activity 42.6 µCi/mg.
Guideline:	Pesticide Assessment Guidelines, -Subdivision O, Residue Chemistry, Series 171-4(a) (1) & (2), US Environmental Protection Agency, Washington, DC.
GLP:	Yes (In part). The biological and analytical phases of this study were conducted prior to the adoption of GLP standards. The final report and metabolite identification were written and conducted under the GLP standards.
Material and methods:	
Test concentration:	123.5 g a.i./ha
Test system:	The study was conducted indoors under greenhouse conditions, with potato seedlings (variety *Red Pontiac*) grown in plastic containers (35 cm diameter x 38 cm depth) containing a sandy loam soil. The soil characteristics were: pH (6.9), sand (60.8%), silt (29.9%), clay (9.2%), CEC (11.2 meq/100 g) and organic matter (0.71%). [Phenyl-^{14}C] difenoconazole, formulated as a 3.5 EC, were applied using a Devilbiss sprayer assembly with nitrogen as a propellant. Artificial lighting was manually activated in the greenhouse on those days that were partially overcast, the relative humidity ranged from 30-100% and the temperature from 11-40°C.
Stage of application:	The first application was made approximately 2 months after planting and the subsequent applications at 7 day intervals thereafter.
No. of applications:	Six
Sampling time points:	Potato plant foliage was harvested following the first application and prior to the third application. Foliage and tubers were harvested prior to the fifth application and at maturity following the final application (11 day PHI).
Method of analysis:	The TRR in each harvested sample was quantified by combustion/LSC. Samples were extracted using either Bligh-Dyer/Ting-Dugger methodology, methanol/water or acetonitrile. Acetonitrile extracts were concentrated to remove the solvent and the remaining aqueous samples were partitioned with ethyl acetate. Identification of the residues in the resulting organic and aqueous phases was carried out by co-chromatography against standard reference compounds using either 1D or 2D-TLC. Aqueous extracts were further analysed by fractionation on Sephadex G-10 gel filtration and Sephadex A-25 anion exchange resins and by enzyme de-conjugation using cellulase. Unextracted radioactivity in the tubers (51% of the TRR) was also treated with

a series of enzymes in an attempt to release bound residues.

Date of experiment: 20 March 1989 to 23 July 1990

Findings: Residue levels in potato plant fractions are shown in Table B.7.1.8-1.

Table B.7.1.8-1. Distribution of radioactive residues in potato plant fractions following six foliar applications of [phenyl-^{14}C] difenoconazole at a rate of 123.5 g a.i./ha.

Plant commodity	Sampling Time[a]	TRR (mg/kg)[b]	CH_3CN extract (%)[c]	Organic phase (%)[d]	Aqueous phase (%)[d]	Non-extracted (%)[c]	Total (%)[c]
Foliage	62 days (post 1st application)	3.48	96.3	--	--	1.9	98.2
Foliage	76 days (prior to 3rd application)	6.0	100.4	--	--	6.2	106.6
Foliage	90 days (prior to 5th application)	9.86	90.9	--	--	5.9	96.8
Tubers	90 days (prior to 5th application)	0.006	51.0	--	--	57.7	108.7
Foliage	108 days (11 day PHI)	12.4	93.9	87.7[d]	6.2[d]	9.7	103.6
Tubers	108 days (11 day PHI)	0.012	50.2	18.1[d]	32.1[d]	51.1	101.3

[a] Days after seeds planting.
[b] mg/kg difenoconazole equivalents.
[c] Percent of the TRR determined by combustion.
[d] Radiolabel distribution in organic and aqueous fractions obtained after partitioning of the acetonitrile extracts (mature foliage and tubers).

The total radioactive residues (TRR) in the potato plant foliage increased from 3.48 to 12.4 mg/kg difenoconazole equivalents, while the TRR in immature and mature tubers was low 0.006 and 0.012 mg/kg, respectively.

In mature foliage, > 90% of the TRR was extractable with acetonitrile and this radioactivity consisted mainly of parent difenoconazole (76.4% TRR) together with at least six metabolites, including metabolite CGA-189138, CGA-205374 and CGA-205375, that individually did not exceed 3% of the TRR (Table B.7.1.8-2) and nine unidentified regions of radioactivity each containing not greater than 6.1% of the TRR. The metabolites observed in the aqueous phase were identified following treatment with cellulase enzyme suggesting that these compounds were present as sugar conjugates in the original plants extract. The unextracted residue was 9.7% of the TRR and was not analysed further.

In mature tubers, 51% (ca 0.006 mg/kg) of the TRR was extractable with acetonitrile and the majority of this extracted radioactivity was present as aqueous soluble components (32.1% of the TRR) with only 18.1% of the TRR extractable into the organic phase following partition. Parent difenoconazole, CGA-205374 and CGA-205375 accounted for 8.7, 3.1 and 3.0% of the TRR respectively. Hydroxy-CGA-205375 was detected as a level of 15.4% of the TRR following treatment with cellulase. Two unidentified regions of radioactivity were also detected accounting for 3.4 and 16.5% of the TRR. Overall levels of residue in the tuber were low and no single region of radioactivity exceeded a level of 0.002 mg/kg. The unextracted residue was 51.1% of the TRR and were subjected to a series of enzyme treatments, however only a relatively small fraction of *ca* 25% of the radioactivity was released, indicating an extensive incorporation of radioactivity into the plant matrix.

Table B.7.1.8-2. Distribution and characterisation of [phenyl-^{14}C] difenoconazole residues in mature potato plant fractions following six foliar applications of at a rate of 123.5 g a.i./ha.

Plant part	Mature foliage		Mature tubers	
	11 day PHI		11 day PHI	
TRR (mg/kg)[b]	12.4		0.012	
Compound	% TRR	mg/kg[b]	% TRR	mg/kg[b]
Difenoconazole	76.4	9.47	8.7	0.0010
CGA-189138	0.5	0.07	n.d.	n.d.
CGA-205374	1.1	0.14	3.1	0.0004
CGA-205375	2.2	0.27	3.0	0.0004
Conjugated OH-difenoconazole[a]	1.0	0.12	n.d.	n.d.
Conjugated OH CGA-205375[a]	0.8	0.10	n.d.	n.d.
Conjugated CGA-205375[a]	3.0	0.37	15.4	0.0018
Unidentified	9.1[c]	1.11	19.9[d]	0.0024
Non-extracted	9.7	1.20	51.1	0.0061
Total	103.8		101.2	

[a] Metabolites present as sugar conjugates, characterised by cellulose enzyme de-conjugation
[b] mg/kg difenoconazole equivalents.
[c] Composed of up to 9 individual radioactive regions, individually not exceeding 6.1% TRR (0.75 mg/kg).
[d] Composed of 2 radioactive regions, containing 3.4% TRR (0.0004 mg/kg) and 16.5% TRR (0.002 mg/kg).
n.d. = Not detected.

Conclusion: At maturity, following six applications of [phenyl-^{14}C] difenoconazole each at a rate of 123.5 g a.i./ha, the TRR in the treated potato foliage and tubers was 12.4 and 0.012 mg/kg, respectively.

Parent difenoconazole was the largest component of the radioactive residue in the mature foliage. Small amounts (≤ 2.2% of the TRR) of each of the ketone, alcohol and acid metabolites CGA-205374, CGA-205375 and CGA-189138 were found in solvent extracts, together with similar amounts (≤ 2.2% TRR) of sugar conjugated CGA-205375, hydroxy-CGA-205375 and hydroxy-difenoconazole. Non-extracted residues were 9.7% TRR.

Mature tubers contained parent difenoconazole (8.7%), CGA-205375 (3.0%) and CGA-205374 (3.1%). An additional 15.4% of the TRR was released by cellulase treatment as CGA-205375. Enzyme treatment of the non-extracted tuber residues (51.1% TRR) indicated that the radioactivity was extensively incorporated into the plant matrix.

The study shows that difenoconazole is metabolised in potato plants via phenyl ring hydroxylation, leading to formation of the difenoconazole ketone (CGA-205374) and alcohol (CGA-205375) metabolites. Cleavage of the triazole ring results in the formation of the corresponding acid (CGA-189138). Secondary metabolism occurs via sugar conjugation of CGA-205375 and the related hydroxyl metabolites.

B.7.1.9 Metabolism of [triazole-^{14}C] difenoconazole in greenhouse grown potatoes

Reference:	**Velagaleti P.R. (1990b)** Metabolism of triazole-^{14}C-CGA-169374 in spray treated potatoes. Battelle, Columbus, Ohio, United States. Unpublished Report N-0964-0500, SAM No. 0489.
Test Material:	Triazole-^{14}C, batch number RAF-VIII-93, radiochemical purity 98.3%, specific activity 44.4 µCi/mg.
Guideline:	Pesticide Assessment Guidelines, -Subdivision O, Residue Chemistry, Series 171-4(a) (1) & (2), US Environmental Protection Agency, Washington, DC.
GLP:	Yes (In part). The biological and analytical phases of this study were conducted prior to the adoption of GLP standards. The final report and metabolite identification were written and conducted under the GLP standards.
Material and methods:	
Test concentration:	123.5 g a.i./ha
Test system:	The study was conducted indoors under greenhouse conditions, with potato seedlings (variety *Red Pontiac*) grown in plastic containers (35 cm diameter x 38 cm depth) containing a sandy loam soil. The soil characteristics were: pH (6.9), sand (60.8%), silt (29.9%), clay (9.2%), CEC (11.2 meq/100 g) and organic matter (0.71%). [Phenyl-^{14}C] difenoconazole, formulated as a 3.5 EC, were applied using a Devilbiss sprayer assembly with nitrogen as a propellant. Artificial lighting was manually activated in the greenhouse on those days that were partially overcast, the relative humidity ranged from 30-100% and the temperature from 11-37°C.
Stage of application:	The first application was made approximately 2 months after planting and the subsequent applications at 7 day intervals thereafter.
No. of applications:	Six
Sampling time points:	Potato plant foliage was harvested following the first application and prior to the third application. Foliage and tubers were harvested prior to the fifth application and at maturity following the final application (11 day PHI).
Method of analysis:	The TRR in each harvested sample was quantified by combustion/LSC. Samples were extracted using either Bligh-Dyer/Ting-Dugger methodology, methanol/water or acetonitrile. Acetonitrile extracts were concentrated to remove the solvent and the remaining aqueous samples were partitioned with ethyl acetate. Identification of the residues in the resulting organic and aqueous phases was carried out by co-chromatography against standard reference compounds using either 1D or 2D-TLC. Aqueous extracts were further analysed by fractionation on Sephadex G-10 gel filtration and Sephadex A-25 anion exchange resins and by enzyme de-conjugation using cellulase.

Date of experiment: 20 March 1989 to 05 July 1990

Findings: Residue levels in potato plant fractions are shown in Table B.7.1.9-1.

Table B.7.1.9-1. Distribution of radioactive residues in potato plant fractions following six foliar applications of [triazole-^{14}C] difenoconazole at a rate of 123.5 g a.i./ha.

Plant commodity	Sampling Time[a]	TRR (mg/kg)[b]	Organic phase (%)[c]	Aqueous phase (%)[c]	Non-extracted (%)[c]	Total (%)
Foliage	62 days (post 1st application)	2.242	100.9	1.8	1.5	104.1
Foliage	76 days (prior to 3rd application)	3.097	86.2	14.3	4.0	104.5
Foliage	90 days (prior to 5th application)	5.494	85.9	14.2	4.5	104.5
Tubers	90 days (prior to 5th application)	0.052	n.d.	92.9	1.8	94.7
Foliage	108 days (11 day PHI)	9.138	80.0	21.7	4.6	106.3
Tubers	108 days (11 day PHI)	0.087	2.1	90.3	1.9	94.3

[a] Days after seeds planting.
[b] mg/kg difenoconazole equivalents.
[c] Percent of the TRR determined by combustion.
n.d.= Not detected.

The total radioactive residues (TRR) in the potato plant foliage increased from 2.24 to 9.14 mg/kg following six foliar applications of [triazole-^{14}C] difenoconazole, while the TRR in immature and mature tubers was low 0.052 and 0.087 mg/kg, respectively.

In mature foliage, 80% of the TRR was organic soluble and consisted mainly of parent difenoconazole (71.3% of the TRR) together with the metabolites CGA-205374, CGA-205375, CGA 131013, that individually did not exceed 2% of the TRR (Table B.7.1.9-2) and at least six unidentified regions of radioactivity each containing not greater than 12.9% of the TRR. The aqueous soluble phase (21.7% of the TRR) contained five unidentified regions that collectively accounted for 21.9% of the TRR and the metabolites CGA-131013 and CGA-142856 (12.9% and 1.3% of the TRR, respectively). The identification of these two metabolites remained tentative because confirmatory TLC analyses was inconclusive due to the matrix interferences. The unextracted residue was 4.6% of the TRR and was not analysed further.

In mature tubers, 90% of the TRR was aqueous soluble radioactivity which consisted mainly of the metabolite CGA-131013 (78.9% of the TRR) together with three minor unidentified compounds that individually did not exceed 5% of the TRR (Table B.7.1.9-2). Further analysis of the tuber aqueous phase showed that the metabolite CGA-142856 was also present in the extract at a level of 16.1% of the TRR. The organic soluble phase accounted for 2.1% of the TRR and consisted predominantly of parent difenoconazole (1.8% of the TRR) together with the metabolite CGA-205374 (0.1% of the TRR) and a small amount of unidentified polar material that accounted for 0.2% of the TRR. The unextracted residue was less than 2% of the TRR and was not analysed further.

Table B.7.1.9-2. Characterisation of radioactive residues in potato plant fractions following six foliar applications of [triazole-^{14}C] difenoconazole at a rate of 123.5 g a.i./ha.

Plant part	Mature foliage		Mature tubers	
	11 day PHI		11 day PHI	
TRR (mg/kg)[a]	9.138		0.087	
Compound	% TRR	mg/kg[a]	% TRR	mg/kg[a]
Difenoconazole	71.3	6.661	1.8	0.0016
CGA-205374	0.8	0.073	0.1	0.0001
CGA-205375	1.9	0.173	n.d.	n.d.
CGA-131013	n.d.	n.d.	78.9[b]	0.0686
Unidentified	21.9[c]	2.043	11.4[b]	0.0099
Polar compounds	2.2	0.208	0.2	0.0001
Non-extracted	4.6	0.420	1.9	0.0017
Total	102.7		94.3	

[a] mg/kg difenoconazole equivalents.
[b] Radioactivity may also contain CGA-142856 at a level of 16.1% TRR (0.014 mg/kg). Unidentified component contains at least 3 individual radioactive regions, individually not exceeding 4.6% TRR (0.004 mg/kg).
[c] Composed of at least 6 radioactive regions, individually not exceeding 12.9% TRR (1.202 mg/kg). Analysis using Sephadex A-25 column chromatography suggested the presence of CGA-131013 and CGA-142856 at levels of 12.9 and 1.3% of the TRR, respectively.
n.d. = Not detected.

Conclusion: At maturity, following six applications of [triazole-^{14}C] difenoconazole each at a rate of 123.5 g a.i./ha, the TRR in treated potato foliage and tubers was 6.138 and 0.087 mg/kg, respectively.

Parent difenoconazole was the largest component of the residue in the mature foliage. Small amounts (<2% of the TRR) of the metabolites CGA-205374 and CGA-205375 were found in solvent extracts. The triazole specific metabolites CGA-131013 (triazole alanine) and CGA-142856 (triazole acetic acid) were also tentatively identified at levels of 12.9% TRR and 1.3% TRR, respectively.

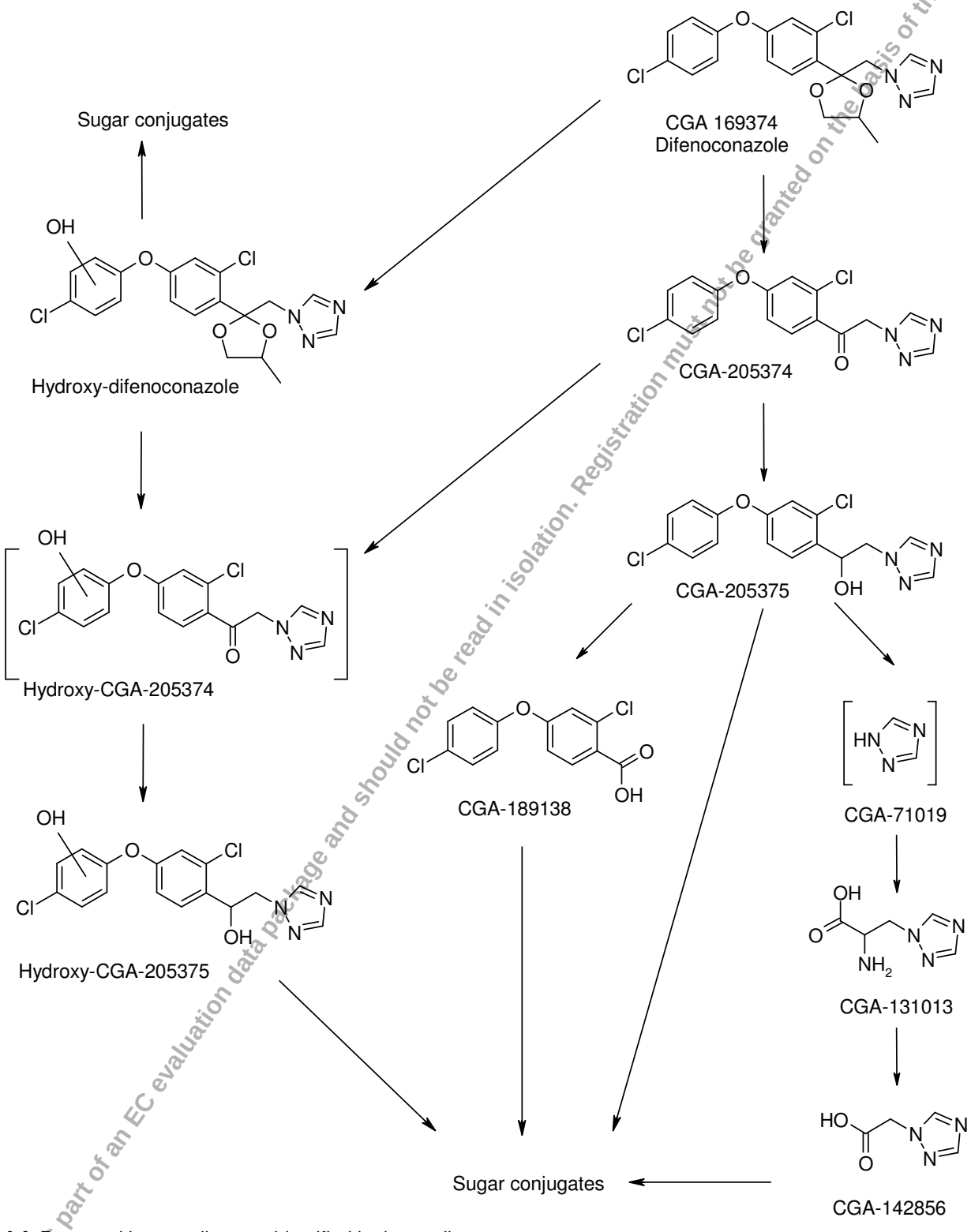
Mature tubers contained predominantly the metabolite CGA-131013 (78.9% of the TRR). A small amount (0.1% TRR) of the metabolite CGA-205374 was also detected in addition to three regions of unidentified radioactivity that individually did not exceed 4.6% of the TRR.

Based on the metabolites detected in foliage and tuber samples, the pathway of metabolism of difenoconazole in potato plants occurred primarily via phenyl ring hydroxylation, formation of the difenoconazole ketone (CGA-205374) via hydrolysis of the dioxolane ring and reduction of CGA-205374 to form the alcohol (CGA-205375). Cleavage of the triazole moiety also occurs leading to the formation of triazole acetic acid (CGA-142856) and triazole alanine (CGA-131013), via amino acid (serine) conjugation of 1,2,4-triazole (CGA-71019).

The proposed pathway of metabolism in potato plants is given in Figure B.7.1.9-1

Comments by RMS: Discussion of storage stability in both potato plant metabolism studies was not reported. However, the lack of this data is not considered to affect the validity of the studies. The application rate in both studies was in accordance with the proposed GAP for carrot in Southern and Northern Europe (125 g a.i./ha). The pre-harvest intervals were shorter (11 days) than the minimum PHI recommended in both European regions (14 days). The interval between applications was 7 days, which was shorter than the minimum of 14 days.

According to the document 7028/VI/95 rev. 3, 22/7/1997, potato and carrot belong to the same category, i.e. root vegetables. However, potato and carrot belong to different plant families, i.e. the *Solanaceae* and Apia*ceae* family, respectively. Thus, to what extent the metabolite CGA-131013 would be formed in carrots (0.068 mg/kg (78.9% TRR) was formed in mature potato tubers), remains unknown.



[ ] Proposed intermediate not identified in the studies.

Figure B.7.1.9-1. Proposed metabolic pathway of difenoconazole in greenhouse grown potato plants following foliar treatment.

B.7.1.10 Metabolism of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole in grape

Reference:	**Capps T. M. (1992)** Uptake and metabolism of ^{14}C-CGA-169374 by grapes from foliar spray-treatment. Metabolism Department, Agricultural Division, Ciba-Geigy Corporation, Greensboro, North Carolina, United States. Unpublished Report ABR-92003, SAM No. 0537.
Test Material:	Phenyl-^{14}C, batch number BPM-XII-4, radiochemical purity 97.1%, specific radioactivity 25.4 µCi/mg. Triazole-^{14}C, batch number JAK-IV-82, radiochemical purity 97.0%, specific radioactivity 27.4 µCi/mg.
Guideline:	Pesticide Assessment Guidelines, -Subdivision O, Residue Chemistry, Series 171-4(a) (1) & (2), US Environmental Protection Agency, Washington, DC.
GLP:	Yes
Material and methods:	
Test concentration:	247 g a.i./ha
Test system:	The study was conducted outdoors under field conditions in caged enclosures on sandy loam soil. The soil characteristics were: pH (6.1), sand (73%), silt (19%), clay (8%), CEC (4.3 meq/100 g) and organic matter (0.9%). The grape variety used was *Chenin Blanc* and one vine was used for each radiolabelled treatment. [Phenyl-^{14}C] and [triazole-^{14}C] difenoconazole, formulated as a 250 EC were applied as a spray.
Stage of application:	The first application was made at the pea berry growth stage (BBCH 75), with the subsequent applications made at 14, 28, 14 and 15 day intervals thereafter. Following the second application to the phenyl-^{14}C labelled vine the plant became diseased and further treatments were terminated. A second vine was selected for treatment (phenyl II) and the remaining three applications were made as described above.
No. of applications:	Five
Sampling time points:	Vine leaves were sampled 35 days after the second application to the first phenyl-^{14}C treated vine (phenyl I) 7 days after the third application to the triazole-^{14}C treated vine and 7 days after the first application to the second phenyl-^{14}C treated vine (phenyl II). Mature fruit and leaves were harvested from the triazole-^{14}C and phenyl-^{14}C (phenyl II) treated vines at a PHI of 20 days.
Method of analysis:	The radioactive residue in each harvested sample was determined by extraction and combustion/LSC. Grape and leaf samples were extracted using Bligh-Dyer/Ting-Dugger methods which gave aqueous, organic and non-extracted fractions. Samples were also extracted with methanol/water (8:2 v/v) and the extract analysed directly by TLC. Methanol/water extracts were also

concentrated to remove the solvent and the remaining aqueous samples were partitioned with chloroform. Identification of the residues in the resulting organic and aqueous phases was carried out by co-chromatography against standard reference compounds using 2D-TLC. Aqueous extracts were further analysed by fractionation on Sephadex A-25 or XAD resins and by enzyme de-conjugation using cellulase and β-glucosidase. Radioactivity in the organic extracts was also derivatised with diazomethane and analysed by HPLC. Identification of selected metabolites was confirmed by MS.

Date of experiment: 23 May 1990 to 29 January 1992

Findings: Residue levels in leaves and grape are shown in Table B.7.1.10-1.

Table B.7.1.10-1. Distribution of radioactive residues in grape plant fractions following five applications of [phenyl^{14}C] and [triazole-^{14}C] difenoconazole at a rate of 247 g a.i./ha.

Labelled form	Plant Commodity	Sampling Time	TRR (mg/kg)[c]	Methanol / water 8:2 (%)[d]	Organic phase (%)[d]	Aqueous phase (%)[d]	Non-extracted (%)[d]
[phenyl-^{14}C] difenoconazole (Phenyl I)[a]	Leaves	Immature	6.560	61.7	39.3	22.4	38.4
	Leaves	Mature 20 day PHI	1.562	67.0	43.9	23.1	33.0
	Grapes	Mature 20 day PHI	0.047	64.0	33.0	31.0	36.0
[phenyl-^{14}C] difenoconazole (Phenyl II)[b]	Leaves	Immature	8.576	87.7	59.2	28.5	12.3
	Leaves	Mature 20 day PHI	9.180	87.0	78.3	8.7	13.0
	Grapes	Mature 20 day PHI	0.127	81.0	67.7	13.3	19.0
[triazole-^{14}C] difenoconazole	Leaves	Immature	8.695	76.5	65.9	10.6	23.5
	Leaves	Mature 20 day PHI	5.782	73.0	46.7	26.3	27.0
	Grapes	Mature 20 day PHI	0.115	83.0	39.8	43.2	17.0

[a] Initial phenyl (following the second application the vine plant became diseased and further treatments were terminated. A second vine plant was selected for treatment; 2x247 g a.i./ha)
[b] Final phenyl (three applications at 247 g a.i./ha).
[c] mg/kg difenoconazole equivalents.
[d] Percent of the residue determined by combustion.

The TRR in leaves from the phenyl I treatments were between 6.560 and 1.562 mg/kg and 0.047 mg/kg in the mature fruit while the TRR in leaves from the phenyl II treatments were between 8.576 and 9.180 mg/kg and 0.127 mg/kg in the mature fruit.

The TRR in leaves treated with [triazole-^{14}C] difenoconazole were between 8.695 and 5.782 mg/kg and 0.115 mg/kg in the mature fruit.

In the phenyl I treated vine plant, leaf samples taken at immature and mature harvest contained 39.4 and 43.9% of the TRR as organic soluble radioactivity and 22.4 and 23.1% as aqueous soluble radioactivity, respectively (Table B.7.1.10-2) while the radioactivity in the mature fruit consisted of 32.9% organic soluble and 31% aqueous soluble. In the phenyl II treated vine plant, leaf samples taken at immature and mature harvest contained 58.9 and 78.8% of the TRR as organic soluble radioactivity and 28.5 and 8.5% as aqueous soluble radioactivity, respectively (Table B.7.1.10-3) while the radioactivity in the mature fruit consisted of 67.8% organic soluble and 13.4% aqueous soluble. In the triazole treated vine plant, leaf samples taken at immature and mature harvest contained 65.9 and 49.4% of the TRR as organic soluble

radioactivity and 10.6 and 26.4% as aqueous soluble radioactivity, respectively (Table B.7.1.10-4). The radioactivity in the mature fruit consisted of 39.8% organic soluble and 43.2% aqueous soluble.

Parent difenoconazole formed the major part of the residue in the mature leaves and fruit treated with [phenyl-^{14}C] difenoconazole (12.3 to 46% and 17.1 to 43.5% of the TRR, respectively). The metabolites CGA-205374, CGA-205375 and CGA-189138 were observed in the leaves at levels between 4.3 and 8.2% and in the grapes at levels between 2.1 and 6.4% of the TRR. These metabolites were also detected as sugar conjugates at levels up to 4.8% of the TRR in leaves and 0.3% of the TRR in the fruit. Sugar conjugates of parent difenoconazole were also observed in leaf and fruit samples at levels up to 5.4 and 7.7% of the TRR, respectively. Unidentified metabolites were observed in leaf and fruit samples, but each individual metabolite or group of metabolites did not exceed 10% of the TRR in either sample, or exceed 0.01 mg/kg in the fruit.

Parent difenoconazole formed also the major part of the residue in leaf and fruit samples treated with [triazole-^{14}C] difenoconazole (25.9 to 28.2% and 25.9% of the TRR, respectively; Table B.7.1.10-4). The metabolites CGA-205374, CGA-205375 and CGA-71019 were observed in the leaves at levels between 2.8 and 10.7% and in the grapes at levels between 1.7 and 3.6% of the TRR. These metabolites were also detected as sugar conjugates at levels up to 1.2% of the TRR in leaves and 3.7% of the TRR in the fruit. Sugar conjugates of parent difenoconazole were also observed in leaf and fruit samples at levels up to 3.3 and 19.2% of the TRR, respectively. Unidentified metabolites were observed in leaf and fruit samples, but none individually exceeded 2.7% of the TRR. Groups of unidentified metabolites were also observed in leaf and fruit samples, with the largest group accounting for 10.1% of the TRR in the fruit (0.012 mg/kg). Separation of this metabolite group using XAD resin divided the sample into two discrete fractions, each containing less than 10% of the TRR (corresponding to less than 0.01 mg/kg).

The non-extracted radioactivity in the [phenyl-^{14}C] difenoconazole treated samples were between 12.3 and 38.4% in the leaf and between 19 and 36% in the fruit. Treatment of the non-extracted residue with cellulase and β-glucosidase released only small amounts of the residue (<1% of the TRR) and the remaining non-extracted radioactivity was not analysed further. The non-extracted radioactivity in the [triazole-^{14}C] difenoconazole treated samples were between 23.5 and 27% in the leaf and 17% in the fruit. Treatment of the non-extracted residue with cellulase and β-glucosidase released up to 2.5% of the TRR from the leaves and up to 6.3% of the TRR from the fruit. Analysis of the extracted radioactivity showed the presence of parent difenoconazole, CGA-205374, CGA-205375, CGA-186138 (phenyl-^{14}C only) and CGA-71019 (triazole-^{14}C only).

Table B.7.1.10-2. Distribution and identification of [phenyl-^{14}C] difenoconazole residues in grape fractions following two applications at a rate of 247 g a.i./ha (Phenyl I treatment)[a].

Extract	Plant Part	Immature leaves		Mature leaves		Mature grapes	
	TRR (mg/kg)[b]	6.560		1.562		0.047	
Organic soluble[c]	Compound	% TRR	mg/kg[b]	% TRR	mg/kg[b]	% TRR	mg/kg[b]
	Difenoconazole	14.2	0.932	12.3	0.192	17.1	0.008
	CGA-205374	3.5	0.230	3.7	0.058	2.5	0.001
	CGA-205375	2.3	0.151	3.2	0.050	4.6	0.002
	CGA-189138	1.5	0.098	2.0	0.031	2.1	0.001
	Unknown group 1 and 2[f]	1.6	0.105	6.3	0.098	4.4	0.002
	Unknown group 7[f]	3.9	0.256	2.2	0.034	--	--
	Unknown group 8[f]	4.8	0.315	4.6	0.072	2.2	0.001
	Non-polar compounds	5.3	0.348	8.4	0.131	--	--
	Polar compounds	2.3	0.151	1.2	0.019	--	--
	%TRR	39.4		43.9		32.9	
Aqueous soluble[d]	Conjugated difenoconazole	5.4	0.354	3.7	0.058	--	--
	Conjugated CGA-205374	1.7	0.112	--	--	--	--
	Conjugated CGA-205375	--	--	4.6	0.072	--	--
	Conjugated CGA-189138	4.8	0.315		--	--	--
	Unknown 1	--	--	1.7	0.027	--	--
	Unknown 2	--	--	--	--	--	--
	Unknown 3	--	--	--	--	--	--
	Unknown 4	--	--	--	--	--	--
	Polar compounds	2.7	0.177	3.0	0.047	19.2	0.009
	Water soluble	7.8	0.512	10.1	0.158	11.8	0.006
	%TRR	22.4		23.1		31.0	
Non-extracted		38.4	2.519	33.0	0.515	36.0	0.017
	Total (%)[g]	100.2		100.0		99.9	
Further analysis of the water soluble fraction[e]	Unknown group 1	3.1	0.203	4.3	0.067	6.4	0.003
	Unknown group 2	--	--	--	--	4.2	0.002
	Unknown	4.7	0.308	5.8	0.091	0.4	<0.001
	Conjugated difenoconazole	1.8	0.118	--	--	1.7	<0.001
	Conjugated CGA-189138	0.7	0.005	--	--	0.4	<0.001
	Unknowns	1.6	0.105	1.9	0.030	2.4	0.001
	Conjugated CGA-205374 / Conjugated CGA-205375	--	--	--	--	0.6	<0.001
	Water soluble	3.7		8.2	0.128	6.7	0.003

[a] following the second application the vine plant became diseased and further treatments were terminated. A second vine plant was selected for treatment.
[b] mg/kg difenoconazole equivalents.
[c] Organic soluble compounds were observed in chloroform extracts following partition.
[d] Aqueous soluble compounds were observed following treatment with cellulase and partition with ethyl acetate.
[e] Water soluble radioactivity was further analysed using separation on Sephadex A-25 resin and treatment with cellulase and β-glucosidase enzymes.
[f] Unknowns separated on HPLC.
[g] Percent determined from the sum of extracted plus non-extracted radioactivity.

Table B.7.1.10-3. Distribution and identification of [phenyl-^{14}C] difenoconazole residues in grape fractions following three applications at a rate of 247 g a.i./ha (Phenyl II treatment)[a].

Extract	Plant Part	Immature leaves		Mature leaves		Mature grapes	
	TRR (mg/kg)[b]	8.576		9.180		0.127	
Organic soluble[c]	Compound	% TRR	mg/kg[b]	% TRR	mg/kg[b]	% TRR	mg/kg[b]
	Difenoconazole	34.9	2.993	46.0	4.223	43.5	0.055
	CGA-205374	4.2	0.360	8.2	0.753	3.8	0.005
	CGA-205375	1.4	0.120	4.3	0.395	6.4	0.008
	CGA-189138	3.8	0.326	5.3	0.487	4.0	0.005
	Unknown group 1 and 2[f]	1.8	0.154	0.5	0.046	4.4	0.006
	Unknown group 7[f]	7.1	0.609	5.2	0.477	2.1	0.003
	Unknown group 8[f]	3.6	0.309	7.6	0.698	2.0	0.003
	Non-polar compounds	2.1	0.180	1.3	0.119	--	--
	Polar compounds	--		0.4	0.037	1.6	0.002
	%TRR	58.9		78.8		67.8	
Aqueous soluble[d]	Conjugated difenoconazole	0.9	0.077	0.4	0.037	7.7	0.010
	Conjugated CGA-205374	--	--	0.1	0.009	0.3	<0.001
	Conjugated CGA-205375	1.2	0.103	--	--	0.2	<0.001
	Conjugated CGA-189138	--	--	--	--	--	--
	Unknown 1	--	--	0.2	0.018	--	--
	Unknown 2	1.1	0.094	0.3	0.028	--	--
	Unknown 3	--	--	--	--	--	--
	Unknown 4	2.0	0.172	--	--	--	--
	Polar compounds	1.9	0.163	4.4	0.404	1.3	0.002
	Water soluble	21.4	1.835	3.1	0.285	3.9	0.005
	%TRR	28.5		8.5		13.4	
Non-extracted		12.3	1.055	13.0	1.193	19.0	0.024
Further analysis of the water soluble fraction[e]	Total (%)[g]	99.7		100.3		100.2	
	Unknown group 1	6.0	0.514	1.2	0.110	3.2	0.004
	Unknown group 2	8.2	0.703	--	--	--	--
	Unknown	7.3	0.626	1.9	0.174	0.9	0.001
	Conjugated difenoconazole	0.1	0.009	--	--	0.4	<0.001
	Conjugated CGA-189138	--	--	--	--	0.1	<0.001
	Unknowns	1.6	0.137	--	--	--	--
	Water soluble	19.7	1.689	3.1	0.285	3.4	0.004

[a] following the second application the vine plant became diseased and further treatments were terminated. A second vine plant was selected for treatment.
[b] mg/kg difenoconazole equivalents.
[c] Organic soluble compounds were observed in chloroform extracts following partition.
[d] Aqueous soluble compounds were observed following treatment with cellulase and partition with ethyl acetate.
[e] Water soluble radioactivity was further analysed using separation on Sephadex A-25 resin and treatment with cellulase and β-glucosidase enzymes.
[f] Unknowns separated on HPLC.
[g] Percent determined from the sum of extracted plus non-extracted radioactivity.

Table B.7.1.10-4. Distribution and identification of [triazole-^{14}C] difenoconazole residues in grape fractions following five applications at a rate of 247 g a.i./ha.

Extract	Plant Part	Immature leaves		Mature leaves		Mature grapes	
	TRR (mg/kg)[a]	8.695		5.782		0.115	
	Compound	% TRR	mg/kg[a]	% TRR	mg/kg[a]	% TRR	mg/kg[a]
Organic soluble[c]	Difenoconazole	28.2	2.452	25.9	1.498	25.9	0.030
	CGA-205374	10.7	0.930	2.7	0.156	1.7	0.002
	CGA-205375	4.5	0.391	3.5	0.202	3.5	0.004
	CGA-71019	2.8	0.243	3.2	0.185	3.6	0.004
	Unknown group 2[e]	3.2	0.278	2.1	0.121	--	--
	Unknown group 5[e]	2.8	0.243	3.0	0.173	--	--
	Unknown group 7[e]	5.7	0.496	2.3	0.133	3.1	0.004
	Unknown group 8[e]	2.4	0.209	--	--	2.0	0.002
	Non-polar compounds	3.4	0.296	4.4	0.254	--	--
	Polar compounds	2.2	0.191	2.3	0.133	--	--
	%TRR	65.9		49.4		39.8	
Aqueous soluble[d]	Conjugated difenoconazole	3.3	0.287	1.1	0.064	19.2	0.022
	Conjugated CGA-205374	0.8	0.070	0.3	0.017	--	--
	Conjugated CGA-205375	1.2	0.104	0.4	0.023	--	--
	Conjugated CGA-71019	--	--	--	--	3.7	0.004
	Unknown 1	0.8	0.070	1.4	0.081	--	--
	Unknown 2	0.9	0.078	0.8	0.046	--	--
	Unknown 3	--	--	1.1	0.064	--	--
	Unknown 4	--	--	--	--	--	--
	Polar compounds	0.5	0.043	2.9	0.168	--	--
	Water soluble	3.1	0.270	18.4	1.064	20.3	0.023
	%TRR	10.6		26.4		43.2	
Non-extracted		23.5	2.043	27.0	1.561	17.0	0.020
Further analysis of the water soluble fraction[d]	Total (%)[f]	100.0		102.8		100.0	
	CGA-71019	--	--	--	--	5.0	0.006
	Unknown group 1	0.8	0.070	7.5	0.434	9.5	0.011
	Unknown group 2	--	--	8.7	0.503	10.1	0.012
	Unknown	2.7	0.235	2.4	0.139	0.7	<0.001
	Conjugated difenoconazole	0.2	0.017	0.4	0.023	4.1	0.005
	Conjugated CGA-71019	--	--	1.6	0.093	--	--
	Conjugated CGA-205374 / Conjugated CGA-205375	--	--	--	--	0.4	<0.001
	Unknowns[g]	0.1	0.009	--	--	0.4	<0.001
	Water soluble	2.8	0.243	6.0	0.347	15.4	0.018

[a] mg/kg difenoconazole equivalents.
[b] Organic soluble compounds were observed in chloroform extracts following partition.
[c] Aqueous soluble compounds were observed following treatment with cellulase and partition with ethyl acetate.
[d] Water soluble radioactivity was further analysed using separation on Sephadex A-25 resin and treatment with cellulase and β-glucosidase enzymes.
[e] Unknowns separated on HPLC.
[f] Percent determined from the sum of extracted plus non-extracted radioactivity.
[g] Possibly CGA-142856 and/or CGA-131013.

Conclusion: At harvest, 20 days after the fifth of five applications of either [phenyl-^{14}C] or [triazole-^{14}C] difenoconazole at 247 g a.i./ha, the TRR in fruit was 0.047 to 0.127 and 0.115 mg/kg, respectively. The TRR in the corresponding leaves was 1.562 to 9.180 and 5.782 mg/kg, respectively.

Difenoconazole was extensively metabolised in grape vine. Parent compound was the largest component of the residue in all plant parts at harvest (12.3 to 43.5% of the TRR) leading to residues of 0.192 to 4.223 mg/kg in leaves and 0.008 to 0.055 mg/kg in the fruit. Sugar conjugated parent difenoconazole accounted for up to 19.2% (0.022 mg/kg) in the [triazole-^{14}C] difenoconazole treated fruit. The metabolites CGA-205374 and CGA-205375 were detected in fruit samples at levels between 1.7 to 3.8% and 3.5 to 6.4% of the TRR, respectively. Small amounts of the metabolites CGA-71019 (3.6% of the TRR) and CGA-189138 (4% of the TRR) were detected in the [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole, leading to residues <0.01 mg/kg.

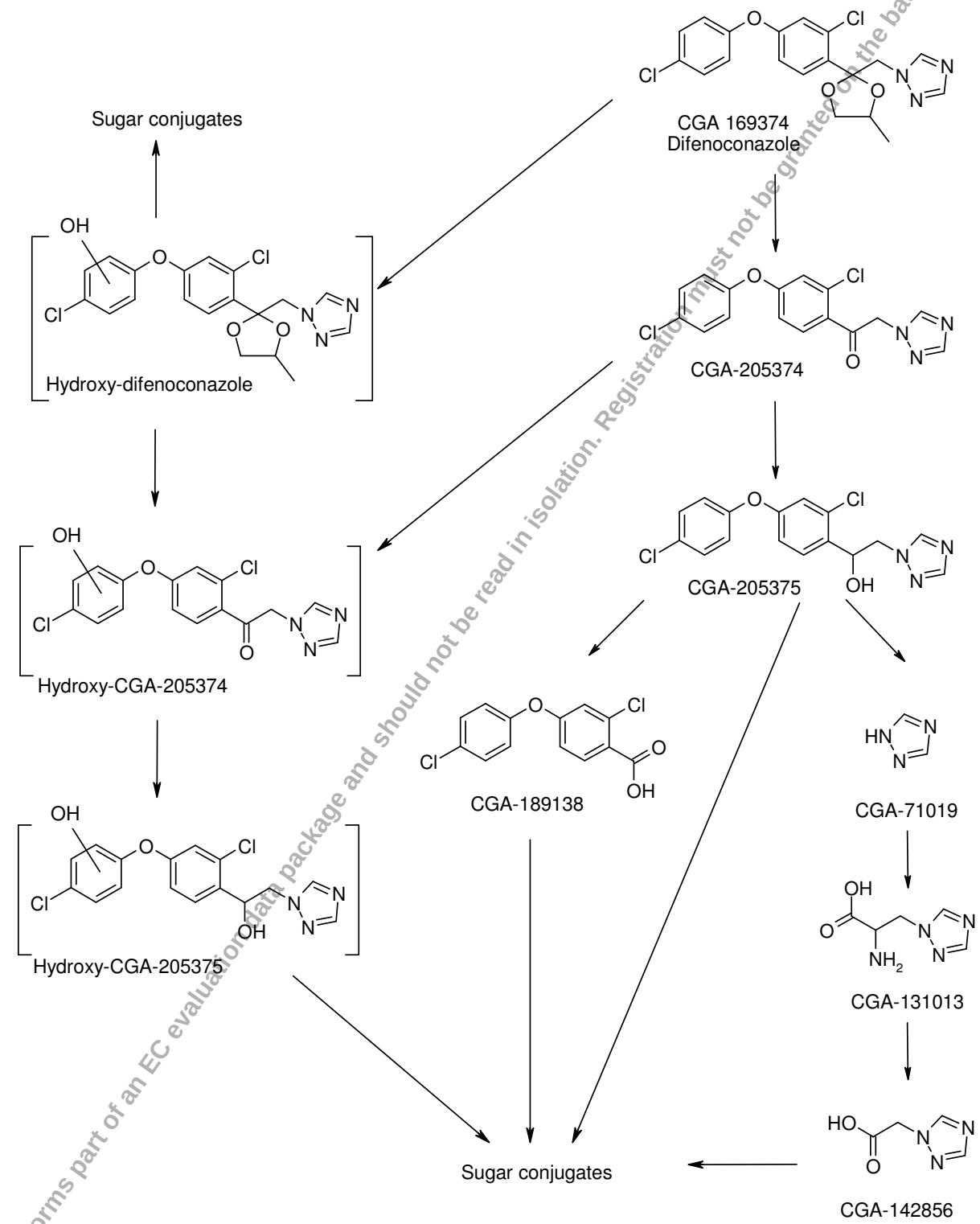
Unidentified metabolites were observed in leaf and fruit samples, but none individually exceeded 10% of the TRR in leaves and fruit or 0.01 mg/kg in fruit.

The unextracted radioactivity accounted for up to 38.4% of the TRR in leaves and up to 36% of the TRR in fruit. A small proportion of these residues were identified as sugar conjugates of either parent compound or the metabolites CGA-205374, CGA 205375, CGA-186138 (phenyl-^{14}C only) and CGA-71019 (triazole-^{14}C only). The presence of remaining unextracted residue indicate that the residue is tightly bound, probably the result of extensive incorporation of radioactivity into the plant matrix.

Based on the metabolites detected in leaf and fruit samples, the pathway of metabolism of difenoconazole in grape vine occurred primarily via formation of the difenoconazole ketone (CGA-205374) and alcohol (CGA-205375). Oxidation of the alcohol (CGA-205375) results in cleavage of the alkyl bridge to form 1,2,4-triazole (CGA-71019) and the corresponding acid (CGA-189138). Further metabolism via sugar conjugation of both difenoconazole and its primary metabolites occurs, resulting in incorporation into unextracted plant residues (see Figure B.7.1.10-1).

Comments by RMS: No weather data and no discussion of storage stability has been included in the report. However, the lack of these data is not considered to affect the validity of the study. The application rate in the present study was 3 to 4 times higher than the critical GAP proposed for pome fruit in Southern and Northern Europe (75 and 56.2 g a.i./ha, respectively). The pre-harvest intervals were longer (20 days) than the minimum recommended in Southern (14 days) and shorter than the minimum recommended in Northern Europe (28 days). The interval between applications was 14 days, which was longer than the minimum of 10 days.

According to the document 7028/VI/95 rev. 3, 22/7/1997, grape and pome fruit belong to the same category. However, grape and pome fruit belong to different plant families, i.e. the *Vitaceae* and *Rosaceae* family, respectively.



[ ] Proposed intermediate not identified in the studies.

Figure B.7.1.10-1. Proposed metabolic pathway of difenoconazole in field grown grapevine following foliar treatment.

B.7.1.11 Metabolism of [phenyl-^{14}C] difenoconazole in field grown spring oilseed rape

Reference:	**Neuman C. (1993a)** Metabolism of [Phenyl-^{14}C]-CGA-169374 in field grown spring rape. Metabolism, Divisional Unit R&D, Plant Protection Division, Ciba-Geigy Limited, Basel, Switzerland. Unpublished Report 11/93, SAM No. 0809.
Test Material:	[Phenyl-^{14}C] difenoconazole, batch number JAK-V-79, radiochemical purity 97.5%, specific radioactivity 23.3 µCi/mg.
Guideline:	Control of Pesticide Regulations Data Requirement Handbook, appendix 6, Rothamsted, Harpenden, October 1992; Pesticide Assessment Guidelines, subdivision O, Residue Chemistry, Series 171-4 (a) (1) & (2), US Environmental Protection Agency, Washington, DC, October 1982; BBA Guidelines "Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln" Series IV 3.2, Biologische Bundesanstalt für Land- und Forst- wirtschaft, Federal Republic of Germany, 1988.
GLP:	Yes
Material and methods:	
Test concentration:	125 g a.i./ha
Test system:	The study was conducted outdoors under field conditions in a plot (3 x 2 m) on a clay loam soil. The soil characteristics were: pH (7.3), sand (24.8%), silt (47.1%), clay (27%) and organic matter (2.26%). Difenoconazole, formulated as a 250 EC, was foliar sprayed to spring oilseed rape (variety *Golda*) using a small plot sprayer KFA with 4 TeeJet nozzles.
Stage of application:	78 days after sowing and 14 days after the first application.
No. of applications:	Two (14 days interval)
Sampling time points:	Plant samples were harvested after the first application, just prior to and immediately after the second application and at maturity 39 days after the second application. The mature plants were separated into straw, pods and seeds.
Method of analysis:	The TRR in each harvested sample was quantified by combustion/LSC. Samples of immature foliage, straw and pod were extracted sequentially with methanol/water (8:2 v/v) and methanol (Soxhlet). Samples of seed were extracted initially with hexane to separate the oil and then sequentially with the solvents described for the other plant fractions. Non-extracted residues greater than 30% TRR or greater than 0.1 mg/kg were further extracted with iso-propanol/water (8:2 v/v) with microwave assistance. Identification of the extracted residues was carried out by co-chromatography against standard reference compounds using 1D and 2D-TLC. Further characterisation of the residue was performed by enzyme de-conjugation using cellulase.

Date of experiment: 04 June 1992 to 13 May 1993.

Findings: Residue levels in rape plant fractions are shown in Table B.7.1.11-1.

Table B.7.1.11-1. Distribution of radioactive residues in oilseed rape plant fractions following two foliar applications of [phenyl-^{14}C] difenoconazole at a rate of 125 g a.i./ha.

Plant Commodity		Sampling Time	TRR (mg/kg)[a]	Extract E1 (%)[b]	Extract E2 (%)[b]	Non Extracted (%)[b]	Total (%)[b]
Foliage		Immature, Day 0	7.037	102	1.7	0.3	104
Foliage		Immature, Day 14[c]	1.51	88.4	4.8	10.1	103.2
Foliage		Immature, Day 14[d]	4.971	96.2	2.5	3.2	101.9
Straw		Mature, 39 day PHI	4.309	68.5	2.4	29.0	99.9
Pods		Mature, 39 day PHI	3.148	61.4	2.7	32.2	96.3
Seeds		Mature, 39 day PHI	0.151	60.7[f]	3.1	33.2	97.0
Processed fractions	Oil	Mature, 39 day PHI	0.102	--	--	--	--
	Meal	Mature, 39 day PHI	0.168	--	--	--	--

E1 = Extract 1, methanol/water (8:2) cold extract.
E2 = Extract 2, methanol Soxhlet hot extract.
[a] mg/kg determined by combustion.
[b] Percent of the TRR determined by combustion.
[c] pre second application (1h before).
[d] post second application (0.5h after).
[f] Sum of radioactivity in hexane extract (oil) and extract of remaining meal.

TRR in mature straw, pods and seeds were 4.309, 3.148 and 0.151 mg/kg, respectively. 18.1% (0.102 mg/kg) of the radioactivity in the seed was detected in the oil and 81.9% (0.168 mg/kg) in the meal. Parent residues were 0.745, 0.523 and 0.022 mg/kg in straw, pods and seeds, respectively (Table B.7.1.11-2).

In mature straw, 70.9% of the TRR was extractable with methanol/water (8:2) and methanol soxhlet. The extracted radioactivity consisted of parent difenoconazole (17.3% of the TRR, Table B.7.11-2) together with the metabolites CGA-205375 and CGA-189138 (13.3 and 0.7% of the TRR, respectively) and a substantial unextracted radioactive residues that comprised 29% of the TRR. This radioactivity was further extracted using microwave extraction and released 19% of the TRR. The majority of this radioactivity consisted of parent difenoconazole (9.7% of the TRR, Table B.7.1.11-2) and the metabolite CGA-205375 (2.6% of the TRR). Also a multi-component polar fraction was observed (21.7% of the TRR, 0.935 mg/kg) containing at least 9 components that individually did not exceed 2.6% of the TRR (0.112 mg/kg).

In mature pods, 61.4% of the TRR was extractable with methanol/water (8:2) and 2.7% with methanol soxhlet. The extracted radioactivity consisted of parent difenoconazole (16.6% of the TRR) together with the metabolites CGA-205375 and CGA-189138 (9.0% and 0.6% of the TRR, respectively). Also a substantial unextracted radioactivity was observed (32.2% of the TRR). This radioactivity was further extracted using microwave extraction and released 18.9% of the TRR. The majority of this radioactivity consisted of parent difenoconazole (9.2% of the TRR) and the metabolites CGA-205375 and CGA-189138 that accounted for 2.0 and 0.2% of the TRR, respectively. A substantial multi-component polar fraction was observed (24.6% of the TRR, 0.774 mg/kg) containing at least 7 components that individually did not exceed 2.6% of the TRR (0.082 mg/kg).

In seeds, 63.8% of the TRR was extractable with methanol/water (8:2) and methanol soxhlet and this radioactivity consisted of parent difenoconazole (14.4% of the TRR) in addition to the metabolites CGA-205375 (7.9% of the TRR) and CGA-189138 (0.3% of the TRR). Polar compounds accounted for 15% of the TRR.

Radioactivity in the meal from mature seeds consisted of unchanged difenoconazole (9.9% of the TRR). In addition, the metabolites CGA 205375 and CGA-189138 were also observed and accounted for 7.9 and 0.3% of the TRR, respectively.

Table B.7.1.11-2. Distribution and identification of [phenyl-^{14}C] difenoconazole residues in oilseed rape fractions following two applications at a rate of 125 g a.i./ha.

Plant part		Mature straw		Mature pods		Oil		Meal		Seeds	
TRR (mg/kg)[a]		4.309		3.148		0.102		0.168		0.151	
Compound		% TRR	mg/kg[a]	% TRR	mg/kg[a]	% TRR	mg/kg[a]	% TRR	mg/kg[a]	% TRR	mg/kg[a]
Difenoconazole		17.3	0.745	16.6	0.523	4.5	0.005	9.9	0.017	14.4[d]	0.022
CGA-205375		13.3	0.573	9.0	0.283	--	--	7.9	0.013	7.9[d]	0.012
CGA-189138		0.7	0.030	0.6	0.019	--	--	0.3	<0.001	0.3[d]	<0.001
Polar compounds		21.7[b]	0.935	24.6[c]	0.774	--	--	15.0	0.025	15.0[d]	0.023
Unresolved[f]		10.7	0.461	7.2	0.227	13.1	0.013	6.5	0.011	19.6[d]	0.030
Soxhlet extract		2.4	0.103	2.7	0.085	--	--	3.1	0.005	3.1[d]	0.005
Microwave extract		19.0	0.819	18.9	0.595	--	--	--	--	--	--
Non-extracted		10.0	0.431	10.0	0.315	--	--	33.2	0.056	33.2[d,e]	0.050
Total		95.1		89.6		17.6		75.9		93.5	
Further analysis of the micro wave extract	Difenoconazole	9.7	0.418	9.2	0.290	--	--	--	--	--	--
	CGA-205375	2.6	0.112	2.0	0.063	--	--	--	--	--	--
	CGA-189138	--	--	0.2	0.006	--	--	--	--	--	--
	Unresolved[f]	6.7	0.289	7.5	0.236	--	--	--	--	--	--

[a] mg/kg determined by combustion.
[b] Contains at least 9 components that individually did not exceed 2.6% of the TRR (0.112 mg/kg) plus unresolved radioactivity accounting for 9.4% of the TRR (0.405 mg/kg).
[c] Contains at least 7 components that individually did not exceed 2.6% of the TRR (0.086 mg/kg) plus unresolved radioactivity accounting for 11% of the TRR (0.346 mg/kg).
[d] Percentages shown are the sum of individual oil and meal analyses.
[e] Further analysis of the non-extractable by microwave was not performed.
[f] Radioactivity streaked or remaining diffuse on the TLC plate.

Conclusion: At harvest, 39 days after the second of two applications of [phenyl-^{14}C] difenoconazole at a rate of 125 g a.i./ha, the TRR in oilseed rape straw, pods and seed was 4.309, 3.148 and 0.151 mg/kg, respectively. Parent compound accounted for 14.4 to 17.3% of the TRR together with the metabolites CGA-205375 and CGA-189138 detected at levels of 7.9 to 13.3% and 0.3 to 0.7% of the TRR, respectively.

A substantial multi-component polar fraction was observed (15 to 24.6% of the TRR) containing individual fractions that individually did not exceed 2.6% of the TRR. Levels of non-extracted radioactivity in the mature straw, pod and seed samples accounted for between 10.0 and 33.2% of the TRR. Further analysis of the straw and pod samples showed the residue contained parent difenoconazole (9.2 to 9.7% of the TRR), CGA-205375 (2.0 to 2.6% of the TRR) and CGA-189138 (0.2% of the TRR). The remaining radioactivity was a multi-component. The presence of remaining unextracted residue indicate that the residue is tightly bound, probably the result of extensive incorporation of radioactivity into the plant matrix.

Based on the metabolites detected, the pathway of metabolism of difenoconazole in oilseed rape plants occurred via hydrolysis of the dioxolane ring to form the ketone (CGA-205374) which is then reduced to the alcohol (CGA-205375). CGA-205375 is then sugar conjugated or the alkyl bridge linkage is cleaved to form 1,2,4-triazole (CGA-71019) and free acid CGA-189138 (see Figure B.7.1.12-1).

B.7.1.12 Metabolism of [triazole-^{14}C] difenoconazole in field grown spring oilseed rape

Reference:	**Neuman C. (1993b)** Metabolism of [triazole-^{14}C] CGA-169374 in field grown spring rape. Metabolism, Divisional Unit R&D, Plant Protection Division, Ciba-Geigy Limited, Basel, Switzerland. Unpublished Report 12/93, SAM No. 0810.
Test Material:	[Triazole-^{14}C] difenoconazole, batch number JAK-V-80, radiochemical purity 97.9%, specific radioactivity 28.1 µCi/mg.
Guideline:	Control of Pesticide Regulations Data Requirement Handbook, appendix 6, Rothamsted, Harpenden, October 1992; Pesticide Assessment Guidelines, subdivision O, Residue Chemistry, Series 171-4 (a) (1) & (2), US Environmental Protection Agency, Washington, DC, October 1982; BBA Guidelines "Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln" Series IV 3.2, Biologische Bundesanstalt für Land- und Forst- wirtschaft, Federal Republic of Germany, 1988.
GLP:	Yes
Material and methods:	
Test concentration:	125 g a.i./ha
Test system:	The study was conducted outdoors under field conditions in a plot (3 x 2 m) on a clay loam soil. The soil characteristics were: pH (7.3), sand (24.8%), silt (47.1%), clay (27%) and organic matter (2.26%). Difenoconazole, formulated as a 250 EC, was foliar sprayed to spring oilseed rape (variety *Golda*) using a small plot sprayer KFA with 4 TeeJet nozzles.
Stage of application:	78 days after sowing and 14 days after the first application.
No. of applications:	Two (14 days interval)
Sampling time points:	Plant samples were harvested after the first application, just prior to and immediately after the second application and at maturity 39 days after the second application. The mature plants were separated into straw, pods and seeds.
Method of analysis:	The TRR in each harvested sample was quantified by combustion/LSC. Samples of immature foliage, straw and pod were extracted sequentially with methanol/water (8:2 v/v) and methanol (Soxhlet). Samples of seed were

extracted initially with hexane to separate the oil and then sequentially with the solvents described for the other plant fractions. Non-extracted residues greater than 30% TRR or greater than 0.1 mg/kg were further extracted with iso-propanol/water (8:2 v/v) with microwave assistance. Identification of the extracted residues was carried out by co-chromatography against standard reference compounds using 1D and 2D-TLC. Further characterisation of the residue was performed by enzyme de-conjugation using cellulase.

Date of experiment: 04 June 1992 to 13 May 1993.

Findings: Residue levels in oilseed rape plant fractions are shown in Table B.7.1.12-1.

Table B.7.1.12-1. Distribution of radioactive residues in oilseed rape plant fractions following two foliar applications of [triazole-^{14}C] difenoconazole at a rate of 125 g a.i./ha.

Plant Commodity		Sampling Time	TRR (mg/kg)[a]	Extract E1 (%)[b]	Extract E2 (%)[b]	Non Extracted (%)[b]	Total (%)[b]
Foliage		Immature, Day 0	5.247	99.5	2.0	0.2	101.7
Foliage		Immature, Day 14[c]	1.197	89.3	2.8	10.2	102.3
Foliage		Immature, Day 14[d]	4.781	94.2	2.4	2.4	99.0
Straw		Mature, 39 day PHI	4.841	72.6	2.6	26.6	101.8
Pods		Mature, 39 day PHI	4.707	73.1	2.3	27.5	102.9
Seeds		Mature, 39 day PHI	2.262	96.9[f]	0.8	8.9	106.6
Processed fractions	Oil	Mature, 39 day PHI	0.167	--	--	--	--
	Meal	Mature, 39 day PHI	2.452	--	--	--	--

E1 = Extract 1, methanol/water (8:2) cold extract.
E2 = Extract 2, methanol Soxhlet hot extract.
[a] mg/kg determined by combustion.
[b] Percent of the TRR determined by combustion.
[c] pre second application (1h before).
[d] post second application (0.5h after).
[f] Sum of radioactivity in hexane extract (oil) and extract of remaining meal.

TRR in mature straw, pods and seeds were 4.841, 4.707 and 2.262 mg/kg, respectively. 2.2% (0.167 mg/kg) of the radioactivity in the seed was detected in the oil and 97.8% (2.452 mg/kg) in the meal. Parent residues were 0.828, 0.645 and 0.093 mg/kg in straw, pods and seeds, respectively (Table B.7.1.12-2).

In mature straw, 75.2 % of the TRR was extractable with methanol/water (8:2) and methanol soxhlet. The extracted radioactivity consisted of parent difenoconazole (17.1% of the TRR, Table B.7.1.12-2) together with the metabolites CGA-205375, CGA-131013, CGA-142856 and CGA-71019 (14.3, 4.1, 3.3 and 1.6% of the TRR, respectively) and a substantial unextracted radioactive residues that comprised 26.6% of the TRR. This radioactivity was further extracted using microwave extraction and released 17.6% of the TRR. The majority of this radioactivity consisted of parent difenoconazole (9.2% of the TRR, Table B.7.1.12-2) and the metabolite CGA-205375 (2.5% of the TRR). Also a multi-component polar fraction was observed (19.6% of the TRR, 0.949 mg/kg) containing at least 6 components that individually did not exceed 2.9% of the TRR (0.140 mg/kg).

In mature pods, 73.1% of the TRR was extractable with methanol/water (8:2) and 2.3% with methanol soxhlet. The extracted radioactivity consisted of parent difenoconazole (13.7% of the TRR) together with the metabolites CGA-205375, CGA-131013 and CGA-142856 (9.0, 12 and 6.7% of the TRR, respectively).

Also a substantial unextracted radioactivity was observed (27.5% of the TRR). This radioactivity was further extracted using microwave extraction and released 19.6% of the TRR. This radioactivity consisted of parent difenoconazole (6.4% of the TRR) and the metabolite CGA-205375 (1.8% of the TRR). A substantial multi-component polar fraction was observed (21.4% of the TRR, 1.007 mg/kg) containing at least 5 components that individually did not exceed 2.8% of the TRR (0.132 mg/kg).

In seeds, 96.9% of the TRR was extractable with methanol/water (8:2) and the majority of this radioactivity consisted of the metabolite CGA-131013 (56.4% of the TRR) together with the metabolites CGA-205375 and CGA-142856 (0.6 and 2.8% of the TRR, respectively) and parent difenoconazole (4.1% of the TRR). Polar compounds accounted for 28.3% of the TRR containing 3 regions that accounted for 10.5, 6.7 and 11.1% of the TRR (0.238, 0.152 and 0.251 mg/kg, respectively).

Table B.7.1.12-2. Distribution and identification of [triazole-^{14}C] difenoconazole residues in oilseed rape fractions following two applications at a rate of 125 g a.i./ha.

Plant part		Mature straw		Mature pods		Oil		Meal		Seeds[e]	
TRR (mg/kg)[a]		4.841		4.707		0.167		2.452		2.262	
Compound		% TRR[b]	mg/kg[a]	% TRR[b]	mg/kg[a]	% TRR[b]	mg/kg[a]	% TRR[b]	mg/kg[a]	% TRR[b]	mg/kg[a]
Difenoconazole		17.1	0.828	13.7	0.645	1.9	0.003	2.3	0.056	4.1	0.093
CGA-205375		14.3	0.692	9.0	0.424	--	--	0.6	0.015	0.6	0.014
CGA-131013		4.1	0.198	12.0	0.565	--	--	56.4	1.383	56.4	1.276
CGA-142856		3.3	0.160	6.7	0.315	--	--	2.8	0.069	2.8	0.063
CGA-71019		1.6	0.077	--	--	--	--	--	--	--	--
Polar compounds		19.6[c]	0.949	21.4[d]	1.007	--	--	28.3[g]	0.694	28.3[g]	0.640
Unresolved[f]		6.5	0.315	8.3	0.391	0.4	<0.001	0.9	0.022	1.3	0.029
Soxhlet extract		2.6	0.126	2.3	0.108	--	--	0.8	0.020	0.8	0.018
Microwave extract		17.6	0.852	19.6	0.923	--	--	--	--	--	--
Non-extracted		7.3	0.353	7.4	0.348	--	--	8.9	0.218	8.9	0.201
Total		94.0		100.4		2.3		101.0		103.3	
Further analysis of the micro wave extract	Difenoconazole	9.2	0.445	6.4	0.301	--	--	--	--	--	--
	CGA-205375	2.5	0.121	1.8	0.085	--	--	--	--	--	--
	Unresolved[f]	5.9	0.286	--	--	--	--	--	--	--	--
	Polar compounds	--	--	9.3	0.438	--	--	--	--	--	--

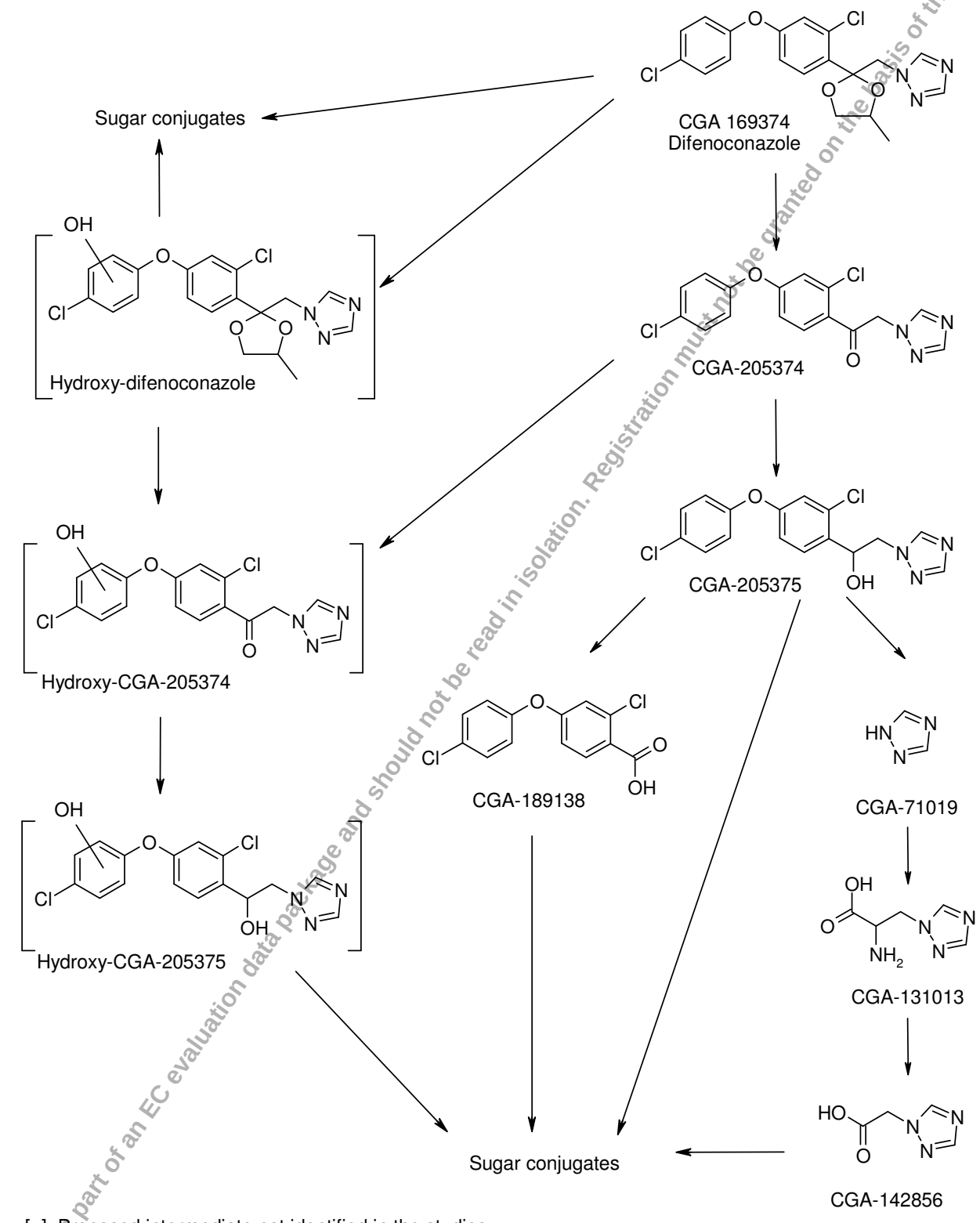
[a] mg/kg determined by combustion.
[b] Percent of the TRR determined by combustion.
[c] Contains at least 6 components that individually did not exceed 2.9% of the TRR (0.140 mg/kg) plus unresolved radioactivity accounting for 7.3% of the TRR (0.353 mg/kg).
[d] Contains at least 5 components that individually did not exceed 2.8% of the TRR (0.132 mg/kg) plus unresolved radioactivity accounting for 11% of the TRR (0.518 mg/kg).
[e] Percentages shown are the sum of individual oil and meal analyses.
[f] Radioactivity streaked or remaining diffuse on the TLC plate.
[g] Contains 3 regions that account for 10.5% of the TRR (0.238 mg/kg), 6.7% of the TRR (0.152 mg/kg) and 11.1% of the TRR (0.251 mg/kg). The regions exceeding 10% contain radioactivity at the origin of the TLC plate and unresolved material and as such are likely to be multi-component in nature.

Conclusion: At harvest, 39 days after the second of two applications of [triazole-^{14}C] difenoconazole at a rate of 125 g a.i./ha, the TRR in oilseed rape straw, pods and seed was 4.841, 4.707 and 2.262 mg/kg, respectively. Parent compound accounted for 4.1 to 17.1% of the TRR together with the metabolites CGA-205375, CGA-131013 and CGA-142856 detected at levels of 0.6 to 14.3%, 4.1 to 56.4% and 2.8 to 6.7% of the TRR, respectively. The metabolite CGA-71019 was detected in straw only at a level of 1.6% of the TRR (0.077 mg/kg).

A substantial multi-component polar fraction was observed (19 to 28.3% of the TRR) containing individual fractions that individually did not exceed 2.9% of the TRR in the straw and pods and 11.1% in the seeds. Levels of non-extracted radioactivity in the mature straw, pod and seed samples accounted for between 8.9 and 27.5% of the TRR. Further analysis of the straw and pod samples showed the residue contained parent difenoconazole (6.4 to 9.2% of the TRR) and CGA-205375 (1.8 to 2.5% of the TRR). The remaining non-extracted radioactivity was a multi-component. The presence of remaining unextracted residue indicate that the residue is tightly bound, probably the result of extensive incorporation of radioactivity into the plant matrix.

Based on the metabolites detected, the pathway of metabolism of difenoconazole in oilseed rape plants occurred via hydrolysis of the dioxolane ring to form the ketone (CGA-205374) which is then reduced to the alcohol (CGA-205375). CGA-205375 can then be sugar conjugated or the alkyl bridge linkage is cleaved to form 1,2,4-triazole (CGA-71019), which in turn can be conjugated with serine to form triazole alanine (CGA-131013). CGA 131013 is further metabolised by oxidative de-amination to triazole lactic acid CGA-205369 and then degraded to the acid CGA-142856 (Figure B.7.1.12-1).

Comments by RMS: The studies were well performed and reported. The application rate in both studies is in accordance with the current GAP that prevails in France and in the UK but lower than in Germany (250 g a.i./ha). However, oilseed rape is not a representative use.



[ ] Proposed intermediate not identified in the studies.

Figure B.7.1.12-1. Proposed metabolic pathway of difenoconazole in field grown spring oilseed rape plant following foliar treatment.

B.7.1.13 Metabolism, distribution and expression of residue in plants - summary and conclusions

The plant metabolism of difenoconazole was carried out in four crops, representing four crop groupings – cereals (wheat), root vegetables (potato), pulses/oilseeds (oilseed rape) and fruits (grape and tomato). The application methods were seed and foliar treatment for wheat and foliar treatment for potato, grape, tomato and oilseed rape. The representative uses for difenoconazole in the Southern and Northern Europe are on cereals (seed treatment), pome fruit (foliar treatment) and carrots (foliar treatment).

Two studies in wheat were conducted under two different conditions, i.e. greenhouse and in field plots. However, no difference was observed in the results. The studies were performed using two radiolabelled forms of difenoconazole. [Phenyl-^{14}C] or [triazole-^{14}C] difenoconazole were applied once as a seed treatment. The application rate in both studies was four times higher than the proposed critical GAP of 6 g a.i./100 kg seed. At maturity, low levels of TRR were found in [phenyl^{14}C] difenoconazole treated grain and straw (0.003 to 0.004 and 0.013 to 0.016 mg/kg, respectively). This radioactivity was not analysed further. Residues in mature grain and straw from the triazole-^{14}C labelled difenoconazole treated seed were 0.024 to 0.183 and 0.011 to 0.069 mg/kg, respectively. The majority of the radioactivity in straw and grain was aqueous soluble (72 to 90% of the TRR, respectively) and was tentatively identified as 1,2,4-triazole (CGA-71019), triazole acetic acid (CGA-142856) or triazole alanine (CGA-131013). The results demonstrate a preferential translocation of triazole related residues to the grains.

In one study in wheat, conducted indoors under greenhouse condition, following four foliar applications of phenyl-^{14}C or triazole-^{14}C labelled difenoconazole, the distribution of the radioactivity between organic and aqueous soluble fractions was similar in mature straw but not in grain. The TRR in the phenyl-^{14}C labelled difenoconazole treated grain was presented as an unextracted residue (81.5% of the TRR, 0.064 mg/kg) while in the corresponding [triazole-^{14}C] difenoconazole labelled treated grain, 69.5% of the TRR (1.05 mg/kg) was present as aqueous soluble radioactivity. This radioactivity consisted of 1,2,4-triazole (CGA-17019, 10% of the TRR, 0.14 mg/kg) and triazole acetic acid (CGA-142856, 20% of the TRR, 0.28 mg/kg) while in the phenyl-^{14}C labelled difenoconazole treated grain, the presence of conjugates accounted for *ca* 35% of the TRR with CGA-189138 or CGA-189138 metabolites.

In two studies in potato (representing root vegetables), at maturity, following six applications of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole each at a rate of 123.5 g a.i./ha, the TRR in the potato tubers was 0.012 and 0.087 mg/kg, respectively. Mature tubers from plants treated with [phenyl-^{14}C] difenoconazole contained parent difenoconazole (8.7%) together with the metabolites CGA-205375 (3.0%) and CGA-205374 (3.1%). An additional 15.4% of the TRR was released by cellulase treatment as sugar conjugated CGA-205375. The corresponding [triazole-^{14}C] difenoconazole treated mature tubers contained predominantly the metabolite triazole alanine, CGA-131013 (78.9% of the TRR, 0.068 mg/kg), demonstrating preferential translocation of triazole related residues to the tubers. The application rate in both studies was in accordance with the proposed GAP for carrots in Southern and Northern Europe (125 g a.i./ha). However, the pre-harvest intervals were shorter (11 days) than the minimum PHI recommended in

both European regions (14 days) and the interval between applications was 7 days, which was shorter than the minimum of 14 days.

In a study in grapes, at maturity, 20 days after the fifth of five applications of either [phenyl-^{14}C] or [triazole-^{14}C] difenoconazole at 247 g a.i./ha, which is 3 to 4 times higher than the proposed critical GAP for pome fruit in Southern and Northern Europe, the TRR in fruit was 0.047 to 0.127 and 0.115 mg/kg, respectively. The PHI was longer than the minimum recommended in Southern (14 days) and shorter than the minimum recommended in Northern Europe (28 days). In the fruit, parent compound was the largest component of the residue (up to 43.5% of the TRR, 0.055 mg/kg). The metabolites CGA-205374 and CGA-205375 were detected in fruit samples at levels between 1.7 to 3.8% and 3.5 to 6.4% of the TRR, respectively. Small amounts of the metabolites CGA-71019 (3.6% of the TRR) and CGA-189138 (4% of the TRR) were also detected, leading to residues less than 0.01 mg/kg. Unidentified metabolites were observed in fruit samples, but none individually exceeded 10% of the TRR. The unextracted radioactivity accounted for up to 36% of the TRR in fruit. A small proportion of these residues were identified as sugar conjugates of either parent compound or the metabolites CGA-205374, CGA 205375, CGA-186138 (phenyl-^{14}C only) and CGA-71019 (triazole-^{14}C only).

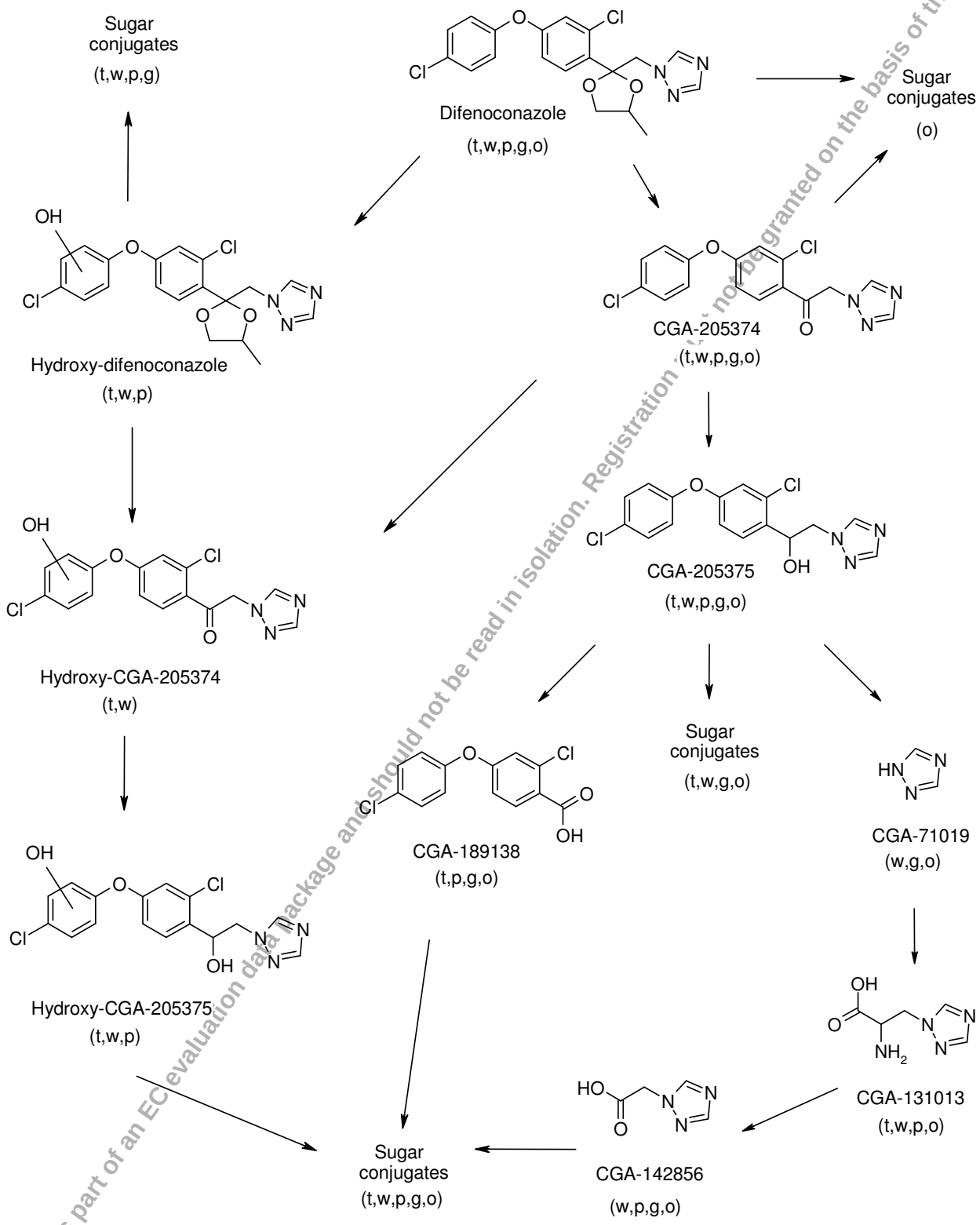
In four studies in tomato, using 3 to 6 foliar applications of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole at a rates of 123 to 247 g a.i./ha, the TRR in mature fruit was 0.037 to 0.170 mg/kg and 122 to 0.267 mg/kg, respectively. Parent difenoconazole was the major residue in fruit accounted for up to 66.3% of the TRR. The major metabolite was CGA-131013 (up to 42.4% of the TRR). The alcohol and ketone metabolites CGA-205375 and CGA-205374 were also identified but at lower levels (<2% of the TRR). The foliar application rates used in the tomato studies were higher than the proposed critical GAP for pome fruit in Southern and Northern Europe (75 and 56.2 g a.i./ha, respectively). The intervals between applications were 7 or 14 days, which were shorter or longer than the minimum of 10 days and the PHI in three of the four available studies were longer (34 or 40 days) than the minimum recommended in Southern and Northern Europe (14 and 28 days, respectively).

In two studies in oilseed rape, [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole were applied on two occasions as a foliar spray at 125 g a.i./ha. The TRR in mature straw and pods contained TRR levels between 3.148 and 4.841mg/kg, whilst TRR levels in the seed were 0.151 and 2.262 mg/kg, respectively. Parent compound accounted for 4.1 to 17.3% of the TRR in each commodity. The metabolites CGA-205375 and CGA-189138 were detected in the [phenyl-^{14}C] treated seed at levels of 7.9 to 0.3% of the TRR, respectively. The metabolites CGA-205375, CGA-131013 and CGA-142856 were detected in the [triazole-^{14}C] treated seed at levels of 0.6, 56.4 and 2.8% of the TRR, respectively. Up to 28.3% of the TRR was present as polar compounds that were multi-component.

The primary metabolic process in all four crop types involved:
-hydrolysis of the dioxolane ring to form the ketone CGA-205374, which is then reduced to the corresponding alcohol CGA-205375.
-oxidation of CGA-205375 occurred resulting in cleavage of the alkyl bridge to form CGA- 189138 and CGA-71019.
-hydroxylation of parent compound and the metabolites CGA-205374 and CGA-205375.

Sugar conjugation of parent compound and hydroxylated metabolites, and conjugation of 1,2,4-triazole was observed as a secondary metabolism process. Conjugation of 1,2,4-triazole resulted in the formation of triazole alanine (CGA-131013), which was further degraded to triazole acetic acid (CGA-142856).

The proposed metabolism of difenoconazole in plants is shown in Figure B.7.1.13-1. A list of the identified compounds in the four crop types is presented in Table B.7.1.13-1 and in Appendix I.



[ ] Proposed intermediate, not observed in studies.

t = tomato, w = wheat, p = potato, g = grape, o = oilseed rape

Figure B.7.1.13-1. Proposed metabolic pathway of difenoconazole in plants.

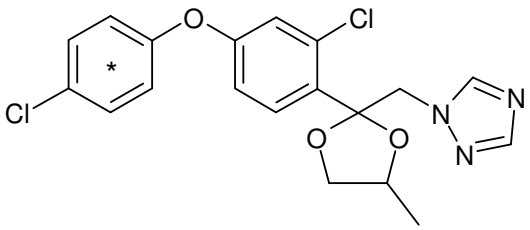
Table B.7.1.13-1. List of identified compounds found in mature wheat, potato, tomato, grape and oilseed rape following foliar applications of [phenyl-^{14}C][1] or [triazole-^{14}C][2] difenoconazole.

Designation	Chemical Name (IUPAC)	Presence in wheat mg/kg[a] (%TRR)[b]		Presence in potato tubers mg/kg[a] (%TRR)[b]	Presence in oilseed rape mg/kg[a] (%TRR)[b]			Presence in tomato fruit mg/kg[a] (%TRR)[b]	Presence in grape mg/kg[a] (%TRR)[b]
		Straw	Grain		Straw	Pods	Seeds[d]		
Difenoconazole CGA-169374	1-[2-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-4-methyl-[1,3]dioxolan-2-ylmethyl]-1H-[1,2,4]triazole or 1H-1,2,4-tiazole, 1-[[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]- (CA)	23.4[1] (50)	--[1]	0.0010[1] (8.7)	0.745[1] (17.3)	0.523[1] (16.6)	0.022[1] (14.4)	0.110[1] (66.3)	0.055[1] (43.5)
		26.9[2] (50)	--[2]	0.0016[2] (1.8)	0.828[2] (17.1)	0.645[2] (13.7)	0.093[2] (4.1)	0.103[2] (50.9)	0.030[2] (25.9)
CGA-205375	1-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-2-[1,2,4]triazol-1-yl-ethanol	--[1]	--[1]	0.0004[1] (3.0)	0.573[1] (13.3)	0.283[1] (9.0)	0.012[1] (7.9)	0.003[1] (1.7)	0.008[1] (6.4)
		2.69[2] (5)	--[2]	--[2]	0.692[2] (14.3)	0.424[2] (9)	0.014[2] (0.6)	0.002[2] (0.7)	0.004[2] (3.5)
CGA-205374	1-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-2-[1,2,4]triazol-1-yl-ethanone	--[1]	--[1]	0.0004[1] (3.1)	--[1]	--[1]	--[1]	0.002[1] (1.4)	0.005[1] (3.8)
		(1.6)[2]	--[2]	0.0001[2] (0.1)	--[2]	--[2]	--[2]	0.001[2] (0.5)	0.002[2] (1.7)
CGA-189138	2-chloro-4-(4-chloro-phenoxy)-benzoic acid	--[1]	--[1]	--[1]	0.030[1] (0.7)	0.019[1] (0.6)	<0.001[1] (0.3)	0.08[1, e] (0.9)	0.005[1] (4)
		--[2]	--[2]	--[2]	--[2]	--[2]	--[2]	--[2]	0.005[2] (4)
CGA-71019	1H-[1,2,4]triazole	--[1]	--[1]	--[1]	--[1]	--[1]	--[1]	--[1]	--[1]
		(5.2)[2]	0.14[2*] (10)	--[2]	0.077[2] (1.6)	--[2]	--[2]	--[2]	0.004[2] (3.6)
CGA-131013	2-amino-3-[1,2,4]triazol-1-yl-propionic acid	--[1]	--[1]	--[1]	--[1]	--[1]	--[1]	--[1]	--[1]
		(1.4)[2]	--[2]	0.0686[2, c] (78.9)	0.198[2] (4.1)	0.565[2] (12)	1.276[2] (56.4)	0.039[2] (19.3)	--[2]
CGA-142856	[1,2,4]triazol-1-yl-acetic acid	--[1]	--[1]	--[1]	--[1]	--[1]	--[1]	--[1]	--[1]
		(1.1)[2]	0.28[2*] (20)	--[2]	0.160[2] (3.3)	0.315[2] (6.7)	0.063[2] (2.8)	--[2]	--[2]
OH-difenoconazole		--[1]	--[1]	--[1]	--[1]	--[1]	--[1]	See study B.7.1.3 and B.7.1.4	--[1]
		0.54[2] (1)	--[2]	--[2]					
OH-CGA-205375		--[1]	--[1]	--[1]	--[1]	--[1]	--[1]	"	--[1]
		0.54[2] (1)	--[2]	--[2]					
Conjugated CGA-205375				0.0018[1] (15.4)				"	<0.001[1] (0.2)
				--[2]					See B.7.1.10

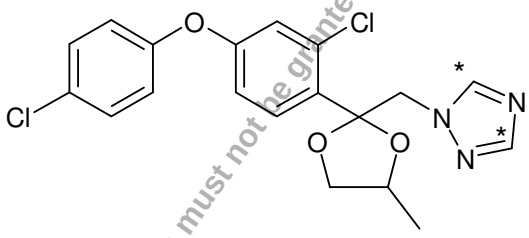
[a] mg/kg difenoconazole equivalents.
[b] Percent of the total radioactive residues.
[c] Radioactivity may also contain CGA-142856 at a level of 16.1% of the TRR (0.014 mg/kg). Unidentified component contains at least 3 individual radioactive regions, individually not exceeding 4.6% of the TRR (0.004 mg/kg).
[d] Sum of individual oil and meal analyses.
[e] was detected in the final harvested foliage.
[*] following four foliar applications of [triazole-^{14}C] difenoconazole at a rate of 247 g a.i./ha (B.7.1.7).

B.7.2 Metabolism, distribution and expression of residues in livestock (Annex IIA 6.2 and Annex III 8.1)

The metabolism and distribution of difenoconazole has been investigated in the lactating goat and laying hens. The studies were performed using two radiolabelled forms of difenoconazole (Figure B.7.2-1).



[Phenyl-^{14}C] difenoconazole



[Triazole-^{14}C] difenoconazole

Figure B.7.2-1. Structure of difenoconazole and position of radiolabels (*).

B.7.2.1. Metabolism of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole in lactating goat

Reference:	**Madrid S. O. (1988)** Metabolism of triazole and phenyl-^{14}C-CGA-169374 in lactating goats dosed daily for ten consecutive days. Biochemistry Department, Agricultural Division, Ciba-Geigy Corporation, Greensboro, North Carolina, United States. Unpublished Report ABR-88087, SAM No. 0234.
Test Material:	Phenyl-^{14}C difenoconazole, batch number GAN-IX-5, radiochemical purity 97%, specific radioactivity 48.6 µCi/mg. Triazole-^{14}C difenoconazole, batch number GAN-IX-7, radiochemical purity 98%, specific radioactivity 48.5 µCi/mg.
Guideline:	Pesticide Assessment Guidelines, Subdivision O, Residue Chemistry, Series 171-4 (b), United States Environmental Protection Agency, Washington D.C., Nature of the Residue - Livestock.
GLP:	No. Conducted prior to the adoption of GLP standards.

Material and methods:

Test concentration:	5 mg/kg feed/day
Test system:	Two lactating goats (31.5 and 32 kg) were individually housed in metabolism cages designed for the separate collection of urine and faeces. The goats were conditioned in the cages 7 days prior to the first radioactive dose. The lactating goats were dosed daily by gelatine capsule with 7.5 mg/capsule/day for ten consecutive days with phenyl-^{14}C or triazole-^{14}C difenoconazole.
Duration:	10 days
Sampling time points:	Urine, faeces and milk were collected daily. Blood was sampled on days 1, 2, 4, 6, 8, 9 and 10. The goats were sacrificed 22 hours and 23 hours after the last dose of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole, respectively. At sacrifice, samples of liver, kidney, tenderloin muscle, omental fat, perirenal fat and eyes were taken. Appropriate

background samples were taken from a control goat.

Method of analysis: TRR in urine and milk was determined directly by LSC. TRR in faeces and tissues was determined by combustion/LSC. Faeces, liver and kidney samples were extracted using Bligh-Dyer/Ting-Dugger methods, which yielded organic, aqueous and non-extractable fractions. Radioactive residues in milk were extracted with acetonitrile and fractioned into fat, whey and casein. Urine was partially purified using XAD-4 resin, then partitioned with dichloromethane at pH 9 and pH 2. Extracts were analysed by 1D-TLC and 2D-TLC.

Number of animals: Two

Date of experiment: 10 June 1986 to 02 February 1987

Findings: Based on feed consumption, the dietary concentrations were 4.17 mg/kg per day and 5.56 mg/kg per day for the phenyl and triazole dosed goats, respectively.

The extractability of the residues in tissues is shown in Table B.7.2.1-1.

Table B.7.2.1-1. Extractability of residues in tissues of lactating goat dosed daily for 10 days with [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole.

Labelled form	Sample	TRR (mg/kg)[a]	Organic phase (%)[b]	Aqueous phase (%)[b]	Non-extracted (%)[b]	Total (%)[b]
[phenyl-^{14}C] difenoconazole	Liver	0.259	52.2	28.6	30.5	111.4
[triazole-^{14}C] difenoconazole	Liver	0.277	51.2	37.5	15.3	104
	Kidney	0.094	24.7	54.4	19.2	98.3

[a] mg/kg difenoconazole equivalents
[b] Percent of total radioactive residues.

Extractability of the radioactive residues from liver and kidney was between 79 and 89% of the TRR.

Following dosing of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole, the overall recovery of the dosed radioactivity was 88.7% and 107%, respectively. The majority of the administered dose was found in the excreta (88% for phenyl and 105% for triazole label). Totals of 0.08% and 0.18% were recovered in the milk and blood (sampled at day 10), respectively. Similar results were observed for the triazole label, 0.13% and 0.50%, respectively. Low levels of administered radioactivity were retained in the tissues (0.4% to 0.9%; 0.385 and 0.524 mg/kg), of which 0.1% to 0.5% was recovered in muscle, 0.27% in liver, 0.04% to 0.11% in fat and 0.009% to 0.012% in kidney.

The highest residue levels were found in liver (0.259 to 0.277 mg difenoconazole equivalents/kg) and kidney (0.064 to 0.094 mg difenoconazole equivalents/kg, Table B.7.2.1-2). Levels in the other edible tissues were lower (<0.1mg difenoconazole equivalents/kg).

The recovery of the radioactive dose in the tissues, milk, blood, urine and faeces is given in Table B.7.2-1-2.

Table B.7.2.1-2. Recovery of administered dose in lactating goat.

Sample	Mean % of dose	Mean residues (mg/kg)	Mean % of dose	Mean residues (mg/kg)
	[phenyl-^{14}C] difenoconazole		[triazole-^{14}C] difenoconazole	
Urine	20.78	--	30.88	--
Faeces	67.24	--	74.60	--
Milk	0.18	--	0.50	--
Blood (Day 10)	0.08	0.025	0.13	0.043
Total tissues	0.442	--	0.897	--
Tenderloin muscle	0.002	0.008	0.006	0.026
Leg muscle	0.114	0.007	0.486	0.028
Liver	0.272	0.259	0.275	0.277
Kidney	0.009	0.064	0.012	0.094
Omental fat	0.042	0.025	0.112	0.064
Perirenal fat	0.003	0.022	0.006	0.035
Eyes	<0.001	0.009	<0.001	0.042
Total	88.72		107.01	

Following dosing of [phenyl-^{14}C] difenoconazole, radioactive residues in milk reached a plateau of between 0.006 and 0.008 mg/kg after 2 days dosing (Figure B.7.2.1-2). Following dosing of [triazole-^{14}C] difenoconazole, radioactive residues in milk reached a plateau of 0.043 mg/kg after 6 days dosing (Figure B.7.2.1-3). In total a very small proportion of dosed radioactivity was secreted into milk during the dosing period (0.18% and 0.50%, respectively, Table B.7.2.1-2). The residues measured in milk during the treatment period are shown in Table B.7.2.1-3.

Table B.7.2.1-3. Total radioactive residues in milk.

Collection Period	[phenyl-^{14}C] difenoconazole		[triazole-^{14}C] difenoconazole	
	% of dose	mg/kg[a]	% of dose	mg/kg[a]
Day 1	0.014	0.005	0.022	0.013
Day 2	0.019	0.007	0.037	0.022
Day 3	0.017	0.006	0.050	0.030
Day 4	0.016	0.006	0.053	0.032
Day 5	0.020	0.008	0.051	0.037
Day 6	0.018	0.007	0.055	0.043
Day 7	0.017	0.007	0.054	0.043
Day 8	0.019	0.008	0.064	0.043
Day 9	0.019	0.007	0.060	0.041
Day 10	0.018	0.007	0.055	0.033
Total	0.177		0.501	

[a] mg/kg difenoconazole equivalents.

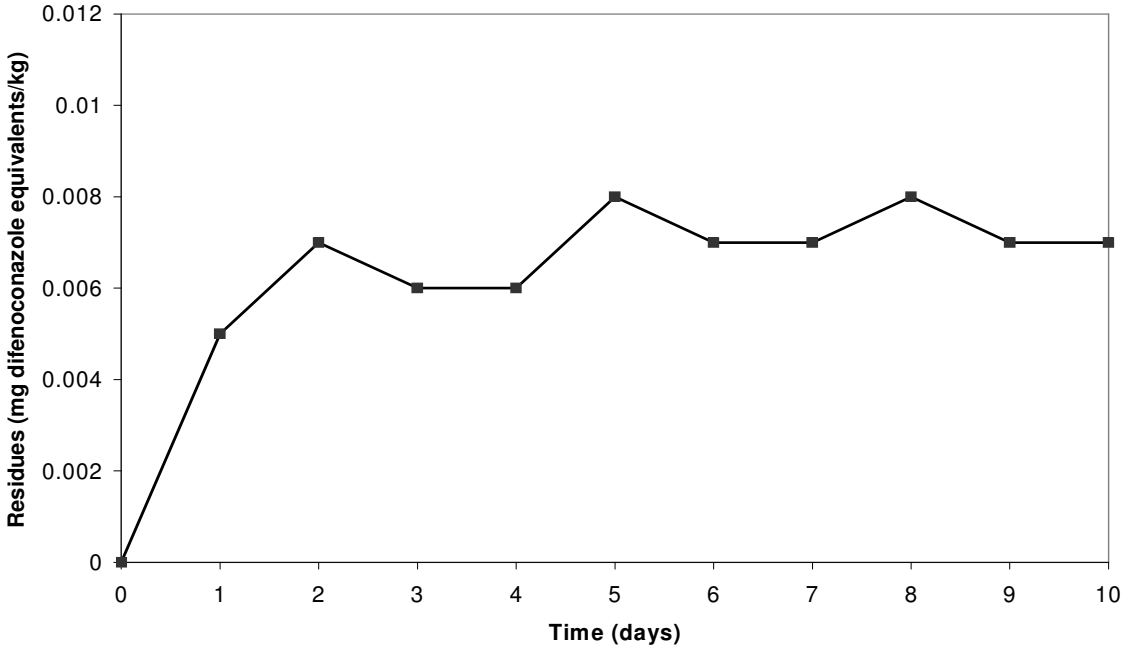


Figure B.7.2.1-2. Residues in milk following [phenyl-^{14}C] difenoconazole administration.

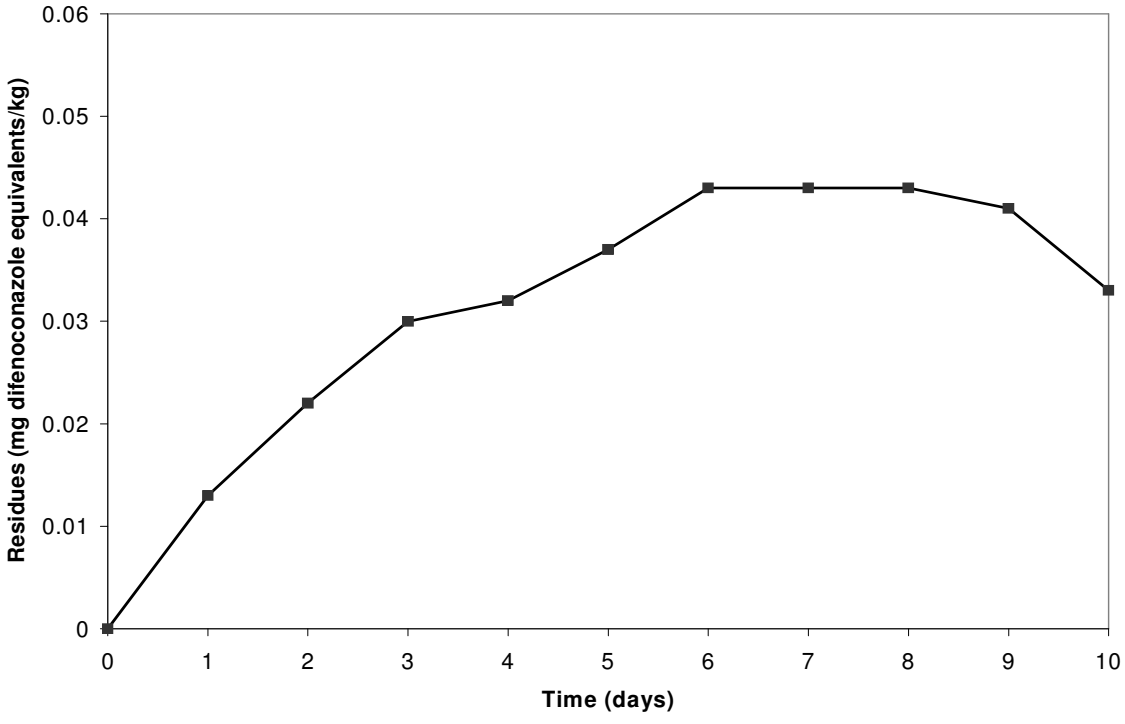


Figure B.7.2.1-3. Residues in milk following [triazole-^{14}C] difenoconazole administration.

Fractionation of milk resulted in 32.1, 42.2 and 12.4% of the radioactivity distributed between fat, whey and casein, respectively, for the [phenyl-^{14}C] difenoconazole treatment. The distribution for the [triazole-^{14}C] difenoconazole treated milk was 18.7, 47.0 and 21.6% of the radioactivity in the fat, whey and casein, respectively. Differences between the amount of radioactivity in the fat and casein for the two radiolabelled positions provided evidence of alkyl bridge cleavage of difenoconazole.

Results from the fractionation of milk samples are summarised in Table B.7.2.1-4.

Table B.7.2.1-4. Fractionation of radioactive residues in milk.

Fraction	[phenyl-^{14}C] difenoconazole	[triazole-^{14}C] difenoconazole
	Day 8 (0.008 mg/kg)	Day 7 (0.043 mg/kg)
	% of the TRR	% of the TRR
Fat	32.1	18.7
Whey	42.2	47.0
Casein	12.4	21.6
Total	86.6	87.3

Parent difenoconazole was detected in liver at levels of *ca* 1.0% of the TRR for both radiolabelled treatment (Table B.7.2.1-5), being CGA-205375 the major metabolite, accounting for 57.7% and 56.8% of the TRR for the [phenyl-^{14}C] and [triazole-^{14}C] liver samples, respectively. The metabolite CGA-71019 was detected in the [triazole-^{14}C] treated liver at levels of 3.2% TRR, while the metabolites CGA-189138 and CGA-205374 were detected at lower levels (<2% of the TRR) in the [phenyl-^{14}C] treated liver sample. Other metabolites occurred at levels between 0.6 to 11.1% of the TRR in both radiolabelled and were not further identified .

Residue levels of difenoconazole were between 0.002 and 0.003 mg/kg in liver from each radiolabelled treatment. Residues of the metabolites CGA-205375, CGA-205374, CGA-189138 and CGA-71019 in the liver were at levels of 0.149 to 0.157, 0.002, 0.004 and 0.009 mg/kg, respectively. Residues of the metabolites CGA-205375 and CGA-71019 in milk were 0.001 and 0.020 mg/kg, respectively. Other metabolites observed in milk and liver did not exceed 0.05 mg/kg.

Parent difenoconazole was not found in the urine samples, but was detected in the faeces at levels of 27.1 and 20.4% TRR for the [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole treated samples, respectively. The metabolite CGA-205375 was the major degradation product observed in both urine and faeces accounting for 18.3 and 28.2%, respectively in the [phenyl-^{14}C] difenoconazole treated sample and 16.0 and 34.8%, respectively in the [triazole-^{14}C] difenoconazole treated sample. The metabolite CGA-71019 was found in the urine and faeces from the goat dosed with [triazole-^{14}C] difenoconazole while the metabolites CGA-205374 and CGA-189138 were found only in faeces from the goat dosed with [phenyl-^{14}C] difenoconazole. All three detected metabolites did not exceed 5.7% of the total radioactivity. Unidentified metabolites were present in the excreta from both radiolabelled treatments, with at least five compounds detected in the urine at levels up to 25.8% of the total radioactivity and at least four compounds detected in the faeces at levels up to 18.0% of the total radioactivity.

Distribution of metabolites and residue levels is summarised in Table B.7.2.1-5.

Table B.7.2.1-5. Distribution of radioactive metabolites in liver, milk, urine and faeces of lactating goat dosed daily for 10 days with [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole.

Labelled form	Compounds detected	Liver 0.259 mg/kg		Milk		Urine[b]	Faeces[c]
		%	mg/kg[a]	%	mg/kg[a]	%	%
[phenyl-^{14}C] difenoconazole	Difenoconazole	1.2	0.003			--	27.1
	CGA-205375	57.7	0.149			18.3	28.2
	CGA-205374	0.9	0.002			--	2.0
	CGA-189138	1.5	0.004			--	1.1
	Metabolite B	1.6	0.004			2.0	9.6
	Metabolite B_1	--	--			2.6	--
	Metabolite C	2.0	0.005			25.1	0.6
	Metabolite D	0.6	0.002			15.9	1.5
	Metabolite G	4.9	0.013			14.5	--
	Other metabolites	10.5	0.027			21.6	18.0
	Non-extracted	30.6	0.079			--	12.0
	Total	111.5		n.a.		100	100.1
[triazole-^{14}C] difenoconazole	TRR	0.277 mg/kg		(day 7) 0.043 mg/kg		Urine[d] %	Faeces[e] %
		%	mg/kg[a]	%	mg/kg[a]		
	Difenoconazole	0.9	0.002	--	--	--	20.4
	CGA-205375	56.8	0.157	3.3	0.001	16.0	34.8
	CGA-205374	n.d.	n.d.	--	--	--	5.7
	CGA-71019	3.2	0.009	45.8	0.020	5.3	2.0
	Metabolite B	1.7	0.005	--	--	1.9	8.7
	Metabolite C	1.1	0.003	--	--	25.8	0.6
	Metabolite D	0.7	0.002	2.9	0.001	15.0	1.6
	Metabolite G	11.1	0.031	--	--	21.5	--
	Other metabolites	8.8	0.024	18.2	0.008	14.5	14.8
	Non-extracted	15.3	0.042	--	--	--	12.0
	Total	99.6		70.2		100	100.6

Results shown are the sum of compounds detected in organic and aqueous extracts and are expressed as a percent of the TRR or total radioactivity where appropriate.
[a] mg/kg difenoconazole equivalents.
[b] Urine sample from day 5 analysed, containing 2.6% of the dose.
[c] Faeces sample from day 5 analysed, containing 6.9% of the dose.
[d] Urine sample from day 6 analysed, containing 2.9% of the dose.
[e] Faeces sample from day 5 analysed, containing 9.2% of the dose.
n.a.= Not analysed; n.d.= Not detected.

Conclusions: Difenoconazole is extensively metabolised and rapidly excreted in goat. Following oral dosing for ten consecutive days with either [phenyl-^{14}C] or [triazole-^{14}C] difenoconazole at levels in the diet equivalent to 5 mg/kg, the majority of the administered dose was found in urine and faeces (>88%). Only small amounts of the administered dose were found in milk (0.18 to 0.5%) and edible tissues (0.4 to 0.9%) demonstrating that difenoconazole and its metabolites do not bio-accumulate and are rapidly excreted.

The highest total radioactive residue in any of the tissues was 0.257 and 0.277 mg/kg in the liver and 0.064 and 0.094 mg/kg in the kidney samples treated with [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole, respectively. In milk, the highest TRR was 0.043 mg/kg detected in the triazole labelled milk sample.

Parent difenoconazole was detected in the liver at a concentration of 0.003 mg/kg in both radiolabelled forms. The major metabolite found was CGA-205375, which occurred at levels of 56.8 to 57.7% of the TRR (0.149 to 0.157 mg/kg) in the liver. Minor amount of the metabolites CGA-205374 (0.9% TRR, 0.002 mg/kg; phenyl only), CGA-189138 (1.5% TRR, 0.004 mg/kg, phenyl only) and CGA-71019 (3.2% TRR, 0.009 mg/kg, triazole only) were also observed, with the presence of 1,2,4-triazole (CGA-71019)

indicating cleavage of the triazole ring. Other metabolites which were not identified also occurred at low levels, less than 12% of the TRR (Metabolite G, maximum 0.031 mg/kg in liver). In milk, parent difenoconazole was not detected. The major metabolite was CGA-71019 accounting for 45.8% of the TRR (0.020 mg/kg).

Following administration of difenoconazole, the proposed metabolic pathway of difenoconazole involves hydrolysis of the dioxolane ring to form the ketone CGA-205374, with subsequent reduction of CGA-205374 to give the corresponding alcohol CGA-205375 as the major metabolite (56.8 and 57.7% of the TRR). Oxidation of CGA-205374 results in cleavage of the alkyl bridge, leading to the formation the acid CGA-186138 and 1,2,4-triazole CGA-71019.

Comments by RMS: The study was well performed and evaluated.

B.7.2.2 Metabolism of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole in lactating goat

Reference:	**Maynard, M.S. (1990a)** [^{14}C]-CGA-169374 Phenyl and triazole label distribution, elimination, and metabolism in goats. Metabolism Department, Agricultural Division, Ciba-Geigy Corporation, Greensboro, North Carolina, United States. Unpublished Report ABR-89100, SAM No. 0379.
Test Material:	Phenyl-^{14}C difenoconazole, batch number JAK-III-7A, radiochemical purity 97.6%, specific radioactivity 14.0 µCi/mg. Triazole-^{14}C difenoconazole, batch number JAK-III-6, radiochemical purity 98.2%, specific radioactivity 13.9 µCi/mg.
Guideline:	Pesticide Assessment Guidelines, Subdivision O, Residue Chemistry, Series 171-4 (b), United States Environmental Protection Agency, Washington D.C., Nature of the Residue - Livestock.
GLP:	Yes (In part). Some of the metabolite characterisation and identification phase of the study was conducted prior to adoption of GLP guidelines. The majority of the practical work and the report were completed in accordance with GLP standards.

Material and methods:

Test concentration:	100 mg/kg feed/day
Test system:	Four lactating goats (two per radiolabelled treatment) were individually housed in metabolism cages designed for the separate collection of urine and faeces. The goats were conditioned in the cages 2 days prior the first radioactive dose. The goats were dosed daily by gelatine capsule containing 150 mg/capsule per day which represents a feeding level of 100 mg/kg.
Duration:	3 days
Sampling time points:	Urine and faeces were collected daily. Milk was collected twice a day in the morning and in the afternoon. For each goat, the afternoon and the following morning milk collections were combined as a daily milk sample.

The animals were sacrificed 4 to 6 hours after the final dose to maximise tissue residues. A whole blood sample was collected prior to sacrifice. Liver, kidney, leg muscle, tenderloin muscle and omental fat were sampled at sacrifice. Appropriate background samples were taken from a control goat.

Method of analysis: TRR in urine and milk was determined directly by LSC. TRR in faeces and solid tissues was determined by combustion/LSC. Samples were extracted using a range of solvents. Identification of the residues was carried out by co-chromatography against standard reference compounds using 2D-TLC and HPLC.

Number of animals: Four

Date of experiment: 20 October 1988 to 01 September 1990

Findings: Extractability of the radioactive residues from tissues, fat and milk was between 83 and 110% of the TRR. The extractability of the residues in tissues and milk is shown in Table B.7.2.2-1. The recovery of the dose in the tissues, milk, blood and excreta is given in Table B.7.2.2-2.

Table B.7.2.2-1. Extractability of residues in tissues and milk of lactating goat dosed daily for 3 days with [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole.

Labelled form	Sample	TRR (mg/kg)[a,c]	Organic phase (%)[b]	Aqueous phase (%)[b]	Non-extracted (%)[b]	Total (%)[b]
[phenyl-^{14}C] difenoconazole	Liver	6.02	72	23	3	98
	Kidney	1.55	42	68	12	122
	Muscle	0.20	77	9	14	100
	Milk	0.14	94	6	18	118
[triazole-^{14}C] difenoconazole	Liver	7.51	60	45	4	109
	Kidney	1.81	58	49	8	115
	Muscle	0.57	55	37	14	106
	Milk	0.38	82	1	6	89

[a] mg/kg difenoconazole equivalents
[b] Percent of total radioactive residues.
[c] Average of 2 animals.

Labelled form	Sample	TRR (mg/kg)[a,c]	Extracted (%)[b]	Non-extracted (%)[b]	Total (%)[b]
[phenyl-^{14}C] difenoconazole	Fat	0.56	95.8	4.2	100
[triazole-^{14}C] difenoconazole	Fat	1.14	94.5	5.5	100

[a] mg/kg difenoconazole equivalents
[b] Percent of total radioactive residues.
[c] Average of 2 animals.

Following dosing of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole, the overall recoveries of the dosed radioactivity was 51.8% to 64.2% and 40.1% to 62.5%, respectively (Table B.7.2.2-2). The majority of the administered dose was found in the excreta (48.3% to 61.2% for phenyl -^{14}C and 35.6% to 57.7% for triazole-^{14}C label). Blood levels were similar for all four goats (0.3% to 0.5% of the dose). Totals of 0.05% to 0.12% and of 0.17% to 0.24% of the dose for phenyl -^{14}C and for triazole-^{14}C label were recovered in the milk. Residues in tissues ranged from 2.55% to 3.0% of the dose for the phenyl -^{14}C label and 4.1% to 4.9% for the triazole-^{14}C label.

The highest residue levels were found in liver (5.465 to 8.487mg difenoconazole equivalents/kg),kidney (1.348 to 1.882 mg difenoconazole equivalents/kg) and fat (0.503 to 1.547 mg difenoconazole equivalents/kg).

Table B.7.2.2-2. Recovery of administered dose in lactating goat.

Sample	[phenyl-^{14}C] difenoconazole				[triazole-^{14}C] difenoconazole			
	Goat 13		Goat 18		Goat 2		Goat 14	
	% dose	mg/kg[a]	% dose	mg/kg[a]	% dose	mg/kg[a]	% dose	mg/kg[a]
Urine[b]	17.7	--	21.8	--	13.6	--	14.2	--
Faeces[b]	43.5	--	26.5	--	21.6	--	43.5	--
Blood	0.32	0.367	0.51	0.520	0.49	0.553	0.44	0.577
Milk[b]	0.12	--	0.05	--	0.17	--	0.24	--
Day 1	--	0.066	--	0.143	--	0.193	--	0.169
Day 2	--	0.109	--	0.163	--	0.428	--	0.329
Total tissues	2.55		2.97		4.94		4.09	
Liver	1.34	6.568	1.47	5.465	1.25	6.533	1.49	8.487
Kidney	0.05	1.348	0.07	1.748	0.07	1.882	0.06	1.745
Muscle	0.92	0.195	1.10	0.207	3.27	0.681	1.89	0.449
Fat	0.24	0.503	0.33	0.618	0.35	1.547	0.65	0.738
Total	64.19		51.83		40.80		62.47	

[a] mg/kg difenoconazole equivalents.
[b] Sum of all samples taken from first dose until sacrifice.

Parent difenoconazole was detected in liver at levels of 6.7 and 8.2% of the TRR for the [phenyl-^{14}C] and [triazole-^{14}C] labels, respectively (Table B.7.2.2-3), being CGA-205375 the major metabolite, accounting for 52.9% and 49.8% of the TRR for the [phenyl-^{14}C] and [triazole-^{14}C] samples, respectively. The metabolites CGA-205374 (2.3% of the TRR) and hydroxylated-CGA-205375 (6.1% of the TRR) were detected in the phenyl-^{14}C treated samples only.

In the kidney, the metabolite CGA-205375 was also the major metabolite detected, accounting for 30.9 to 51.0% of the TRR in both labelled samples. Parent difenoconazole accounted for 1.5 to 5.2% of the TRR in both samples and a small amount (2.0% of the TRR) of hydroxylated metabolite hydroxy-CGA-205375 was also detected in the phenyl-^{14}C treated sample only.

In muscle, the major metabolite was CGA-205375, accounting for 68.5 and 43.2% of the TRR for the phenyl-^{14}C and triazole-^{14}C labels, respectively. Parent difenoconazole comprised 3.5 and 3.7% of the TRR. Hydroxylated CGA-205375, CGA-205374 and CGA-71019 were found only in the triazole-^{14}C labelled sample at levels of 2.3, 1.4 and 1.8% of the TRR, respectively.

The residue profile in fat was very similar to that observed in muscle, with CGA-205375 the major (73.5 to 75.3% TRR) compound detected in both labelled samples. Parent difenoconazole accounted for 3.2 to 6.5% of the TRR and hydroxylated-CGA-205375 and hydroxylated-difenoconazole were present at levels of 1.2 to 4.1% of the TRR.

In milk, following dosing of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole, the major component of the residue was CGA-205375, ranging from 21.3% to 34.4% of the TRR, respectively. Ring-hydroxylated difenoconazole accounted for 15.2% of the TRR for the phenyl-^{14}C labelled sample. Parent difenoconazole

was present at 8.6 and 6.2% of the TRR for the phenyl-^{14}C and triazole-^{14}C labels, respectively. The metabolite CGA-71019 was found in the triazole-^{14}C labelled sample at 5.8% TRR and the corresponding ring cleavage acid CGA-189138 was found in the phenyl-^{14}C labelled sample at 6.3% TRR. Ring hydroxylated CGA-205375 was present at levels up to 4.4% of the TRR.

Parent difenoconazole was detected in urine at very low levels (0.3% TRR), but was present in the faeces at levels of 27.6 and 41.7% for the [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole treated samples, respectively. The metabolite CGA-205375 was the major degradation product observed in both urine and faeces accounting for 30.0 and 10.8%, respectively in the [phenyl-^{14}C] difenoconazole treated sample and 48.1 and 16.0%, respectively in the [triazole-^{14}C] difenoconazole treated sample. Hydroxylated-CGA-205375 was also a major metabolite in urine and faeces detected at levels up to 27.9 and 8.0% of the TRR, respectively. Other metabolites included CGA-205374, CGA-189138 and CGA-71019 at levels up to 8.7% of the TRR, and hydroxylated parent difenoconazole at a level of 6.4% TRR in the urine and 1.2% TRR in the faeces.

Distribution of metabolites and residue levels is summarised in Tables B.7.2.2-3 and B.7.2.2-4.

Table B.7.2.2-3. Distribution of radioactive metabolites in tissues, milk, urine and faeces of lactating goat dosed daily for 3 days with [phenyl-^{14}C] difenoconazole.

Compounds detected	Milk		Liver		Kidney		Muscle		Fat		Urine	Faeces
	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	%
Difenoconazole	8.6	0.012	6.7	0.403	1.5	0.023	3.5	0.007	3.2	0.018	0.3	27.6
CGA-205375	21.3	0.029	52.9	3.183	30.9	0.478	68.5	0.138	73.5	0.412	30.0	10.8
CGA-205374	--	--	2.3	0.138	--	--	--	--	--	--	1.4	3.5
CGA-189138	6.3	0.009	--	--	--	--	--	--	--	--	8.7	1.6
OH-CGA-205375-isomer	4.4	0.006	2.2	0.132	2.0	0.031	--	--	3.4	0.019	12.4	6.3
OH-CGA-205375-isomer	--	--	3.9	0.235	--	--	--	--	--	--	4.0	--
OH-difenoconazole-isomer	15.2	0.021	--	--	--	--	--	--	4.1	0.023	6.4	--
OH-difenoconazole-isomer	--	--	--		--	--	--	--	1.8	0.010	--	--
Unknown	31.4	--	--	--	51.7	--	--	--	--	--	6.5	3.1
Unknown	--	--	--	--	2.8	--	--	--	--	--	14.4	2.3
Unknown	--	--	--	--	--	--	--	--	--	--	7.0	--
Unresolved	6.9	--	6.7	--	6.8	--	1.3	--	2.2	--	0.3	1.9
Not analysed	6.0	--	27.0	--	7.0	--	14.0	--	8.0	--	--	34.0
Non-extracted	18.0	--	3.0	--	12.0	--	14.0	--	4.0	--	--	10.0
Total	118		104.7		114.7		101.3		100.2		91.4	101.1

Data shown are the mean of two animals.

Table B.7.2.2-4. Distribution of radioactive metabolites in tissues, milk, urine and faeces of lactating goat dosed daily for 3 days with [triazole-^{14}C] difenoconazole.

Compounds detected	Milk		Liver		Kidney		Muscle		Fat		Urine	Faeces
	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	%
Difenoconazole	6.2	0.023	8.2	0.616	5.2	0.095	3.7	0.021	6.5	0.074	0.3	41.7
CGA-205375	34.4	0.130	49.8	3.740	51.0	0.925	43.2	0.244	75.3	0.860	48.1	16
CGA-205374	--	--	--	--	--	--	1.4	0.008	--	--	0.6	5.1
CGA-71019	5.8	0.022	--	--	--	--	1.8	0.010	--	--	7.6	5.1
OH-CGA-205375-isomer	3.0	0.011	--	--	--	--	2.3	0.013	1.2	0.014	27.9	8.0
OH-CGA-205375-isomer	--	--	--	--	--	--	--	--	--	--	1.4	--
OH-difenoconazole-isomer	--	--	--	--	--	--	--	--	1.8	0.021	<0.1	1.2
Unknown	33.2	--	--	--	39.8	--	--	--	--	--	3.4	--
Unknown	--	--	--	--	2.8	--	--	--	--	--	--	3.3
Unresolved	2.1	--	0.3	--	1.5	--	1.0	--	1.2	--	0.8	4.8
Not analysed	1.0	--	47.0	--	1.0	--	41.0	--	9.0	--	--	23.5
Non-extracted	6.0	--	4.0	--	8.0	--	14.0	--	6.0	--	--	10.5
Total	91.7		109.3		109.3		108.4		101		90.1	118.7

Data shown are the mean of two animals.

Conclusions: Difenoconazole is extensively metabolised and rapidly excreted in goat. Following oral dosing for three consecutive days with either [phenyl-^{14}C] or [triazole-^{14}C] difenoconazole at levels in the diet equivalent to 100 mg/kg, the majority of the administered dose was found in urine (14 to 22% of the TRR) and faeces (22 to 44%). Only small amounts of the administered dose were found in milk (0.05 to 0.12%) and edible tissues (2.6 to 3.0% for the phenyl-^{14}C treatment and 0.17 to 0.24% for the triazole-^{14}C treatment) demonstrating that difenoconazole and its metabolites do not bio-accumulate and are rapidly excreted.

The highest residue levels were found in liver (5.465 to 8.487mg difenoconazole equivalents/kg), kidney (1.348 to 1.882mg difenoconazole equivalents/kg) and fat (0.503 to 1.547mg difenoconazole equivalents/kg).

Parent difenoconazole was detected in all tissues, milk and excreta at levels less than 9% of the TRR. The major primary metabolite found was CGA-205375 which occurred at levels up to 49.8% TRR (3.740 mg/kg) in liver, 51% TRR (0.925 mg/kg) in kidney, 43.2% TRR (0.244 mg/kg) in muscle and 34.4% TRR (0.130 mg/kg) in milk. Minor amount of the metabolites CGA-205374 in liver and muscle were also observed (2.3% TRR, 0.138 mg/kg and 1.4% TRR, 0.008 mg/kg, respectively) as well as isomers of hydroxy-CGA-205375.

Following administration of difenoconazole, the proposed metabolic pathway of difenoconazole involves hydrolysis of the dioxolane ring to form the ketone CGA-205374, with subsequent reduction of CGA-205374 to give the corresponding alcohol CGA-205375 as a major metabolite. Oxidation of CGA-205374 results in cleavage of the alkyl bridge, leading to the formation of the acid CGA-186138 and 1,2,4-triazole CGA-71019. A secondary pathway involves hydroxylation of difenoconazole to form the hydroxylated analogue CGA-205375 via hydroxylated CGA-205374.

Comments by RMS: The study was well performed and evaluated.

B.7.2.3 Metabolism of [phenyl-^{14}C] difenoconazole in lactating goat

Reference:	**Ray W. J. (1996)** Metabolism of phenyl-^{14}C-CGA-169374 in lactating goats. Biochemistry Department, Ciba Crop Protection, Ciba-Geigy Corporation, Greensboro, North Carolina, United States. Unpublished Report ABR-95099, SAM No. 1232.
Test Material:	Phenyl-^{14}C difenoconazole, batch number TYP-IV-17, radiochemical purity 98.3%, specific radioactivity 41.9 µCi/mg.
Guideline:	Pesticide Assessment Guidelines, Subdivision O, Residue Chemistry, Series 171-4 (b), United States Environmental Protection Agency, Washington D.C., Nature of the Residue - Livestock.
GLP:	Yes

Material and methods:

Test concentration:	100 mg/kg feed/day
Test system:	Two lactating goats (*capra hircus*, 45.9 and 51.3 kg, approximately 2 years old) were individually housed in a metabolism cage designed for the separate collection of urine and faeces. Treatment room lights were on a 12-hour on/off cycle each 24-hour period. During the 5-7-days acclimation and 4-day dosing period, room temperatures ranged from 16-26 °C and humidity from 5-51%. The goats were dosed over a period of 4 consecutive days in the morning. Bottled water was provided *ad libitum*. The goats were sacrificed 6 hours after the final dose.
Duration:	4 days
Sampling time points:	Urine and faeces were collected daily. Milk was collected twice a day, AM and PM. A whole blood sample was taken just prior to sacrifice. At sacrifice, samples of liver, kidney, bile, leg muscle, tenderloin muscle, perirenal fat, omental fat and gastrointestinal (GI) tract were taken. Appropriate background samples were taken from a control goat.
Method of analysis:	TRR in urine and milk was determined directly by LSC. TRR in faeces and solid tissues was determined by combustion/LSC. Liver and kidney samples were extracted with acetonitrile/water (9:1 v/v), fat was extracted with acetonitrile/hexane (10:1 v/v) and milk was extracted with acetonitrile followed by acetone. Identification of the extracted residues was carried out by co-chromatography against standard reference compounds using 2D-TLC and HPLC. Confirmation of structural identity was obtained by MS analysis.
Number of animals:	Two
Date of experiment:	18 January 1995 to 31 October 1995

Findings: Based on the mean goat weight and mean dietary dry matter consumed during the dosing period, the actual dose rates were 74 ppm and 69 ppm, respectively.

The extractability of the residues in tissues and milk is shown in Table B.7.2.3-1.

Table B.7.2.3-1. Extractability of residues in tissues and milk of lactating goat dosed daily for 4 days with [phenyl-^{14}C] difenoconazole.

Sample	TRR (mg/kg)[a]	Extracted		Non-extracted		Total	
		%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Liver	9.790	95.3	9.330	4.87	0.477	100.2	9.807
Kidney	2.731	95.7	2.614	2.04	0.056	97.7	2.670
Muscle	0.463	101.0	0.468	0.75	0.004	101.8	0.472
Fat	1.035	106.0	1.097	2.39	0.025	108.4	1.122
Milk	0.317	108.0	0.342	4.88	0.016	112.9	0.358

[a] mg/kg difenoconazole equivalents
[b] Percent of total radioactive residues.

Extractability of the radioactive residues from tissues and milk was high (95.3 to 106% of the TRR for tissues and 108% of the TRR for milk).

The overall recovery of the dosed radioactivity was 70.5% (Table B.7.2.3-2). The majority of the administered dose was found in the excreta (66.3%) and only small amounts in bile (0.18%). Totals of 0.42% and 0.04% were recovered in the blood and milk, respectively. Low levels of administered radioactivity were retained in the tissues (3.3%), of which 1.43% was recovered in muscle, 1.47% in liver, 0.35% in fat and 0.06% in kidney.

The highest residue levels were found in liver (9.76 mg/kg) and kidney (2.55 mg/kg, Table B.7.2.3-1). Levels in the other edible tissues were lower, with 1.1 mg/kg found in fat and 0.45 mg/kg in muscle.

The recovery of the radioactive dose in the tissues, milk, blood, urine and faeces is given in Table B.7.2-3-2.

Table B.7.2.3-2. Recovery of administered dose in lactating goat.

Sample	Goat 345		Goat 346		Mean	
	% dose	mg/kg[a]	% dose	mg/kg[a]	% dose	mg/kg[a]
Urine[b]	30.51	--	27.68	--	29.09	--
Faeces[b]	35.61	--	38.81	--	37.20	--
Blood	0.40	0.733	0.45	0.723	0.42	0.728
Bile	0.24	40.4	0.13	75.4	0.18	57.954
Milk[c]						
Day 1	0.04	0.177	0.03	0.168	0.03	0.173
Day 2	0.05	0.228	0.04	0.247	0.05	0.237
Day 3	0.05	0.242	0.04	0.264	0.05	0.253
Day 4	0.04	0.327	0.03	0.328	0.04	0.328
Liver	1.32	9.790	1.62	9.729	1.47	9.760
Kidney	0.07	2.731	0.06	2.371	0.06	2.551
Muscle	1.39	0.463	1.47	0.430	1.43	0.447
Fat	0.31	1.035	0.39	1.131	0.35	1.083
Total	70.16		70.85		70.50	

[a] mg/kg difenoconazole equivalents.
[b] Cumulative total over the 4 day dosing period.
[c] Day 1 to 3 results are the mean of measurements made AM and PM.

The major residue in milk was the metabolite CGA-205375 (39.2% TRR, 0.124 mg/kg; Table B.7.2.3-3), the glycine conjugate of CGA-189138 (35.8% of the TRR, 0.114 mg/kg)), the sulphate conjugate of CGA-205375 (10.7% of the TRR, 0.034 mg/kg) and parent difenoconazole (8.8% of the TRR, 0.028 mg/kg). Smaller amounts (<4.5% of the TRR) of the sulphate conjugate of hydroxy-CGA-205375, CGA-189138, hydroxy-CGA-205375 and the sulphate conjugate of hydroxy-difenoconazole were also identified.

The major metabolite detected in liver was CGA-205375, occurring at levels of 72.8% of the TRR. Parent difenoconazole was detected at a level of 9.1% of the TRR and various minor metabolites that individually did not exceed 1.8% of the TRR (Table B 7.2.3-3). The glucuronide conjugate of CGA-205375 was observed at a level of 7.5% of the TRR.

The major metabolites observed in kidney were CGA-205375, the glucuronide conjugate of CGA-205375 and glycine conjugate of CGA-189138 occurring at levels of 43.2, 17.0 and 11.1% of the TRR, respectively. Parent difenoconazole accounted for only 0.5% of the TRR, with small amounts (< 4.0% TRR) of the sulphate conjugate of hydroxy-CGA-205375, CGA-189138, hydroxy-CGA-205375 and the glucuronide conjugates of CGA-205374, and hydroxy-CGA-189138 were also identified.

In muscle and fat > 91% of the TRR was present as the metabolite CGA-205375. The remainder of the residue comprised small amounts (< 10% TRR) of parent difenoconazole, hydroxy-CGA-205375, CGA-205374 (fat only), the glycine conjugate of CGA-189138 (muscle only) and sulphate and glucuronide conjugates of CGA-205375 (muscle only).

TRR in urine was mainly composed of glucuronide, sulphate or glycine conjugates of CGA-205375, hydroxy-CGA-205375, CGA-189138 or hydroxy-CGA-189138 (2.1 to 44.3 of the TRR). Hydroxy acetic acid-difenoconazole and glutamic acid/threonine conjugates of CGA-189138 (<2% of the TRR) were observed as urine specific metabolites. Only 0.4% of the TRR was present as parent difenoconazole.

Distribution of metabolites and residue levels is summarised in Table B.7.2.3-3.

Table B.7.2.3-3. Distribution of radioactive metabolites in tissues and milk of lactating goat dosed daily for 4 days with [phenyl-^{14}C] difenoconazole.

Compounds detected	Milk		Liver		Kidney		Muscle		Fat	
	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg
Difenoconazole	8.8	0.028	9.1	0.891	0.5	0.014	4.4	0.020	9.2	0.095
CGA-205375	39.2	0.124	72.8	7.127	43.2	1.180	91.4	0.423	91.7	0.949
Glycine-CGA-189138	35.8	0.114	0.4	0.039	11.1	0.303	0.9	0.004	--	--
Glucuronide-CGA-205375	0.5	0.002	7.5	0.734	17.0	0.464	1.4	0.006	--	--
Sulphate-CGA-205375	10.7	0.034	0.7	0.068	10.0	0.273	0.5	0.002	--	--
Sulphate-OH-CGA-205375	4.1	0.013	1.8	0.176	3.0	0.082	--	--	--	--
CGA-189138	1.0	0.003	0.2	0.020	2.1	0.057	0.5	0.002	--	--
OH-CGA-205375	0.4	0.001	1.0	0.098	0.6	0.016	1.9	0.009	1.9	0.020
Glucuronide-OH-difenoconazole	--	--	1.6	0.157	0.7	0.019	--	--	--	--
Sulphate-OH-difenoconazole	2.9	0.009	--	--	--	--	--	--	--	--
Glucuronide-OH-CGA-205375	--	--	--	--	1.0	0.027	--	--	--	--
Glucuronide-OH-CGA-189138	--	--	--	--	3.2	0.087	--	--	--	--
CGA-205374	--	--	--	--	--	--	--	--	3.2	0.033
Unknown 9	1.6	0.005	--	--	1.0	0.027	--	--	--	--
Unknown 11	2.8	0.009	--	--	--	--	--	--	--	--
Unknown 15	--	--	--	--	2.5	0.068	--	--	--	--
Non-extracted	4.9	0.016	4.9	0.477	2.0	0.056	0.8	0.004	2.4	0.025
Total	112.7		100		97.9		101.8		108.4	

As the radioactive residues in tissue and milk samples were comparable for both goats, data shown here is from one goat (goat 345) only.

Conclusions: Difenoconazole is extensively metabolised and rapidly excreted in goat. Following oral dosing for four consecutive days with [phenyl-^{14}C] difenoconazole at levels in the diet equivalent to 100 mg/kg, the majority of the administered dose was found in urine and faeces (66.3%). Only small amounts of the administered dose were found in milk (up to 0.05%) and edible tissues (0.06 to 1.5%).

Difenoconazole was detected in liver, muscle, fat and in milk and at very low levels in kidney (0.5%). CGA-205375 was detected as a major metabolite in all tissues, milk and urine and conjugates (glucuronide and/or sulphate) of CGA-205375 were observed in liver, kidney, muscle and milk samples. The metabolite CGA-189138 was detected in kidney, milk, liver, muscle and urine as a glycine conjugate. Phenyl ring hydroxylated analogues of the major primary metabolites and the corresponding glucuronide and/or sulphate conjugates were also observed as minor metabolites in tissues, milk and urine.

Following administration of difenoconazole, the proposed metabolic pathway of difenoconazole in the goat involves hydrolysis of the dioxolane ring to form the ketone CGA-205374, with subsequent reduction of CGA-205374 to give the corresponding alcohol CGA-205375 as a major metabolite. Oxidation of CGA-205374 results in cleavage of the alkyl bridge, leading to the formation the acid CGA-186138 and 1,2,4-triazole CGA-71019 (not observed in this study due to the radiolabel position). A second pathway involves hydroxylation of difenoconazole, to form the hydroxylated metabolites CGA-205374 and

CGA-205375. Secondary metabolism processes involve the formation of glucuronide, sulphate and amino acid conjugates of the primary metabolites (see Figure B.7.2.3-1).

Comments by RMS: The study was well performed and evaluated.

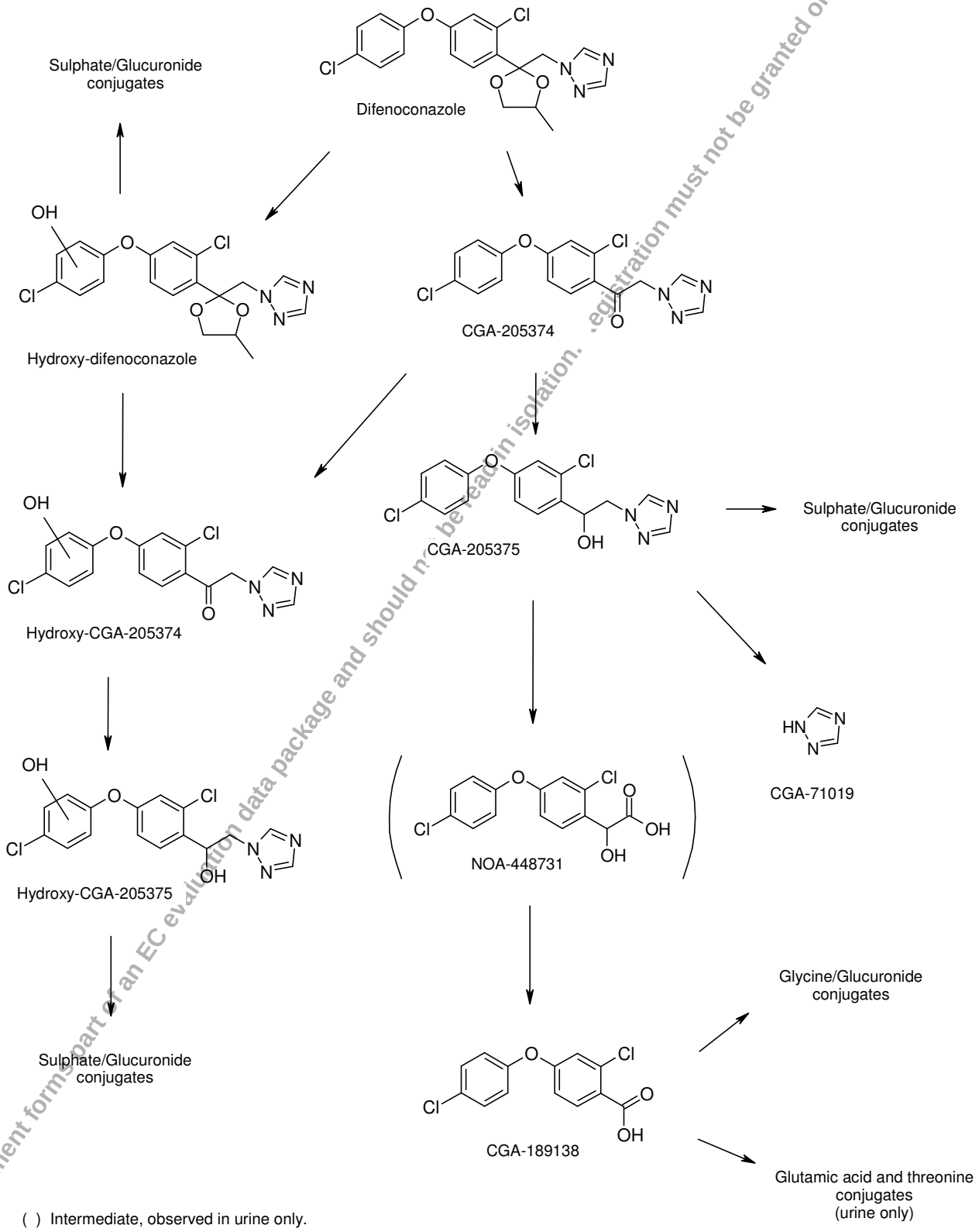


Figure B.7.2.3-1. Proposed metabolic pathway of difenoconazole in the lactating goat.

B.7.2.4 Metabolism of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole in laying hens

Reference:	**Madrid S. O. (1989)** Metabolism of triazole and phenyl-^{14}C-CGA-169374 in laying hens dosed daily for fourteen consecutive days. Metabolism Department, Agricultural Division, Ciba-Geigy Corporation, Greensboro, North Carolina, United States. Unpublished Report ABR-89051 (plus Amendment 1), SAM No. 0270.
Test Material:	[Phenyl-^{14}C] difenoconazole, batch GAN-IX-5, radiochemical purity 97%, specific activity 98.6 µCi/mg. [Triazole-^{14}C] difenoconazole, batch GAN-IX-7, radiochemical purity 98%, specific activity 48.5 µCi/mg.
Guideline:	Pesticide Assessment Guidelines, Subdivision O, Residue Chemistry, Series 171-4 (b), United States Environmental Protection Agency, Washington D.C., Nature of the Residue - Livestock.
GLP:	Yes (In part). The work was conducted and completed prior to the adoption of GLP standards; however the final report was audited to GLP standards.

Material and methods:

Test concentration:	5 mg/kg feed/day
Test system:	One gelatine capsule per day (containing 0.55 mg of the test material) was administered perorally to *Leghorn* hens (two hens in each treatment group) over a period of fourteen consecutive days. The hens were sacrificed 22 hours after the last dose.
Duration:	14 days
Sampling time points:	Eggs and excreta were collected daily and the eggs separated into yolk and white. Blood was sampled immediately prior to sacrifice. After sacrifice, lean meat, skin plus attached fat, peritoneal fat, kidney and liver were taken. Appropriate background samples were taken from control hens.
Method of analysis:	Samples of each commodity were extracted and fractionated to determine the quantitative and qualitative nature of the residue. Radioactive residues in the eggs (egg white and yolk) and in tissues were determined by combustion/LSC. Excreta was extracted initially with acetonitrile/water and the non-extracted residue analysed by combustion/LSC. Selected samples of all matrices were extracted by the Bligh-Dyer/Ting-Dugger procedure, which yielded an organic and aqueous fractions and a non-extractable fraction. Identification of the extracted residues was carried out by co-chromatography against standard reference compounds using 1D and 2D-TLC.
Number of animals:	Four
Date of experiment:	July 1986 to February 1987

Findings: Based on feed consumption, the dietary concentrations were 5.19 to 5.73 mg/kg per day and 4.66 to 4.91 mg/kg per day for the phenyl-^{14}C and triazole-^{14}C dosed hens, respectively.

The overall recovery of the dosed radioactivity was 91.5 to 97.5% and 92.1 to 93.5% for the phenyl-^{14}C and triazole-^{14}C dosed hens, respectively (Table B.7.2.4-1). The majority of the administered dose was found in the excreta (94.5% for the phenyl-^{14}C and 93% for the triazole-^{14}C label, average value).

For the phenyl-^{14}C label, totals of 0.01 to 0.06%, 0.53 to 0.72% and 0.04 to 0.07% were recovered in the blood, the egg yolk and egg white, respectively. Low levels of administered radioactivity were retained in the tissues (0.15 to 0.28%), of which 0.01 to 0.04% was recovered in skin plus attached fat, 0.02 to 0.03% in peritoneal fat, 0.02 to 0.05% in lean muscle, 0.03 to 0.08% in liver and 0.07 to 0.09% in kidney.

For the triazole-^{14}C label, totals of 0.11 to 0.15%, 0.65% and 0.68 to 0.80% were recovered in the blood, the egg yolk and egg white, respectively. Low levels of administered radioactivity were retained in the tissues (0.52 to 0.62%), of which 0.01% was recovered in peritoneal fat, 0.05% in skin plus attached fat, 0.05 to 0.06% in liver, 0.06 to 0.07% in kidney and 0.35 to 0.43% in lean muscle.

Tissue residues for the phenyl-^{14}C label were 0.004 to 0.011 mg/kg in the lean muscle, 0.008 to 0.024 mg/kg in the skin plus attached fat, 0.034 to 0.046 mg/kg in the peritoneal fat, 0.101 to 0.150 mg/kg in the liver and 0.449 to 0.522 mg/kg in the kidney. Residues in blood were 0.011 to 0.048 mg/kg.

Tissue residues for the triazole-^{14}C label were 0.07 to 0.09 mg/kg in the lean muscle, 0.02 mg/kg in the peritoneal fat, 0.031mg/kg in the skin plus attached fat, 0.13 mg/kg in the liver and 0.352 to 0.517 mg/kg in the kidney. Residues in blood were 0.085 to 0.120 mg/kg.

Table B.7.2.4-1. Recovery of the administered dose and residue levels from laying hens dosed with [phenyl-^{14}C] or [triazole-^{14}C] difenoconazole for 14 consecutive days.

Sample	[phenyl-^{14}C] difenoconazole				[triazole-^{14}C] difenoconazole			
	Hen 3B		Hen 4B		Hen 1A		Hen 2A	
	% dose	mg/kg[b]	% dose	mg/kg[b]	% dose	mg/kg[b]	% dose	mg/kg[b]
Tissues[a]	0.336	--	0.166	--	0.635	--	0.784	--
Skin plus attached fat	0.036	0.024	0.012	0.008	0.049	0.030	0.049	0.031
Lean meat (light and dark)	0.050	0.011	0.019	0.004	0.346	0.072	0.427	0.093
Peritoneal fat	0.030	0.046	0.022	0.034	0.014	0.019	0.013	0.019
Liver	0.075	0.150	0.033	0.101	0.050	0.118	0.060	0.134
Kidney	0.087	0.522	0.066	0.449	0.058	0.352	0.075	0.517
Blood	0.058	0.048	0.014	0.011	0.114	0.085	0.154	0.120
Eyes	<0.001	0.010	<0.001	0.005	0.004	0.082	0.006	0.081
Egg yolks	0.718	--	0.529	--	0.648	--	0.650	--
Egg whites	0.066	--	0.039	--	0.677	--	0.802	--
Excreta	90.39	--	96.77	--	91.55	--	89.84	--
Total	91.51		97.50		93.51		92.08	

[a] Lean meat (dark plus light), skin attached fat, peritoneal fat, and blood total weight were calculated using factors of 23.3%, 8.0%, 3.5%, and 6.5%, respectively.
[b] mg/kg difenoconazole equivalents.

Levels of radioactivity in egg white reached a plateau after *ca* 5 days. The total radioactivity in the egg white from the triazole-^{14}C treated hens (0.677 to 0.802% of the dose; Table B.7.2.4-2) was significantly higher than the corresponding white from the phenyl-^{14}C treated hens (0.039 to 0.066% of the dose). The result indicated alkyl bridge cleavage and preferential transfer of triazole-related residues to the egg white.

Plateau levels of residue in phenyl-^{14}C and triazole-^{14}C treated egg whites were *ca* 0.01 and 0.14 mg/kg, respectively (Figure B.7.2.4-1).

Levels of radioactivity in egg yolk reached a plateau after *ca* 7 days. The total radioactivity in the egg yolk from the triazole-^{14}C treated hens (0.648 to 0.650% of the dose) was of the same magnitude as the corresponding white from the phenyl-^{14}C treated hens (0.529 to 0.718% of the dose; Table B.7.2.4-3). The plateau level of residue in both the phenyl-^{14}C and triazole-^{14}C treated yolk was *ca* 0.3 mg/kg (Figure B.7.2.4-2).

Table B.7.2.4-2. Radioactivity in egg white from laying hens dosed with [phenyl-^{14}C] or [triazole-^{14}C] difenoconazole for 14 consecutive days.

Sampling time	[phenyl-^{14}C] difenoconazole				[triazole-^{14}C] difenoconazole			
	Hen 3B		Hen 4B		Hen 1A		Hen 2A	
	% dose	mg/kg[a]	% dose	mg/kg[a]	% dose	mg/kg[a]	% dose	mg/kg[a]
Day 1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 2	0.005	0.012	0.003	0.008	0.041	0.101	0.047	0.107
Day 3	0.005	0.013	0.003	0.009	0.057	0.131	0.062	0.141
Day 4	0.005	0.013	0.003	0.009	0.057	0.135	0.064	0.148
Day 5	0.005	0.014	0.003	0.007	0.062	0.153	0.069	0.157
Day 6	0.005	0.012	0.003	0.008	0.055	0.133	0.067	0.152
Day 7	0.005	0.013	0.002	0.007	0.052	0.127	0.065	0.150
Day 8	0.005	0.013	0.003	0.008	0.050	0.121	0.063	0.147
Day 9	0.005	0.013	0.003	0.007	0.049	0.117	0.065	0.141
Day 10	0.006	0.016	0.003	0.009	0.038	0.129	0.067	0.143
Day 11	0.006	0.014	0.004	0.010	0.050	0.124	0.058	0.139
Day 12	0.006	0.014	0.004	0.010	0.065	0.155	0.067	0.144
Day 13	0.004	0.011	0.003	0.008	0.058	0.133	0.043	0.184
Day 14	0.004	0.011	0.002	0.006	0.043	0.103	0.065	0.145
Total	0.066		0.039		0.677		0.802	

[a] mg/kg difenoconazole equivalents.
n.d.= Not detected, above either the limit of detection or quantitation.

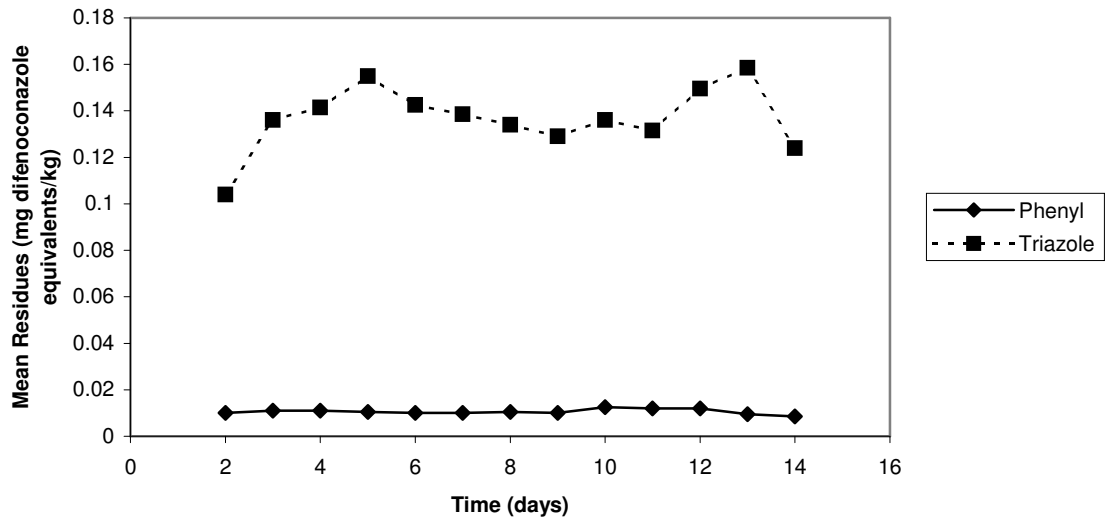


Figure B.7.2.4-1. Mean residues in egg white.

Table B.7.2.4-3. Radioactivity in egg yolk from laying hens dosed with [phenyl-^{14}C] or [triazole-^{14}C] difenoconazole for 14 consecutive days.

Sampling time	[phenyl-^{14}C] difenoconazole				[triazole-^{14}C] difenoconazole			
	Hen 3B		Hen 4B		Hen 1A		Hen 2A	
	% dose	mg/kg[a]	% dose	mg/kg[a]	% dose	mg/kg[a]	% dose	mg/kg[a]
Day 1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 2	0.003	0.012	0.003	0.015	0.013	0.052	0.013	0.061
Day 3	0.013	0.053	0.014	0.069	0.027	0.116	0.027	0.132
Day 4	0.027	0.130	0.024	0.126	0.035	0.150	0.042	0.199
Day 5	0.046	0.191	0.037	0.194	0.049	0.236	0.052	0.246
Day 6	0.072	0.275	0.046	0.237	0.060	0.277	0.061	0.267
Day 7	0.066	0.308	0.042	0.246	0.058	0.299	0.065	0.294
Day 8	0.078	0.339	0.054	0.248	0.064	0.295	0.061	0.287
Day 9	0.070	0.324	0.050	0.240	0.059	0.285	0.058	0.285
Day 10	0.069	0.334	0.051	0.244	0.058	0.285	0.064	0.289
Day 11	0.075	0.345	0.051	0.250	0.059	0.268	0.062	0.282
Day 12	0.075	0.351	0.054	0.271	0.058	0.281	0.024	0.283
Day 13	0.060	0.326	0.050	0.253	0.056	0.246	0.062	0.292
Day 14	0.064	0.339	0.053	0.266	0.052	0.246	0.059	0.291
Total	0.718		0.529		0.648		0.650	

[a] mg/kg difenoconazole equivalents.
n.d.= Not detected, above either the limit of detection or quantitation.

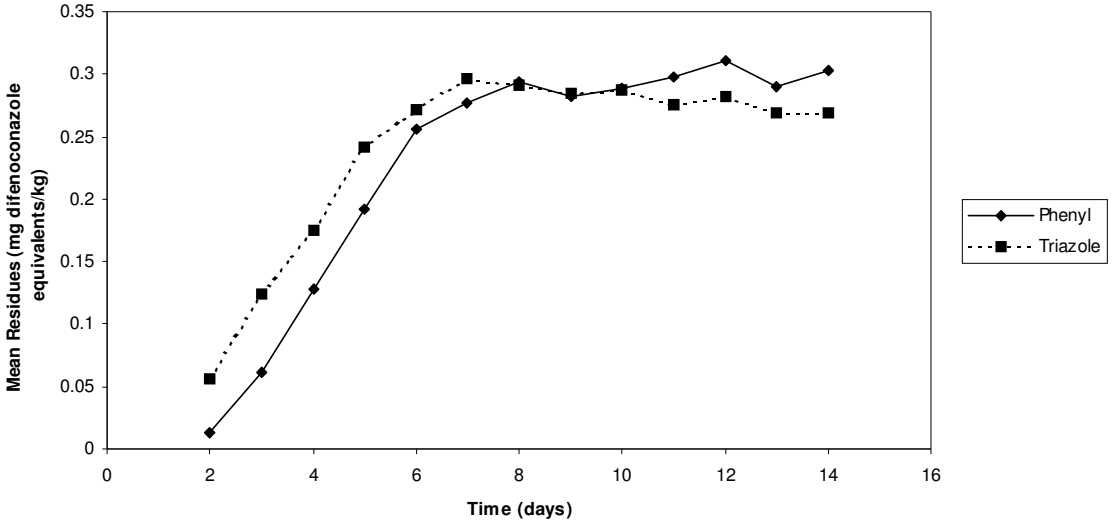


Figure B.7.2.4-2. Mean residues in egg yolk.

The extractability of the radioactive residues from tissues and blood was between 56 and 94% of the TRR. This radioactivity was higher for the triazole-^{14}C label than for the phenyl-^{14}C label (Table B.7.2.4-4). The extractable radioactivity of the triazole-^{14}C label was more to aqueous soluble while the phenyl-^{14}C label more to organic soluble. Similar extraction pattern was observed for the egg yolks and egg whites and excreta (see Table B.7.2.4-5 and B.7.2.4-6), indicating that an alkyl bridge cleavage had occurred.

Table B.7.2.4-4. Distribution of radioactivity in tissues and blood of laying hens dosed with [phenyl-^{14}C] or [triazole-^{14}C] difenoconazole for 14 consecutive days.

Extract	Liver	Kidney	Liver	Kidney	Blood	Lean meat	Lean meat
	[phenyl-^{14}C] difenoconazole						
Hen	3B	3B	4B	4B	4B	--	
TRR (mg/kg)	0.147	0.522	0.101	0.449	0.011	--	--
Organic soluble (%)	51.38	8.48	53.88	9.59	55.87	--	--
Aqueous soluble (%)	13.48	60.84	7.19	72.60	<1.0	--	--
Non-extracted (%)	23.62	62.32	31.37	28.51	23.64	--	--
Total (%)	88.48	134.64	92.44	110.70	79.51		
	[triazole-^{14}C] difenoconazole						
Hen	1A	1A	2A	2A	--	1A	1A
TRR (mg/kg)	0.118	0.352	0.134	0.517	--	0.072	0.093
Organic soluble (%)	27.79	11.86	27.98	13.29	--	8.71	7.52
Aqueous soluble (%)	50.83	69.14	63.50	76.26	--	82.99	86.07
Non-extracted (%)	11.23	11.48	13.59	11.12	--	9.29	7.00
Total (%)	89.85	92.48	105.07	100.67		100.99	100.59

Results expressed as a percent of the TRR.

Table B.7.2.4-5. Distribution of radioactivity in eggs of laying hens dosed with [phenyl-^{14}C] or [triazole-^{14}C] difenoconazole for 14 consecutive days.

Extract	Egg white			Egg yolk		
	[phenyl-^{14}C] difenoconazole					
Collection period	Day 4	Day 10	Day 11	Day 3	Day 10	Day 11
Hen	3B	3B	4B	3B	3B	4B
TRR (mg/kg)	0.013	0.016	0.010	0.053	0.334	0.250
Organic soluble (%)	81.4	63.73	72.95	69.84	47.28	44.32
Aqueous soluble (%)	<1.00	34.51	<1.00	21.41	22.12	18.94
Non-extracted (%)	7.86	8.82	15.22	13.50	21.31	28.99
Total (%)	88.90	107.06	88.17	104.75	90.71	92.25
	[triazole-^{14}C] difenoconazole					
Collection period	Day 3	Day 12	Day 13	Day 3	Day 12	Day 13
Hen	2A	1A	2A	2A	1A	2A
TRR (mg/kg)	0.141	0.155	0.184	0.132	0.281	0.292
Organic soluble (%)	11.14	17.88	13.81	11.11	39.92	20.01
Aqueous soluble (%)	80.74	73.14	77.14	69.89	45.56	76.43
Non-extracted (%)	12.45	12.33	18.17	7.91	12.52	9.47
Total (%)	104.33	103.35	109.12	88.91	98.00	105.91

Results expressed as a percent of the TRR.

Table B.7.2.4-6. Distribution of radioactivity in excreta of laying hens dosed with [phenyl-^{14}C] or [triazole-^{14}C] difenoconazole for 14 consecutive days.

Extract	[phenyl-^{14}C] difenoconazole			[triazole-^{14}C] difenoconazole		
Collection period	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
Hen	4B	3B	3B	1A	2A	1A
% of the dose	5.93	6.82	6.55	5.00	6.76	6.34
Organic soluble (%)	30.76	22.31	25.70	40.92	36.18	26.24
Aqueous soluble (%)	40.25	47.65	48.60	51.26	57.04	55.67
Non-extracted (%)	21.01	19.74	23.76	12.64	12.81	7.45
Total (%)	92.02	89.70	98.06	104.82	106.03	89.36

Results expressed as a percent of the TRR in each sample.

Characterisation of the extractable radioactivity in the excreta revealed that parent difenoconazole was present in both samples at *ca* 12% of the TRR, CGA-205375 was present in the phenyl-^{14}C sample at 2.4% TRR and in the triazole-^{14}C treatment at 4.7% and CGA-71019 was present in the triazole-^{14}C treated sample at 22.9% TRR (Table B.7.2.4-7), indicating alkyl bridge cleavage had occurred. Three other zones were common to both treatments. Six additional zones were present only in the phenyl-treated sample.

Table B.7.2.4-7. Characterisation of the extractable radioactivity in excreta of laying hens dosed with [phenyl-^{14}C] or [triazole-^{14}C] difenoconazole for 14 consecutive days.

	[phenyl-^{14}C] difenoconazole	[triazole-^{14}C] difenoconazole
Collection period	Day 7	Day 7
Hen	3B	2A
% of sample radioactivity in extract	67.6	75.5
Compounds Detected		
Difenoconazole	11.63	11.94
CGA-205375	2.37	4.71
CGA-71019	--	22.91
Zone B	5.58	3.13
Zone D	3.43	2.29
Zone F	9.06	9.46
Zone P	1.99	n.d.
Zone P_1	2.81	n.d.
Zone P_2	4.82	n.d.
Zone P_3	3.84	n.d.
Zone P_4	1.39	n.d.
Zone P_5	2.32	n.d.
Other zones	18.79	21.06

n.d.= Not detected.

Conclusions: Following oral dosing for fourteen consecutive days with either [phenyl-^{14}C] or [triazole-^{14}C] difenoconazole at levels in the diet equivalent to 5 mg/kg, the majority of the administered dose was found in the excreta (94.5% for the phenyl-^{14}C and 93% for the triazole-^{14}C label). Only small amounts of the administered dose were found in egg whites and egg yolks (0.04 to 0.8%) and in edible tissues (0.02 to 0.78%) demonstrating that difenoconazole did not accumulate.

Tissue residues were low for both radiolabelled forms of difenoconazole. Tissue residues were 0.004 to 0.09 mg/kg in the lean muscle, 0.008 to 0.031 mg/kg in the skin plus attached fat, 0.02 to 0.046 mg/kg in the peritoneal fat, 0.101 to 0.150 mg/kg in the liver and 0.352 to 0.522 mg/kg in the kidney. Residues in blood were 0.011 to 0.120 mg/kg. Phenyl-^{14}C treated egg white and yolk contained 0.039 to 0.066 mg/kg and 0.529 to 0.718 mg/kg, respectively. The triazole-^{14}C treated egg white and yolk contained 0.677 to 0.802 mg/kg and 0.648 to 0.650 mg/kg, respectively.

Parent difenoconazole and the metabolite CGA-205375 were detected in the excreta of hens dosed with either [phenyl-^{14}C] or [triazole-^{14}C] difenoconazole. The metabolite CGA-71019 was only detected in the [triazole-^{14}C] dosed hens. The presence of CGA-71019 in the [triazole-^{14}C] dosed hens and the occurrence of six radioactive zones exclusive to the [phenyl-^{14}C] label indicate cleavage of the alkyl bridge connecting the phenyl and triazole rings.

Following administration of difenoconazole, the proposed metabolic pathway of difenoconazole in the hen involves hydrolysis of the dioxolane ring to form the ketone CGA-205374, with subsequent reduction of

CGA-205374 to give the corresponding alcohol CGA-205375 as a major metabolite. Oxidation of CGA-205374 results in cleavage of the alkyl bridge, leading to the formation the acid CGA-186138 and 1,2,4-triazole CGA-71019.

Comments by RMS: The study was well performed and reported.

B.7.2.5 Metabolism of [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole in laying hens

Reference:	**Maynard M. S. (1990b)** [^{14}C]-CGA-169374 phenyl and triazole label distribution, elimination, and metabolism in Hens. Metabolism Department, Agricultural Division, Ciba-Geigy Corporation, Greensboro, North Carolina, United States. Unpublished Report ABR-89101 (plus Amendment 1), SAM No. 0364.
Test Material:	[Phenyl-^{14}C] difenoconazole, batch JAK-111-7, radiochemical purity > 97.6%, specific activity 14.0 µCi/mg). [Triazole-^{14}C] difenoconazole, batch JAK-111-6, radiochemical purity > 98.2%, specific activity 13.9 µCi/mg.
Guideline:	Pesticide assessment Guidelines, Subdivision O, Residue Chemistry, Series 171-4 (b), United States Environmental Protection Agency, Washington D.C. (Nature of the Residue - Livestock).
GLP:	Yes (In part). Some of the metabolite characterisation and identification phase of the study was conducted prior to adoption of GLP guidelines. The majority of the work however was conducted and reported in accordance with GLP standards.

Material and methods:

Test concentration:	68 mg/kg feed/day
Test system:	One gelatine capsule per day (containing 7.5 mg of the test material) was administered perorally to *Leghorn* hens (ten hens in each treatment group). The hens were separately dosed in the morning over a period of three consecutive days. After the acclimation period and two days before dosing the hens were kept in individual metabolic cages to enable the collection of eggs and excreta. The hens were sacrificed 4 to 6 hours after the last dose.
Duration:	3 days
Sampling time points:	Eggs and excreta were collected daily and the eggs separated into yolk and white. Blood was sampled immediately prior to sacrifice. After sacrifice, muscle, skin plus attached fat, kidney and liver were taken. Appropriate background samples were taken from control hens.
Method of analysis:	Excreta were extracted with acetonitrile and fat with dichloromethane. Both liquid and solid (non-extractable) portions were analysed. Extracts were analysed by direct LSC. Blood, solid samples, and samples following extraction were analysed by combustion/LSC. Identification of the extracted residues was carried out by co-chromatography against standard reference compounds using 1D and 2D-TLC and

HPLC.

Number of animals: Twenty

Date of experiment: 18 November 1988 to 15 January 1990

Findings: The overall recovery of the dosed radioactivity was 76% for both radiolabelled forms of difenoconazole (Table B.7.2.5-1). Recoveries for the first two days were 89 to 92% of the administered dose.

For the phenyl-^{14}C label, totals of 0.3%, <0.01% and < 0.01 % were recovered in the blood, the egg yolk and egg white, respectively. Low levels of administered radioactivity were retained in the tissues (1.7%), of which 0.1% was recovered in kidney, 0.4% in muscle, 0.6% in skin plus attached fat and 0.6% in liver. For the triazole-^{14}C label, totals of 0.7%, 0.02% and 005% were recovered in the blood, the egg yolk and egg white, respectively. Low levels of administered radioactivity were retained in the tissues (3.4%), of which 0.1% was recovered in kidney, 0.5% in liver, 0.7% in skin plus attached fat and 2.1% in muscle.

Tissue residues for the phenyl-^{14}C label were 0.102 mg/kg in muscle, 0.454 mg/kg in the skin plus attached fat, 2.247 mg/kg in kidney and 4.660 mg/kg in the liver. Residues in blood were 0.297 mg/kg. Tissue residues for the triazole-^{14}C label were 0.464 mg/kg in the skin plus attached fat, 0.509 mg/kg in muscle, 1.886 mg/kg in kidney and 4.259 mg/kg in the liver. Residues in blood were 0.649 mg/kg.

The recovery of the radioactive dose in the tissues, eggs, blood and excreta is shown in Table B.7.2.5-1.

Table B.7.2.5-1. Recovery of the administered dose and residue levels from laying hens dosed with [phenyl-^{14}C] or [triazole-^{14}C] difenoconazole for 3 consecutive days.

Sample	[phenyl-^{14}C] difenoconazole		[triazole-^{14}C] difenoconazole	
	% dose	mg/kg[c]	% dose	mg/kg[c]
Excreta[a]	76.1		76.1	
Tissues Total	1.7		3.4	
Liver	0.6	4.660	0.5	4.259
Kidney	0.1	2.247	0.1	1.886
Muscle[b]	0.4	0.102	2.1	0.509
Skin plus attached fat[b]	0.6	0.454	0.7	0.464
Blood[b]	0.3	0.297	0.7	0.649
Egg white	<0.01		0.05	
Day 1		0.015		0.062
Day 2		0.023		0.269
Day 3		0.011		0.413
Egg yolks	<0.01		0.02	
Day 1		<0.003		0.014
Day 2		0.037		0.132
Day 3		0.047		0.272

[a] Values for days 1 and 2 were 89-92% of total dose; values reported above were lower due to sacrifice 4 to 6 hours after last dose.
[b] Muscle, skin plus attached fat and blood total weight were calculated using factors of 23.3%, 8.0% and 6.5%, respectively.
[c] mg/kg difenoconazole equivalents.

Extraction of the Day 2 excreta with acetonitrile resulted in removal of 75 and 80% of the radioactive residue in the phenyl-^{14}C and triazole-^{14}C labelled samples, respectively. The non-extractable portion of the Day 2 excreta was subjected to an additional acetonitrile/water extraction which removed most of the

residual radioactivity for both labels, leaving non-extracted residues of 9.3 and 0.4% for the phenyl-^{14}C and triazole-^{14}C labels, respectively.

Dichloromethane removed 85 and 63% of the phenyl-^{14}C and triazole-^{14}C radioactive residue in fat, respectively.

The distribution of radioactivity in excreta and fat is shown in Table B.7.2.5-2.

Table B.7.2.5-2. Extractability of residues in excreta and fat from hens dosed daily for 3 days with [phenyl-^{14}C] or [triazole-^{14}C] difenoconazole.

Labelled form	Sample	Sampling time	Extracted (%)[b]	Non-extracted (%)[b]
[phenyl-^{14}C] difenoconazole	Excreta[a]	Day 1	72.1	27.9
		Day 2	75.0	25.0
		Day 2[c]	17.5	9.3
		Day 2 total	92.5	9.3
		Day 3	82.8	17.2
[triazole-^{14}C] difenoconazole	Excreta[a]	Day 1	80.2	19.8
		Day 2	80.0	20.0
		Day 2[c]	19.6	0.4
		Day 2 total	99.6	0.4
		Day 3	83.5	16.5

[a] Acetonitrile extraction.
[b] Percent of total radioactive residues.
[c] Extraction of non-extracted portion.

Labelled form	Sample	Extracted (%)[b]	Non-extracted (%)[b]
[phenyl-^{14}C] difenoconazole	Fat[a]	85.3	14.7
[triazole-^{14}C] difenoconazole	Fat[a]	63.0	37.0

[a] Dichloromethane extraction.
[b] Percent of total radioactive residues.

Parent difenoconazole was detected in liver, skin plus attached fat, egg white and egg yolk at levels ranging from 0.9% TRR in egg yolk up to 4.3% TRR in liver (Table B.7.2.5-3) for the phenyl-^{14}C treatment. The major metabolite found was CGA-205375, which occurred at levels ranging from 21.8% TRR in kidney up to 84.7% TRR in egg white. Other metabolites occurred at low levels (<10% of the TRR).

Residue levels of difenoconazole for the phenyl-^{14}C treated hens ranged from 0.001mg/kg in egg white up to 0.200 mg/kg in liver. Residues of CGA- 205375 ranged from 0.019 mg/kg in egg white up to 1.640mg/kg in liver. Residues of metabolite hydroxy-CGA-205375 were 0.452 mg/kg in liver and less than 0.08 mg/kg in other tissues.

Parent difenoconazole was detected in the kidney, muscle, skin plus attached fat, egg white and egg yolk at levels ranging from 0.8% TRR in egg yolk up to 4.9% TRR in egg white (Table B.7.2.5-4) for the triazole-^{14}C treatment. Two major metabolites were found, CGA-205375, which occurred at levels ranging from 0.8% TRR in egg yolk up to 45.6% TRR in skin plus attached fat and the metabolite CGA-71019, which occurred at levels ranging from 0.9% TRR in skin + fat up to 67.7% TRR in egg white. Other metabolites occurred at low levels (<9% of the TRR).

Residue levels of difenoconazole for the triazole-^{14}C treated hens ranged from 0.001mg/kg in egg yolk up to 0.032 mg/kg in liver. Residues of CGA- 205375 ranged from 0.021 mg/kg in egg white up to 1.278 mg/kg in liver and residues of CGA-71019 ranged from 0.004 mg/kg in skin + fat up to 0.230 mg/kg in liver. Residues of the metabolite hydroxy-CGA-205375 were 0.349 mg/kg in liver and less than 0.1 mg/kg in other tissues.

The major residue in excreta was parent difenoconazole, ranging from 46.2% of the TRR for the phenyl-^{14}C treatment to 60.1% of the TRR for the triazole-^{14}C treatment. Other identified residues were CGA-205375 at 5.1 and 0.4% for the phenyl-^{14}C and triazole-^{14}C labels, respectively, and hydroxy-CGA-205375 at 2.5 and 8.7%, of the phenyl-^{14}C and triazole-^{14}C total residue, respectively.

The distribution of metabolites and residue levels is summarised in Table B.7.2.5-3 and Table B.7.2.5-4.

Table B.7.2.5-3. Distribution of radioactive metabolites in edible tissues, eggs and excreta of laying hens dosed daily for 3 days with [phenyl-^{14}C] difenoconazole.

Compounds Detected	Muscle		Skin + fat		Liver		Kidney		Egg white		Egg yolk		Excreta
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	%
Difenoconazole	--	--	1.6	0.007	4.3	0.200	--	--	2.7	0.001	0.9	n.d.	46.2
CGA-205375	34.8	0.035	63.5	0.288	35.2	1.640	21.8	0.490	84.7	0.019	72.9	0.027	5.1
OH-CGA-205375	4.3	0.004	8.2	0.037	9.8	0.457	3.2	0.072	2.9	0.001	0.7	n.d.	2.5
OH-CGA-difenoconazole	--	--	--	--	2.9	0.135	4.8	0.108	12.5	0.003	12.4	0.005	--
CGA-205374	--	--	1.7	0.008	1.8	0.084	5.2	0.117	--	--	--	--	--
CGA-189138	--	--	--	--	2.7	0.126	--	--	--	--	--	--	--
Unknown[a]	--	--	--	--	--	--	64.4	--	--	--	--	--	13.8
Unknown	--	--	--	--	1.8	--	--	--	--	--	--	--	5.0
Unknown	--	--	--	--	--	--	6.7	--	--	--	--	--	0.7
Unresolved	2.0	--	1.7	--	1.0	--	2.0	--	7.8	--	7.0	--	7.7
Unanalysed	46.0	--	4.0	--	19.0	--	40.0	--	--	--	--	--	11.0
Non-extracted	97.0	--	12.0	--	35.0	--	30.0	--	31.0	--	19.0	--	9.0
Total	184.1		92.7		113.5		178.1		141.6		112.8		101.0

n.d. = Not detected.
[a] May contain CGA-189138.

Table B.7.2.5-4. Distribution of radioactive metabolites in edible tissues, eggs and excreta of laying hens dosed daily for 3 days with [triazole-^{14}C] difenoconazole.

Compounds Detected	Muscle		Skin + fat		Liver		Kidney		Egg white		Egg yolk		Excreta
	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg	%
Difenoconazole	0.9	0.005	1.5	0.007	--	--	1.7	0.032	4.9	0.013	0.8	0.001	60.1
CGA-205375	8.9	0.045	45.6	0.212	30.0	1.278	19.7	0.372	7.7	0.021	35.2	0.047	0.4
OH-CGA-205375	1.4	0.007	5.7	0.026	8.2	0.349	4.9	0.092	0.7	0.002	2.0	0.003	8.7
OH-CGA-difenoconazole	--	--	--	--	--	--	3.2	0.060	1.0	0.003	4.2	0.006	--
CGA-205374	--	--	0.9	0.004	1.4	0.060	2.1	0.040	--	--	--	--	--
CGA-71019	4.9	0.025	0.9	0.004	5.4	0.230	6.9	0.130	67.7	0.182	32.3	0.043	--
Unknown[a]	--	--	--	--	--	--	27.5	--	--	--	--	--	12.6
Unknown	--	--	--	--	1.7	--	2.0	--	--	--	--	--	4.7
Unknown	--	--	--	--	4.7	--	11.1	--	--	--	--	--	1.2
Unresolved	2.0	--	0.8	--	1.0	--	2.0	--	5.9	--	26.9	--	1.2
Unanalysed	76.0	--	4.0	--	20.0	--	44.0	--	--	--	--	--	15.0
Non-extracted	25.0	--	37.0	--	31.0	--	9.0	--	4.0	--	19.0	--	<1.0
Total	119.1		96.4		103.4		134.1		91.9		120.4		103.9

n.d. = Not detected.
[a] May contain CGA-71019.

Conclusions: Difenoconazole is extensively metabolised and rapidly excreted in hens. Following oral dosing for three consecutive days with either [phenyl-^{14}C] or [triazole-^{14}C] difenoconazole at levels in the diet equivalent to 68 mg/kg, the majority of the administered dose (76.1%) was found in the excreta. Only very small amounts of the administered dose were found in egg whites and egg yolks (<0.01 to 0.05%) and edible tissues (1.7 to 3.4%) demonstrating that difenoconazole and its metabolites do not accumulate and are rapidly excreted.

Total tissue residues accounted for 1.7 and 3.4% of the phenyl-^{14}C and triazole-^{14}C treatment, respectively. Tissue residues were 0.102 to 0.509 mg/kg in the muscle, 0.454 to 0.464 mg/kg in the skin plus attached fat, 1.886 to 2.247 mg/kg in the kidney and 4.259 to 4.660 mg/kg in the liver. Residues in blood were 0.297 and 0.649 mg/kg. Day 3 egg white contained 0.01 and 0.41 mg/kg and egg yolk contained 0.05 and 0.27 mg/kg, for the phenyl-^{14}C and triazole-^{14}C treatments, respectively.

The metabolism of difenoconazole occurred via the hydrolysis of the dioxolane ring and subsequent reduction of the ketone to an alcohol (CGA-205375) and by hydroxylation of the phenyl ring. Both ring hydroxylated difenoconazole and CGA-205375 were observed. CGA-205375 was the major metabolite found. The majority of degradation occurred through hydrolysis of the dioxolane carbon followed by reduction to the alcohol and less so by ring hydroxylation. Consistent with this observation, levels of CGA-205375 were greater than levels of hydroxy-difenoconazole and hydroxy-CGA-205375. Cleavage of the alkyl bridge between the triazole and biphenyl portions of the molecule occurred, resulting in the formation of CGA-71019 and CGA-189138. Both products were identified in hen samples, but based on the amounts found (< 10% of the TRR) this was a minor pathway in hen tissues, but the major pathway in eggs.

Comments by RMS: The study was well performed and evaluated.

B.7.2.6 Metabolism of [triazole-^{14}C] difenoconazole in laying hens

Reference:	**Ray W.J. (2004)** [Triazole-^{14}C] CGA-169374: Nature of the residue in laying hens. Dietary Safety Department, Syngenta Crop Protection Inc, Greensboro, North Carolina, United States. Unpublished Report 786-02, SAM No. 2441.
Test Material:	[Triazole-^{14}C] difenoconazole, Lot number CDC-IX-66-1, radiochemical purity 98.6%, specific activity 43.7 µCi/mg.
Guideline:	Residue Chemistry Test Guidelines, OPPTS 860.1300, Nature of the Residue – Livestock.
GLP:	Yes

Material and methods:

Test concentration:	121 mg/kg feed/day
Test system:	One gelatine capsule per day (containing 12.5 mg of the test material) was administered perorally and by hand to *Gallus gallus domestica* hens. The hens were

separately dosed over a period of four consecutive days in the morning at approximately 7:00 AM after feeding and collection of eggs and excreta. During acclimation the hens were kept in individual metabolic cages to enable the collection of eggs and excreta. Treatment room light was on 24 hours per day. During the acclimation and dosing period, room temperatures ranged from 23-28°C and humidity from 15-51%. Preweighed feed was given individually and the remaining feed was reweighed to calculate the daily feed intake. Tap water was available *ad libitum*. The hens were sacrificed *ca* 6 hours after the last dose.

Duration: 4 days

Sampling time points: Eggs and excreta were collected daily and the eggs separated into yolk and white. Blood was sampled immediately prior to sacrifice. After sacrifice, muscle, peritoneal fat, skin plus attached fat, kidney and liver were taken. Appropriate background samples were taken from control hens.

Method of analysis: Samples of egg white, egg yolk, muscle and fat were extracted with acetonitrile/water (8:2 v/v). Non-extracted residues were determined by combustion/LSC. Identification of the extracted residues was carried out by co-chromatography against standard reference compounds using 1D and 2D-TLC and HPLC. Structural confirmation of metabolites was obtained by MS and nuclear magnetic resonance spectrometry (NMR).

Number of animals: Five

Date of experiment: 28 March 2002 to 24 September 2003

Findings: Extractability of the radioactive residues from tissues, egg whites and egg yolks was high (92 to 100.7% of the TRR for tissues and 99.9 to 103.5% TRR in egg white and egg yolk). The unextracted residue was low (<5% of the TRR) and was not analysed further

The extractability of the residues in tissues, egg white and egg yolk is shown in Table B.7.2.6-1.

Table B.7.2.6-1. Extractability of residues in tissues and eggs of laying hens dosed with [triazole-^{14}C] difenoconazole for 4 consecutive days.

Sample	Sampling day	TRR (mg/kg)	CH_3CN/H_2O extract[a]		Non-extractable		Total	
			% TRR	mg/kg	% TRR	mg/kg	% TRR	mg/kg
Egg white	4	3.962	99.86	3.957	0.38	0.015	100.24	3.972
Egg yolk	4	7.735	103.49	8.005	0.68	0.053	104.17	8.058
Egg yolk	3[b]	4.458	99.9	4.453	1.7	0.076	101.6	4.529
Liver	4	13.282	97.11	12.898	2.72	0.361	99.83	13.259
Muscle	4	4.853	91.79	4.454	4.78	0.232	96.57	4.686
Fat	4	10.393	100.74	10.470	0.74	0.077	101.48	10.547

[a] Total extracted radioactivity.
[b] Day 3 yolk sample extracted following loss of Day 4 sample during processing.

The overall recovery of the dosed radioactivity was 91.5% (Table B.7.2.6-2). The majority of the administered dose was found in the excreta (65.5%) and carcass (16.85%). Totals of 1.2% and 1.5 % were recovered in eggs and blood, respectively. Low levels of administered radioactivity were retained in the tissues (6.5%), of which 0.53% was recovered in skin plus attached fat, 1.1% in peritoneal fat, 1.3% in liver, and 3.5% in muscle.

Tissue residues were 4.010 to 7.231 mg/kg in muscle, 3.812 to 10.298 mg/kg in the skin plus attached fat, 7.674 to 12.353 mg/kg in the peritoneal fat and 9.234 to 20.409 mg/kg in the liver. Residues in egg white, egg yolk and blood were 3.007 to 6.210 mg/kg, 5.186 to 9.396 and 4.931 to 9.259 mg/kg, respectively.

The residue levels in the tissues, eggs and blood is given in Table B.7.2.6-3.

Table B.7.2.6-2. Recovery of the administered dose from laying hens dosed with [triazole-^{14}C] difenoconazole for 4 consecutive days.

Sample	Recovery (% of administered dose)					
Hen number	998	999	1000	1001	1002	**Mean**
Total excreta	67.89	71.17	68.16	54.91	68.97	65.53
Tissue Total	6.03	5.82	6.50	7.63	6.93	6.50
Liver	1.34	0.97	1.31	1.66	1.22	1.32
Muscle	3.27	3.16	3.32	4.38	3.36	3.53
Skin plus attached fat	0.51	0.61	0.51	0.47	1.12	0.53
Peritoneal fat	0.91	1.08	1.36	1.12	1.23	1.12
Blood	1.36	1.08	1.79	1.56	1.23	1.45
Carcass	15.60	13.70	15.40	22.70	15.30	16.85
Eggs Total	1.25	0.85	1.11	1.17	0.15	1.18
Egg white						
Day 1	0.03	0.02	0.01	0.12	0.11	0.06
Day 2	0.22	0.14	0.15	n.s.	n.s.	0.17
Day 3	0.23	0.18	0.21	0.31	n.s.	0.23
Day 4	0.27	0.21	0.22	0.38	n.s.	0.27
Egg yolk						
Day 1	n.d.	n.d.	n.d.	0.01	0.04	0.01
Day 2	0.10	0.04	0.07	n.s.	n.s.	0.07
Day 3	0.17	0.10	0.17	0.11	n.s.	0.14
Day 4	0.23	0.16	0.28	0.24	n.s.	0.23
Total recovery	92.13	92.62	92.96	87.97	92.58	91.51

n.s. = No sample.
n.d. = Not detected.

Table B.7.2.6-3. Residue levels in tissues and eggs from laying hens dosed with [triazole-^{14}C] difenoconazole for 4 consecutive days.

Sample / Hen number	Residue levels (mg/kg)					
	998	999	1000	1001	1002	Composite
Liver	10.852	9.234	12.571	20.409	12.737	13.282
Muscle	4.130	4.010	4.213	7.231	4.565	4.853
Skin plus attached fat	3.812	4.429	4.586	5.126	10.298	n.a.
Peritoneal fat	7.674	9.112	11.448	12.353	11.083	10.393
Whole blood	6.149	4.931	8.153	9.259	5.968	n.a.
Egg white						
Day 1	0.386	0.336	0.142	1.842	0.947	0.758
Day 2	2.463	1.957	2.111	n.s.	n.s.	2.217
Day 3	3.395	2.647	3.025	4.719	n.s.	3.436
Day 4	3.836	3.007	3.246	6.210	n.s.	3.962
Egg yolk						
Day 1	0.096	0.076	0.032	0.549	0.569	0.320
Day 2	2.091	1.330	2.256	NS	n.s.	1.910
Day 3	5.619	2.973	5.409	4.075	n.s.	4.458
Day 4	8.387	5.186	9.396	8.343	n.s.	7.735

n.s. = No sample.
n.a.= Non applicable.

Except for egg white, parent difenoconazole was detected in all other tissues analysed at levels ranging from 2.2% TRR in muscle up to 18.4% TRR in fat (Table B.7.2.6-4).

The major metabolite found in egg whites was CGA-71019, which occurred at levels of 75.1% of the TRR. An unidentified compound Metabolite A, which was found to degrade readily to CGA-71010, was detected at levels of 18.5% TRR. A small amount (2.6% TRR) of the metabolite CGA-205375 was also found, in addition to 0.7% TRR of a second unidentified compound labelled Unknown 2. Further characterisation by ion exchange resin and MS showed that Metabolite A was acidic and probably of low molecular weight. Degradation to 1,2,4-triazole (CGA-71019) during sample preparation showed the molecule was unstable and the formation of 1,2,4-triazole suggested the presence of an unstable triazole conjugate.

The major metabolites in Day 3 egg yolks were CGA-205375 and CGA-71019 at 54.0 and 31.7% of the TRR, respectively. Small amounts of the unidentified compounds, Metabolite A and Unknown 6 and 7 were also detected (≤ 2% TRR).

In the liver, the major metabolites were CGA-205375 and CGA-71019 (55 and 18% of the TRR, respectively). The remainder of the extracted residue was composed of small amounts of the unidentified compounds Metabolite A, Unknowns 1 and Unknowns 3, 4 and 5 (≤ 5% TRR).

The metabolites CGA-71019 and CGA-205375 formed the majority of the residue in muscle with levels of 54.7 and 25.5% of the TRR detected, respectively. Unknown 7 occurred at levels less than 3% of the TRR.

The major residue in fat was CGA-205375 at levels of 60.4% of the TRR followed by parent difenoconazole with 18.4% of the TRR. The metabolite CGA-71019 was also detected at a level of 4.6% of the TRR. Unidentified compounds were present at low levels, with Unknowns 4, 6 and 7 present at levels of 0.5, 1.4 and 5.4% of the TRR, respectively.

The distribution of metabolites and residue levels is summarised in Table B.7.2.6-4.

Table B.7.2.6-4. Distribution of radioactive metabolites in edible tissues and eggs and from laying hens dosed daily for 4 days with [triazole-^{14}C] difenoconazole.

	Egg white		Egg yolk[a]		Liver		Muscle		Fat	
	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg	%TRR	mg/kg
Difenoconazole	n.d.	n.d.	5.3	0.236	5.9	0.784	2.2	0.107	18.4	1.912
CGA-205375	2.6	0.103	54.0	2.407	55.3	7.345	25.5	1.238	60.4	6.277
CGA-205374	n.d.	n.d.	n.d.	n.d.	1.8	0.239	n.d.	n.d.	n.d.	n.d.
CGA-71019	75.1	2.976	31.7	1.413	18.0	2.391	54.7	2.655	4.6	0.478
Metabolite A	18.5	0.733	0.3	0.013	0.7	0.093	n.d.	n.d.	n.d.	n.d.
Unknown 1	n.a.	n.a.	n.a.	n.a.	1.4	0.186	n.a.	n.a.	n.a.	n.a.
Unknown 2	0.7	0.028	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Unknown 3	n.a.	n.a.	n.a.	n.a.	5.0	0.664	n.a.	n.a.	n.a.	n.a.
Unknown 4	n.a.	n.a.	n.a.	n.a.	2.9	0.385	n.a.	n.a.	0.5	0.052
Unknown 5	n.a.	n.a.	n.a.	n.a.	3.5	0.465	n.a.	n.a.	n.a.	n.a.
Unknown 6	n.a.	n.a.	0.9	0.040	n.a.	n.a.	n.a.	n.a.	1.4	0.146
Unknown 7	n.a.	n.a.	2.0	0.089	n.a.	n.a.	2.4	0.116	5.4	0.561
Unresolved	1.6	0.063	1.9	0.085	2.4	0.319	4.8	0.233	7.2	0.748
Total identified	77.7	3.079	91.0	4.056	79.2	10.759	82.4	4.000	83.4	8.667
Non-extracted	0.38	0.015	1.70	0.076	2.72	0.361	4.78	0.232	0.74	0.077
Total characterised	96.58	3.827	93.00	4.145	84.42	11.213	87.18	4.232	84.14	8.744

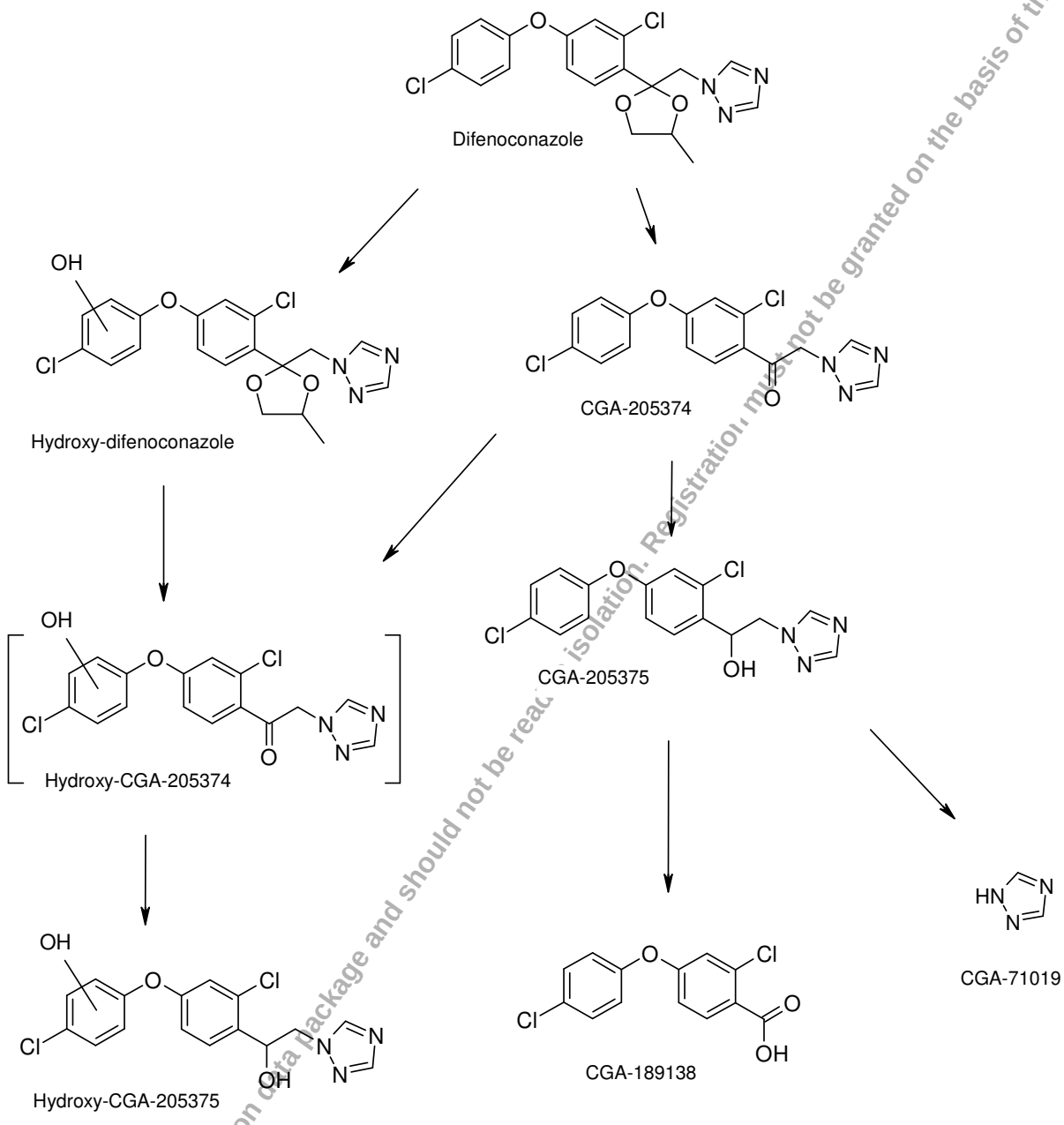
[a] Day 4 egg yolk was inadvertently spilled, therefore Day 3 egg yolk was used for characterisation/identification.
n.d. = Not detected.
n.a. = Not applicable.

Conclusions: Following oral dosing for four consecutive days with [triazole-^{14}C] difenoconazole at levels in the diet equivalent to 121mg/kg, the majority of the administered dose was found in the excreta (55 to 71%). Total tissue residues accounted for between 5.8 and 7.6% of the treatment, representing up to 4.853 mg/kg in muscle, 3.812 to 10.298 mg/kg in skin plus attached fat and 13.282 mg/kg in liver. Blood contained 4.931 to 9.259 mg/kg, Day 4 egg whites and egg yolks contained 3.962 and 7.735 mg/kg.

Following administration of difenoconazole, the proposed metabolic pathway of difenoconazole in the hen involves hydrolysis of the dioxolane ring to form the ketone CGA-205374, with subsequent reduction of CGA-205374 to give the corresponding alcohol CGA-205375. Cleavage of the alkyl bridge between the triazole and biphenyl portions of the molecule occurred, leading to the formation of CGA-71019. Unidentified metabolites that individually did not exceed 5% of the TRR were observed in tissues and eggs.

The proposed metabolic pathway of difenoconazole in hens is shown in Figure B.7.2.6-1.

Comments by RMS: The study was well performed and reported.



[ ] Proposed metabolite not identified in the study.

Figure B.7.2.6-1. Proposed metabolic pathway of difenoconazole in laying hens.

B.7.2.4 Metabolism, distribution and expression of residue in livestock - summary and conclusions

Metabolism studies of difenoconazole were carried out in lactating goats and laying hens. The metabolism studies were performed using two radiolabelled forms of difenoconazole, [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole.

Capsules containing the test substance were administered orally to lactating goats and laying hens with concentrations corresponding to doses of 5 to 100 ppm in feed to the lactating goats and 5, 68 and 121 ppm in feed to the laying hens. Difenoconazole was rapidly metabolised, with the majority of the administered radioactivity excreted in the urine and faeces (up to 96.8% in hen and up to >88% in goat).

Maximum residue levels were present in the liver and kidney, at 9.790 and 2.731mg/kg, respectively, in lactating goats and up to 4.660 and 2.247 mg/kg, respectively, in laying hens. Higher tissue residues (up to 20.409 mg/kg in liver) were observed in the hen following an extremely high dose of difenoconazole (121 mg/kg for 4 days) and sampling immediately after the final dose.

In lactating goats and laying hens, maximum residues of parent difenoconazole were detected in the liver and fat, at concentrations up to 0.891 (9.1% TRR) and 1.912 mg/kg (18.4% TRR), respectively. In other edible tissues, residues of parent difenoconazole were ≤ 0.107 mg/kg (2.2% TRR). In milk, residues of parent difenoconazole were up to 0.028 and mg/kg (8.8% TRR) and up to 0.236 mg/kg (5.3% TRR) in egg yolk .

Plateau level of residues in milk was reached after *ca* 2 and 6 days for the [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole treated goats, respectively, with residues in the [triazole-^{14}C] group being 2 – 3 times higher than the [phenyl-^{14}C] group (see Figure B.7.2.1-2 and B.7.2.1-3). Plateau level of residues in egg whites and egg yolks was reached slowly, i.e. after ca 5 and 7 days, respectively (see Figure B.7.2.4-1 and B.7.2.4-2). The TRR in the [triazole-^{14}C] egg whites were 10 – 15 times lower than the [phenyl-^{14}C] egg whites while the TRR in egg yolks was very similar for the two labels.

CGA-205375 was the major metabolite in the goats and hens, occurring at levels up to 7.127 mg/kg (72.8% TRR) in liver, 1.180 mg/kg (43.2% TRR) in kidney, 0.949 mg/kg (91.7% TRR) in fat, 0.423 mg/kg (91.4% TRR) in muscle and up to 0.130 mg/kg (34.4% TRR) in milk, egg white and egg yolk. 1,2,4-triazole CGA-71019 was transported preferentially to eggs and milk, occurring at levels of 0.182 mg/kg (67.7% TRR) and 0.043 mg/kg (32.3% TRR) in egg white and yolk, respectively and levels up to 0.022 mg/kg (5.8% TRR) in milk. Ring hydroxylated difenoconazole, CGA-205374 and CGA-205375 were observed in the goats at levels up to 0.235 mg/kg (3.9% TRR) in liver and 0.021 mg/kg (15.2% TRR) in milk. Ring hydroxylated difenoconazole and CGA-205375 were observed in the hen at levels up to 0.457 mg/kg (9.8% TRR) in the liver.

Glucuronide and sulphate conjugates of difenoconazole and CGA-205375 and a glycine conjugate of CGA-189138 were also major metabolites in the goats. The highest residues of these individual metabolites

in the tissues and milk were 0.734 mg/kg (7.5% TRR) in liver (CGA-205375 glucuronide conjugate), 0.114 mg/kg (35.8% TRR) in milk (CGA-189138 glycine conjugate), 0.273 mg/kg (10.0% TRR) in kidney (CGA-205375 sulphate conjugate), 0.176 mg/kg (1.8% TRR) in liver (OH-CGA-205375 sulphate conjugate), 0.157 mg/kg (1.5% TRR) in liver (OH-difenoconazole glucuronide conjugate) and 0.009 mg/kg (2.9% TRR) in milk (OH-difenoconazole sulphate conjugate).

The primary metabolic processes in each animal involves:
- hydrolysis of the dioxolane ring to form the ketone CGA-205374, with subsequent reduction of CGA-205374 to give the corresponding alcohol CGA-205375 as a major metabolite.
- Oxidation of CGA-205374 resulted in cleavage of the alkyl bridge, leading to the formation of the acid CGA-186138 and 1,2,4-triazole CGA-71019.
A second pathway involves hydroxylation of difenoconazole to form the hydroxylated CGA-205374 and CGA-205375. Sulphate ester, glycine and glucuronide conjugation were observed as secondary metabolism processes in the lactating goats. Hydroxy acetic acid difenoconazole and amino acid (glutamic acid/threonine) conjugates of CGA-189138 were also observed as urine specific metabolites.

As the metabolic pattern in ruminants does not significantly differ compared to rats, a pig study is not required (Guideline document 7030/VI/95-rev.3, 22/7/1997). For details to the metabolism studies performed with difenoconazole in rats please see Volume 3, Annex B.6: Toxicology and Metabolism.

The proposed metabolism of difenoconazole in lactating goat, laying hen and rat is shown in Figure B.7.2.4-1. A list of the identified compounds in lactating goats and laying hens dosed daily for 3 days with [phenyl-^{14}C] or [triazole-^{14}C] difenoconazole is presented in Table B.7.2.4-1.

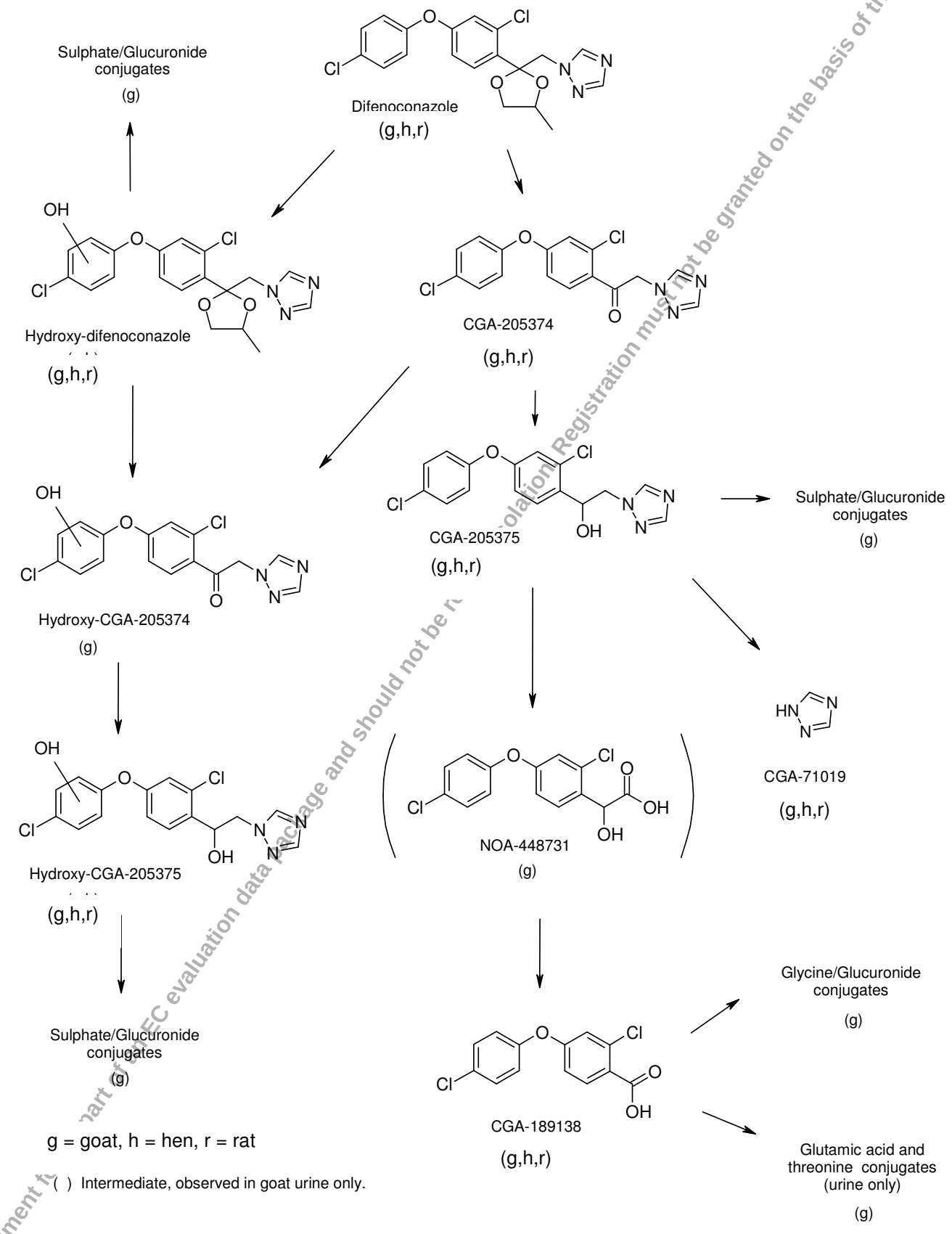


Figure B.7.2.4-1. Proposed metabolic pathway of difenoconazole in lactating goat, laying hen and rat.

Table B.7.2.4-1. List of identified compounds found in goats and laying hens dosed daily 3 days with [phenyl-^{14}C][1] or [triazole-^{14}C][2] difenoconazole.

Designation	Chemical Name (IUPAC)	Presence in goat mg/kg[a] (%TRR)[b]							Presence in hen mg/kg[a] (%TRR)[b]						
		Milk	Liver	Kidney	Muscle	Fat	Urine	Faeces	Muscle	Fat + skin	Liver	Kidney	Egg white	Egg yolk	Excreta
Difenoconazole CGA-169374	1-[2-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-4-methyl-[1,3]dioxolan-2-ylmethyl]-1H-[1,2,4]triazole or 1H-1,2,4-tiazole, 1-[[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]- (CA)	0.012[1] (8.6)	0.403[1] (6.7)	0.023[1] (1.5)	0.007[1] (3.5)	0.018[1] (3.2)	(0.3)[1]	(27.6)[1]	--[1]	0.007[1] (1.6)	0.200[1] (4.3)	--[1]	0.001[1] (2.7)	n.d. (0.9)[1]	(46.2)[1]
		0.023[2] (6.2)	0.616[2] (8.2)	0.095[2] (5.2)	0.021[2] (3.7)	0.074[2] (6.5)	(0.3)[2]	(41.7)[2]	0.005[2] (0.9)	0.007[2] (1.5)	--[2]	0.032[2] (1.7)	0.013[2] (4.9)	0.001[2] (0.8)	(60.1)[2]
CGA-205375	1-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-2-[1,2,4]triazol-1-yl-ethanol	0.029[1] (21.3)	3.183[1] (52.9)	0.478[1] (30.9)	0.138[1] (68.5)	0.412[1] (73.5)	(30.0)[1]	(10.8)[1]	0.035[1] (34.8)	0.288[1] (63.5)	1.640[1] (35.2)	0.490[1] (21.8)	0.019 (84.7)	0.027[1] (72.9)	(5.1)[1]
		0.130[2] (34.4)	3.740[2] (49.8)	0.925[2] (51.0)	0.244[2] (43.2)	0.860[2] (75.3)	(48.1)[2]	(16)[2]	0.045[2] (8.9)	0.212[2] (45.6)	1.278[2] (30.0)	0.372[2] (19.7)	0.021[2] (7.7)	0.047[2] (35.2)	(0.4)[2]
CGA-205374	1-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-2-[1,2,4]triazol-1-yl-ethanone	--[1]	0.138[1] (2.3)	--[1]	--[1]	--[1]	(1.4)[1]	(3.5)[1]	--[1]	0.008[1] (1.7)	0.084[1] (1.8)	0.117[1] (5.2)	--[1]	--[1]	--[1]
		--[2]	--[2]	--[2]	0.008[2] (1.4)	--[2]	(0.6)[2]	(5.1)[2]	--[2]	0.004[2] (0.9)	0.060[2] (1.4)	0.040[2] (2.1)	--[2]	--[2]	--[2]
CGA-189138	2-chloro-4-(4-chloro-phenoxy)-benzoic acid	0.009[1] (6.3)	--[1]	--[1]	--[1]	--[1]	(8.7)[1]	(1.6)[1]	--[1]	--[1]	0.126[1] (2.7)	--[1]	--[1]	--[1]	--[1]
CGA-71019	1H-[1,2,4]triazole	0.022[2] (5.8)	--[2]	--[2]	0.010[2] (1.8)	--[2]	(7.6)[2]	(5.1)[2]	0.025[2] (4.9)	0.004[2] (0.9)	0.230[2] (5.4)	0.130[2] (6.9)	0.182[2] (67.7)	0.043[2] (32.3)	--[2]
OH-CGA difenoconazole		--	--	--	--	--	--	--	--[1]	--[1]	0.135[1] (2.9)	0.108[1] (4.8)	0.003[1] (12.5)	0.005[1] (12.4)	--[1]
									--[2]	--[2]	--[2]	0.060[2] (3.2)	0.003[2] (1.0)	0.006[2] (4.2)	--[2]
OH-difenoconazole-isomer		0.021[1] (15.2)	--[1]	--[1]	--[1]	0.23[1] (4.1)	(6.4)[1]	--[1]							
		--[2]	--[2]	--[2]	--[2]	0.021[2] (1.8)	(<0.1)[2]	(1.2)[2]							

Designation	Chemical Name (IUPAC)	Presence in goat mg/kg[a] (%TRR)[b]							Presence in hen mg/kg[a] (%TRR)[b]						
		Milk	Liver	Kidney	Muscle	Fat	Urine	Faeces	Muscle	Fat + skin	Liver	Kidney	Egg white	Egg yolk	Excreta
OH-difenoconazole-isomer		--[1]	--[1]	--[1]	--[1]	0.010[1] (1.8)	--[1]	--[1]							
OH-CGA-205375									0.004[1] (4.3)	0.037[1] (8.2)	0.457[1] (9.8)	0.072[1] (3.2)	0.001[1] (2.9)	n.d. (0.7)[1]	(2.5)[1]
									0.007[2] (1.4)	0.026[2] (5.7)	0.349[2] (8.2)	0.092[2] (4.9)	0.002[2] (0.7)	0.003[2] (2.0)	(8.7)[2]
OH-CGA-205375 isomer		0.006[1] (4.4)	0.132[1] (2.2)	0.031[1] (2.0)	--[1]	0.019[1] (3.4)	(12.4)[1]	(6.3)[1]							
		0.011[2] (3.0)	--[2]	--[2]	0.013[2] (2.3)	0.014[2] (1.2)	(27.9)[2]	(8.0)[2]							
OH-CGA-205375 isomer		--[1]	0.235[1] (3.9)	--[1]	--[1]	--[1]	(4.0)[1]	--[1]							
		--[2]	--[2]	--[2]	--[2]	--[2]	(1.4)[2]	--[2]							
Glycine-CGA-189138[c]		0.114[1] (35.8)	0.039[1] (0.4)	0.303[1] (11.1)	0.004[1] (0.9)	--[1]	--	--							
Glucuronide-CGA-205375[c]		0.002[1] (0.5)	0.734[1] (7.5)	0.464[1] (17.0)	0.006[1] (1.4)	--[1]	--	--							
Sulphate-CGA-205375[c]		0.034[1] 10.7	0.068[1] 0.7	0.273[1] 10.0	0.002[1] 0.5	--[1]	--	--							
Sulphate-OH-CGA-205375[c]		0.013[1] (4.1)	0.176[1] (1.8)	0.082[1] (3.0)	--[1]	--[1]	--	--							
Glucuronide-OH-difenoconazole[c]		--[1]	0.157[1] (1.6)	0.019[1] (0.7)	--[1]	--[1]	--	--							
Sulphate-OH-difenoconazole[c]		0.009[1] (2.9)	--[1]	--[1]	--[1]	--[1]	--	--							
Glucuronide-OH-CGA-205375[c]		--[1]	--[1]	0.027[1] (1.0)	--[1]	--[1]	--	--							
Glucuronide-OH-CGA-189138[c]		--[1]	--[1]	0.087[1] (3.2)	--[1]	--[1]	--	--							

[a] mg/kg difenoconazole equivalents.
[b] Percent of the total radioactive residues.
[c] See study B.7.2.3.

B.7.3 Definition of the residue (Annex IIA 6.7; Annex IIIA 8.6)

Proposed residue definition (plants, plant products):

CGA-71019 (1,2,4-triazole)

1,2,4-triazole has a moderate acute oral toxicity. The LD_{50} value for 1,2,4-triazole in rats was 1,648 mg/kg bw when administered by the oral route and should be classified as Xn, R22 (harmful if swallowed) according to 67/548/EEG. Teratogenic effects of 1,2,4-triazole (an increased incidence of cleft palate) were observed in one of two teratogenicity studies presented in Annex B.6: Toxicology and Metabolism.
Due to its teratogenic effects, 1,2,4-triazole is classified as R63 "Possible risk of harm to the unborn child".
Because only a single Ames has been conducted, new studies on the genotoxicity of 1,2,4-triazole are being initiated. The RMS suggest that these studies could be included in an Addendum.

CGA-142856 (triazole acetic acid)

CGA-142856 has a low acute oral toxicity. The LD_{50} value for CGA-142856 in rats was >5,000 mg/kg bw when administered by the oral route. This metabolite is considered to be toxicologically non-relevant (see Annex B.6: Toxicology and Metabolism).

Following foliar application to spring wheat, CGA-142856 and CGA-71019 were found at levels up to 0.28 mg/kg (20% TRR) and at up to 0.14 mg/kg (10% TRR) in wheat grain, respectively. Following seed treatment, which is the intended application, at 2-4 times higher the proposed critical GAP of 6 g a.i./100 kg seed or 12 g a.i./ha, the majority of the radioactivity was aqueous soluble and was either identified as CGA-71019 or CGA-142856. In grape, CGA-71019 occurred at low levels (0.004 mg/kg, 3.6% TRR).

CGA-131013 (triazole alanine)

CGA-131013 was the major metabolite in oilseed rape seeds and in mature potato tubers, occurring at levels up to 1.276 mg/kg (56.4% TRR) and up to 0.068 mg/kg (78.9% TRR), respectively. This metabolite is considered to be toxicologically non-relevant (see Annex B.6: Toxicology and Metabolism).

CGA-205369 (triazole lactic acid)

CGA-205369 was only identified in a rotational crop study following difenoconazole application to bare soil. This metabolite was found at levels up to 0.025 mg/kg (21.2% TRR) in wheat stalks, 0.020 mg/kg (9.7% TRR) in maize grain, 0.019 mg/kg (12.1% TRR) in wheat husks and 0.017 mg/kg (54.3% TRR) in sugar beet tops. Studies on the genotoxicity of triazole lactic acid are being initiated. The RMS suggest that these studies could be included in an Addendum.

The levels of the triazole metabolites observed in the available plant metabolism studies, indicate that they are unlikely to exceed the proposed MRL when difenoconazole is used as the proposed GAP.

Based on the results of the metabolism studies in cereals (wheat), root vegetables (potatoes), fruits (tomatoes, grapevine) and pulses/oilseeds (oilseed rape), the proposed residue definition in plants is difenoconazole alone for both monitoring and risk assessment purposes. If, however, the intended use within the EU is to expand the use pattern to include foliar application to cereals and/or for use in oilseed rape, this conclusion should be re-evaluated.

Proposed residue of definition (animals, products of animal origin):

CGA-205375

The difenoconazole specific metabolite, CGA-205375, was the major metabolite in the goats and hens following doses of 100 and 68 mg difenoconazole/kg in the diet, respectively.

In dairy cattle, following administration of 10 mg difenoconazole/kg diet (i.e., 43 times higher than the realistic estimated feeding rate of 0.23 mg difenoconazole/kg diet calculated in B.7.8.1.1-3) CGA-205375 levels were 0.3 mg/kg in liver, 0.08 mg/kg in fat, 0.04 mg/kg in kidney, 0.02 mg/kg in muscle and 0.007 mg/L in milk.

CGA-71019 (1,2,4-triazole)

In hens dosed with 68 mg difenoconazole/kg diet (i.e., 4250 times higher than the realistic estimated feeding rate of 0.016 mg difenoconazole/kg diet calculated in B.7.8.2-5), CGA-71019 was found at levels of 0.182 mg/kg (67.7% TRR) and 0.043 mg/kg (32.3% TRR) in egg white and egg yolk, respectively.
In goats dosed with 100 mg difenoconazole/kg diet, levels up to 0.022 mg/kg (5.8% TRR) were found in milk.

It can be concluded that following the use of difenoconazole according to the proposed GAP, negligible exposure to difenoconazole and its metabolites in edible animal commodities is expected.

Based on the results of metabolism studies in the lactating goats and laying hens, the proposed definition of the residue in animals and products of animal origin is parent difenoconazole for risk assessment purposes. For the purposes of monitoring, the proposed definition in animals and products of animal origin is parent difenoconazole plus metabolite CGA-205375.

B.7.4 Use pattern

Difenoconazole (1-[2-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-4-methyl-[1,3]dioxolan-2-ylmethyl]-1H-[1,2,4] triazole) is a systemic triazole fungicide used for long-lasting preventative and curative broad-spectrum control of cereal, fruit and vegetable diseases including powdery mildew, rust, scab and leaf spots.

The compound is formulated either as a 250 g active ingredient (a.i.)/L emulsifiable concentrate (EC) or as a flowable concentrate (FS). The intended use in the EU regions is in the cultivation of cereals (wheat, barley, triticale, rye, oats; seed treatment), carrot and pome fruits (foliar application).

In Southern Europe, difenoconazole will be applied on pome fruit at a maximum of 75 g a.i./ha per application at a maximum of 4 applications per season and with a pre-harvest interval (PHI) of 14 days. In Northern Europe, the application rate will be lower (56.25 g a.i./ha per application) with a maximum of 4 applications per season and with a PHI of 28 days. In Northern and Southern Europe, difenoconazole will be applied on carrots at a maximum of 125 g a.i./ha per application with a maximum of three applications per season and with a PHI of 14 days. In Northern and Southern Europe, difenoconazole will be applied once to seeds of cereals at a maximum of 6 g a.i./100 kg seeds.

Table B.7.4-1. Use pattern of difenoconazole.

Crop	Country	Formulation type (code) & content of a.i. (g/kg)	Application					PHI days
			Method	kg a.i./ha	kg a.i./hl	Water L/ha	Number	
Pome fruit	EU (N/S)	EC 250	Spray	Northern 0.01875 – 0.05625 Southern 0.0375 – 0.0750	Northern 0.00375 Southern 0.0075	500 - 1500	1 - 4	Northern 28 Southern 14
Carrot	EU (N/S)	EC 250	Spray	0.125	--	100 - 500	1 – 3	14
Cereals (wheat, barley, triticale, rye, oats)	EU (N/S)	FS 30	Seed treatment	0.005 – 0.012	0.03 – 0.06 kg as/tonne	--	1	--

B.7.5 Identification of critical GAPs

Pesticide(s) (common name) : Difenoconazole
CCPR No(s) :
Trade name(s) : Score / Dividend
Main uses (e.g. insecticide, fungicide) : Fungicid

1		2	3	4	5	6				7			8	9
Crop and/or situation (a)	Member State or country	F or G (b)	Pest or group of pest controlled (c)	Formulation rate Type (d)	Conc. of a.i. (e)	Application method, kind (f-g)	growth stage (h)	number (range)	interval between application (min)	Application rate per treatment kg a.i./hl min max	water L/ha min max	kg a.i./ha min max	PHI (days) (k)	Remarks (l)
Pome fruit	Northern and Southern Europe	F	*Podosphaera leucotricha* *Venturia inaequalis*	EC	250 g/l	High volume spray or mist blower	BBCH 61 (flowering stage)	1 – 4	10 – 14	0.00375	500 - 1500	0.01875 - 0.05625	28	Northern EU
										0.0075	500 - 1000	0.0375 - 0.0750	14	Southern EU
Carrot	Northern and Southern Europe	F	*Alternaria dauci* *Erysiphe heracli*	EC	250 g/l	High volume spray	BBCH 42/43	1 – 3	14	--	100 - 500	0.125	14	
Cereals	Northern and Southern Europe	F	*Fusarium spp.* *Septoria spp.* *Tilletia spp.* *Ustilago spp.* *Cochliobolus sativus*	EC	30 g/l	Seed treatment	BBCH 00	1	--	0.03 - 0.06 kg a.i./tonne	--	0.005 – 0.012	--	kg a.i./ha rate depends on seeding rate

(a) in case of group of crops the Codex classification should be used
(b) outdoor or field use (F), or glasshouse application (G)
(c) e.g. biting and sucking insects, soil born insects, foliar fungi
(d) e.g. wettable powder (WP), emulsifiable concentration (EC), granulate (GR)
(e) g/kg or g/l
(f) method e.g. high volume spraying, low volume spraying, spreading, dusting, drench
(g) kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants
(h) growth stage at last treatment
(k) PHI = Pre-harvest interval
(l) remarks may include: Extent of use / economic importance / restrictions (e.g. feeding, grazing)

B.7.6 Residues resulting from supervised trials (Annex IIA 6.3; Annex IIIA 8.2)

A total of 25 individual trials in countries of the Northern EU region and 25 individual trials in countries of the Southern EU region, were conducted in spring wheat, pome fruit and carrot over eight seasons. There were 14 trials in spring wheat (Table B.7.6-3), 20 trials in pome fruit (Table B.7.6-4) and 16 trials in carrot (Table B.7.6-5). The locations, seasons, the commercial products used in the trials are summarised in Table B.7.6-1 (Northern EU) and B.7.6-2 (Southern EU).

Table B.7.6-1. Residue trials in Northern European regions.

Reference	Location	Year	No. of trials	Commercial product (name)	Content of a.i. (g/l)	Formulation	Adjuvant	Other a.i. in formulation	Crop
Pointurier R. (2000a,b)	France F-45480 F-49125	1998	2	A-9996A	12.5	FS	N/A	Thiamethoxam (131.25 g/l) Fludioxonil (12.5 g/l) Tefluthrin (50 g/l)	Spring wheat
Pointurier R. (2000c,d)	France F-45480 F-49125	1998	2	A-9996A	25	FS	N/A	Thiamethoxam (262.5 g/l) Fludioxonil (25 g/l)	Spring wheat
Simon P. (2002a,b)	Germany D-23821 D-19347	2001	2	A9142G	30	FS	N/A	None	Spring wheat
Krainz A. (2003a,b)	United Kingdom	2002	2	A-9142G	30	FS	N/A	None	Spring wheat
Pointurier R. (2001a)	France F-45370	2000	1	A-7402 G	250	EC	N/A	None	Apple
Kühne-Thu H. (2001a)	Switzerland CH-1907	2000	1	A-7402 G	250	EC	N/A	None	Pear
Kühne-Thu H. (2001b)	Switzerland CH-1907	2000	1	A-7402 G	250	EC	N/A	None	Apple
Kühne-Thu H. (2002a)	Switzerland CH-1880	2001	1	A-7402 G	250	EC	N/A	None	Apple
Kühne-Thu H. (2002b)	Switzerland CH-1907	2001	1	A-7402 G	250	EC	N/A	None	Pear
Pointurier R. (2002a)	France F-45370	2001	1	A-7402 G	250	EC	N/A	None	Apple
Krainz A.	France	2002	1	A-7402 G	250	EC	N/A	None	Apple

Reference	Location	Year	No. of trials	Commercial product (name)	Content of a.i. (g/l)	Formulation	Adjuvant	Other a.i. in formulation	Crop
(2003e)	F-45560								
Krainz A. (2003f,g)	France F-71570	2002	2	A-7402 G	250	EC	N/A	None	Apple
Kühne-Thu H. (1988)	Switzerland CH-1896	1987	1	EC 250	250	EC	N/A	None	Carrot
Kühne-Thu H. (1989)	Switzerland CH-1896	1987	1	EC 250	250	EC	N/A	None	Carrot
Maffezoni M. (1993a,b)	France F-02	1991	2	A-7402A	250	EC	N/A	None	Carrot
Maffezoni M. (1993c)	France F-49	1992	1	A-7402A	250	EC	N/A	None	Carrot
Maffezoni M. (1995)	France F-49	1993	1	F70464 EC250	250	EC	N/A	None	Carrot
Pointurier R. (2001c)	France F-37270	2000	1	A-7402 G	250	EC	N/A	None	Carrot
Pointurier R. (2001d)	France F-51240	2000	1	A-7402 G	250	EC	N/A	None	Carrot

Table B.7.6-2. Residue trials in Southern European regions.

Reference	Location	Year	No. of trials	Commercial product (name)	Content of a.i. (g/l)	Formulation	Adjuvant	Other a.i. in formulation	Crop
Pointurier R. (2000 e, f)	France F-34590 F-82700	1998	2	A-9996 A	12.5	FS	N/A	Thiamethoxam (131.25 g/l) Fludioxonil (12.5 g/l) Tefluthrin (50 g/l)	Spring wheat
Pointurier R. (2000 g, h)	France F-34590 F-82700	1998	2	A-9997 A	25	FS	N/A	Thiamethoxam (262.5 g/l) Fludioxonil (25 g/l)	Spring wheat
Krainz A. (2003c,d)	France F-01190 F-01380	2002	2	A-9142	30	FS	N/A	None	Spring wheat
Pointurier R.	France	2000	1	A-7402 G	250	EC	N/A	None	Apple

Reference	Location	Year	No. of trials	Commercial product (name)	Content of a.i. (g/l)	Formulation	Adjuvant	Other a.i. in formulation	Crop
(2001b)	F-11700								
Kühne-Thu H. (2001c)	Greece	2000	1	A-7402 G	250	EC	N/A	None	Apple
Kühne-Thu H. (2001d)	Italy I-37067	2000	1	A-7402 G	250	EC	N/A	None	Apple
Kühne-Thu H. (2001e)	Spain E-25262	2000	1	A-7402 G	250	EC	N/A	None	Apple
Kühne-Thu H. (2001f)	Spain E-25334	2000	1	A-7402 G	250	EC	N/A	None	Apple
Solé C. (2002a)	Italy	2001	1	A-7402 G	250	EC	N/A	None	Apple
Solé C. (2002b)	Greece	2001	1	A-7402 G	250	EC	N/A	None	Apple
Solé C. (2002c)	Greece	2001	1	A-7402 G	250	EC	N/A	None	Pear
Pointurier R. (2002b)	France F-11700	2001	1	A-7402 G	250	EC	N/A	None	Apple
Solé C. (2002d)	Spain	2001	1	A-7402 G	250	EC	N/A	None	Apple
Krainz A. (2003h)	France F-86300	2002	1	A-7402 G	250	EC	N/A	None	Pear
Maffezoni M. (1993)	France F-34	1992	1	A-7402A	250	EC	N/A	None	Carrot
Maffezoni M. (1995)	France F-30	1993	1	F70464 EC250	250	EC	N/A	None	Carrot
Maffezoni M. (1999a)	France F-31840	1996	1	A-7402 G	250	EC	N/A	None	Carrot
Maffezoni M. (1999b)	France F-30200	1996	1	A-7402 G	250	EC	N/A	None	Carrot
Maffezoni M. (1999c)	France F-34130	1996	1	A-7402 G	250	EC	N/A	None	Carrot
Pointurier R. (2001e)	France F-11700	2000	1	A-7402 G	250	EC	N/A	None	Carrot
Pointurier R. (2001f)	France F-30470	2000	1	A-7402 G	250	EC	N/A	None	Carrot
Pointurier R. (2001g)	France F-34590	2000	1	A-7402 G	250	EC	N/A	None	Carrot

Table B.7.6-3. Results of residue trials performed with difenoconazole on wheat (data used for calculations of proposed MRLs are underlined, i.e. those corresponding or approximating to critical GAP).

Reference Report No Trial No Location (a)	Commodity / Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment: g a.i. /hl	water l/ha	g a.i./100 kg seed	Dates of treatment(s) or number of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed	Residues (mg/kg)	PHI (days) (f)	Remarks: (g)
Pointurier R. (2000a) 9840501 169374 / 2012 Izy, France (Northern EU)	Spring wheat/ Furio	1. 03 March 98 2. -- 3. 28 July 98	Seed treatment	--	--	5.6	05 Feb 98	BBCH 0	Whole plant Whole plant Grain Straw	<0.04[a] <0.04[a] <u><0.02[a]</u> <u><0.04[a]</u>	71 92 <u>153</u> <u>153</u>	Method AG-575A[1] LOQ=0.02 mg/kg (grain) LOQ=0.04 mg/kg (plant, straw) Analysis Nov 98 – Jan 99 Seed rate: 140 kg/ha
			Control	--	--	--	--	--	Whole plant Whole plant Grain Straw	<0.04 <0.04 <0.02 <0.04	71 92 153 153	
Pointurier R. (2000b) 9840502 169374 / 2013 Tiercé, France (Northern EU)	Spring wheat/ Florence Aurore	1. 24 Feb 98 2. -- 3. 30 July 98	Seed treatment	--	--	5.7	06 Oct 98	BBCH 0	Whole plant Whole plant Grain Straw	<0.04[a] <0.04[a] <u><0.02[a]</u> <u>0.04[a]</u>	65 91 <u>156</u> <u>156</u>	Method AG-575A LOQ=0.02 mg/kg (grain) LOQ=0.04 mg/kg (plant, straw) Analysis Nov 98 – Jan 99 Seed rate: 80.5 kg/ha
			Control	--	--	--	--	--	Whole plant Whole plant Grain Straw	<0.04 <0.04 <0.02 0.04	65 91 156 156	
Pointurier R. (2000c) 9840601 2016 Izy, France (Northern EU)	Spring wheat/ Furio	1. 03 March 98 2. -- 3. 01 Aug 98	Seed treatment	--	--	6.0	05 Feb 98	BBCH 0	Grain Straw	<u><0.02[a]</u> <u><0.04[a]</u>	<u>151</u> <u>151</u>	Method AG-575A LOQ=0.02 mg/kg (grain) LOQ=0.04 mg/kg (straw) Analysis Jan 99 Seed rate: 140 kg/ha
			Control	--	--	--	--	--	Grain Straw	<0.02 <0.04	151 151	

Reference Report No Trial No Location (a)	Commodity / Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment: g a.i. /hl	water l/ha	g a.i./100 kg seed	Dates of treatment(s) or number of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed	Residues (mg/kg)	PHI (days) (f)	Remarks: (g)
Pointurier R. (2000d) 9840602 2017 Tiercé, France (Northern EU)	Spring wheat/ Prinqual	1. 24 Feb 98 2. -- 3. 30 July 98	Seed treatment	--	--	6.1	06 Oct 98	BBCH 0	Grain Straw	<u><0.02[a]</u> <u>0.05[a]</u>	<u>156</u> <u>156</u>	Method AG-575A LOQ=0.02 mg/kg (grain) LOQ=0.04 mg/kg (straw) Analysis Jan 99 Seed rate: 61.2 kg/ha
			Control	--	--	--	--	--	Grain Straw	<0.02 0.08	156 156	
Simon P. (2002a) gwh14101 2300 Rohlstorf, Germany (Northern EU)	Spring wheat/ Thasos	1. 09 Apr 01 2. 02 – 12 July 01 3. 23 Aug 01	Seed treatment	--	--	5.29	03 Apr 01	BBCH 0	Grain Straw	<u><0.02[b]</u> <u><0.02[b]</u>	<u>136</u> <u>136</u>	Method AG-575A LOQ=0.02 mg/kg (grain, straw) Analysis 21 – 29 May 02 Seed rate: 168 kg/ha
			Control	--	--	--	--	--	Grain Straw	<0.02 <0.02	136 136	
Simon P. (2002b) Gwh94101 2301 Gross Niendorf, Germany (Northern EU)	Spring wheat/ Thasos	1. 11 Apr 01 2. 01 – 11 July 01 3. 18 Aug 01	Seed treatment	--	--	5.29	03 Apr 01	BBCH 0	Grain Straw	<u><0.02[b]</u> <u><0.02[b]</u>	<u>129</u> <u>129</u>	Method AG-575A LOQ=0.02 mg/kg (grain, straw) Analysis 21 – 29 May 02 Seed rate: 190 kg/ha
			Control	--	--	--	--	--	Grain Straw	<0.02 <0.02	129 129	

Reference Report No Trial No Location (a)	Commodity / Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment: g a.i. /hl	water l/ha	g a.i./100 kg seed	Dates of treatment(s) or number of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed	Residues (mg/kg)	PHI (days) (f)	Remarks: (g)
Krainz A. (2003a) 847415 2413 Barrow upon Trent, UK (Northern EU)	Spring wheat/ Paragon	1. 14 May 02 2. -- 3. 24 Sept 02	Seed treatment	--	--	4.64	09 May 02	BBCH 0	Whole plant Grain Straw	<0.01 <u><0.01</u> <u><0.01</u>	36 <u>133</u> <u>133</u>	Method AG-575A LOQ=0.01 mg/kg (plant, straw, grain) Analysis June – Aug 03 Seed rate: 250 kg/ha
			Control	--	--	--	--	--	Whole plant Grain Straw	<0.01 <0.01 <0.01	36 133 133	
Krainz A. (2003b) 847416 2414 Wilson, Derbys, UK (Northern EU)	Spring wheat/ Paragon	1. 14 May 02 2. -- 3. 17 Sept 02	Seed treatment	--	--	4.64	09 May 02	BBCH 0	Whole plant Grain Straw	<0.01 <u><0.01</u> <u><0.01</u>	36 <u>126</u> <u>126</u>	Method AG-575A LOQ=0.01 mg/kg (plant, straw, grain) Analysis 20 June – 22 Aug 03 Seed rate: 250 kg/ha
			Control	--	--	--	--	--	Whole plant Grain Straw	<0.01 <0.01 <0.01	36 126 126	
Pointurier R. (2000 e) 9840503 169374 / 2014 Marsillargues, France (Southern EU)	Spring wheat/ Florence Aurore	1. 09 Feb 98 2. -- 3. 06 July 98	Seed treatment	--	--	6.2	06 Oct 97	BBCH 0	Whole plant Whole plant Grain Straw	<0.04[a] <0.04[a] <u><0.02[a]</u> <u><0.04[a]</u>	64 92 <u>147</u> <u>147</u>	Method AG-575A LOQ=0.02 mg/kg (grain) 0.04 mg/kg (plant, straw) Analysis Nov 98 – Jan 99 Seed rate: 230 kg/ha
			Control	--	--	--	--	--	Whole plant Whole plant Grain Straw	<0.04 <0.04 <0.02 <0.04	64 92 147 147	

Reference Report No Trial No Location (a)	Commodity / Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment: g a.i. /hl	water l/ha	g a.i./100 kg seed	Dates of treatment(s) or number of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed	Residues (mg/kg)	PHI (days) (f)	Remarks: (g)
Pointurier R. (2000 f) 9840504 169374 / 2015 Escatalens, France (Southern EU)	Spring wheat/ Furio	1. 13 Feb 98 2. -- 3. 09 July 98	Seed treatment	--	--	5.9	06 Oct 97	BBCH 0	Whole plant Whole plant Grain Straw	<0.04[a] <0.04[a] <u><0.02[a]</u> <u><0.04[a]</u>	60 101 <u>145</u> <u>145</u>	Method AG-575A LOQ=0.02 mg/kg (grain) LOQ=0.04 mg/kg (plant, straw) Analysis 30 Nov 98 – 18 Jan 99 Seed rate: 180 kg/ha
			Control	--	--	--	--	--	Whole plant Whole plant Grain Straw	<0.04 <0.04 <0.02 <0.04	60 101 145 145	
Pointurier R. (2000 g) 9840603 169374 / 2018 Marsillargues, France (Southern EU)	Spring wheat/ Florence Aurore	1. 09 Feb 98 2. -- 3. 06 July 98	Seed treatment	--	--	6	06 Oct 97	BBCH 0	Grain Straw	<u><0.02[a]</u> <u>0.05[a]</u>	<u>147</u> <u>147</u>	Method AG-575A LOQ=0.02 mg/kg (grain) LOQ=0.04 mg/kg (straw) Analysis 20 – 28 Jan 99 Seed rate: 230 kg/ha
			Control	--	--	--	--	--	Grain Straw	<0.02 <0.04	147 147	
Pointurier R. (2000 h) 9840604 169374 / 2019 Escatalens, France (Southern EU)	Spring wheat/ Furio	1. 13 Feb 98 2. -- 3. 08 July 98	Seed treatment	--	--	6.3	06 Oct 97	BBCH 0	Grain Straw	<u><0.02[a]</u> <u><0.04[a]</u>	<u>145</u> <u>145</u>	Method AG-575A LOQ=0.02 mg/kg (grain) LOQ=0.04 mg/kg (straw) Analysis 20 – 28 Jan 99 Seed rate: 180 kg/ha
			Control	--	--	--	--	--	Grain Straw	<0.02 <0.04	145 145	

Reference Report No Trial No Location (a)	Commodity / Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment: g a.i. /hl	water l/ha	g a.i./100 kg seed	Dates of treatment(s) or number of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed	Residues (mg/kg)	PHI (days) (f)	Remarks: (g)
Krainz A. (2003c) 847413 2411 Sermoyer, France (Southern EU)	Spring wheat/ Paragon	1. 15 May 02 2. -- 3. 22 Aug 02	Seed treatment	--	--	4.64	09 May 02	BBCH 0	Whole plant Grain Straw	<0.01 <u><0.01</u> <u><0.01</u>	40 <u>99</u> <u>99</u>	Method AG-575A LOQ=0.01 mg/kg (plant, straw, grain) Analysis June – Aug 03 Seed rate: 280 - 300 kg/ha
			Control	--	--	--	--	--	Whole plant Grain Straw	<0.01 <0.01 <0.01	40 99 99	
Krainz A. (2003d) 847414 2412 Bage-la Ville, France (Southern EU)	Spring wheat/ Paragon	1. 15 May 02 2. -- 3. 28 Aug 02	Seed treatment	--	--	4.64	09 May 02	BBCH 0	Whole plant Grain Straw	<0.01 <u><0.01</u> <u><0.01</u>	40 <u>105</u> <u>105</u>	Method AG-575A LOQ=0.01 mg/kg (plant, straw, grain) Analysis 20 June – 22 Aug 03 Seed rate: 250 kg/ha
			Control	--	--	--	--	--	Whole plant Grain Straw	<0.01 <0.01 <0.01	40 105 105	

(a) According to the EU and Codex class classification (both) should be used
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc; overall broadcast, aerial spraying, row, individual plant, between plants - type of equipment used must be indicated.
(d) Year must be indicated
(e) BBCH monograph, growth stages of plants
(f) Minimum days after last treatment
(g) Remarks may include: Climatic conditions; reference to analytical method; information concerning metabolites included
[a] One sample taken and analysed in duplicate; mean result presented
[b] Two samples taken and analysed separately; mean result presented
[1] Method AG-575A involves extraction using methanol/ammonium hydroxide, partitioning with hexane and back partition with acetonitrile. Clean-up is performed on Sep-Pak silica, phenyl bond-elute and finally by partition into hexane ether. Difenoconazole is determined by GC using electron capture detection or mass spectrometry detection.
<u>Recoveries:</u> Straw (78-125%), whole plant (92-125%), grain (84-124%). The methods are acceptable.

Table B.7.6-4. Results of residue trials performed with difenoconazole on apple and pear (data used for calculations of proposed MRLs are underlined, i.e. those corresponding or approximating to critical GAP).

Reference Report No Trial No Location (a)	Commodity / Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment: g a.i. /hl	water l/ha	g a.i./ha	Dates of treatment(s) or number of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed	Residues (mg/kg)	PHI (days) (f)	Remarks: (g)
Pointurier R. (2001a) 0012101 2129 Clery St André, France (Northern EU)	Apple/ Idared	1. 1987 2. -- 3. 10 Oct 00	Foliar spray	3.75 3.75 3.75 3.75	1458 1411 1388 1522	55 53 52 57	11 Aug 00 22 Aug 00 31 Aug 00 11 Sept 00	BBCH 78-79	Fruit	<u>0.02</u>[a]	<u>29</u>	Method AG-575A[1] LOQ=0.01 mg/kg Analysis 05 – 09 Jan 01
			--	--	--	--	--	--	Fruit	<0.01	29	
Kühne-Thu H. (2001a) 2089/00 2155 Saxon, Switzerland (Northern EU)	Pear/ Williams	1. 1976 2. -- 3. 14 Aug 00	Foliar spray	5.9 5.9 5.9 5.9	960 960 960 960	56.25 56.25 56.25 56.25	13 June 00 26 June 00 06 July 00 17 July 00	BBCH 81	Fruit	<u>0.05</u>[a]	<u>28</u>	Method AG-575A LOQ=0.01 mg/kg Analysis March 01
			--	--	--	--	--	--	Fruit	<0.01	29	
Kühne-Thu H. (2001b) 2088/00 2156 Saxon, Switzerland (Northern EU)	Apple/ Golden Smoothy	1. 1986 2. -- 3. 19 Sept 00	Foliar spray	6.3 6.3 6.3 6.3	896 896 896 896	56.25 56.25 56.25 56.25	13 July 00 27 July 00 09 Aug 00 22 Aug 00	BBCH 84	Fruit Fruit Fruit Fruit	0.16 0.15 0.10 <u>0.07</u>[a]	0 7 14 <u>28</u>	Method AG-575A LOQ=0.01 mg/kg Analysis 09 – 16 March 01
			--	--	--	--	--	--	Fruit Fruit Fruit	<0.01 <0.01 <0.01	0 14 28	

Reference Report No Trial No Location (a)	Commodity / Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment: g a.i. /hl	water l/ha	g a.i./ha	Dates of treatment(s) or number of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed	Residues (mg/kg)	PHI (days) (f)	Remarks: (g)
Kühne-Thu H. (2002a) 2004/01 2213 Bex/Vaud, Switzerland (Northern EU)	Apple/ Primerouge	1. 1985 2. -- 3. 08 Aug 01	Foliar spray	7.2 7.2 7.2 7.2	784 784 784 784	56.25 56.25 56.25 56.25	08 June 01 19 June 01 29 June 01 09 July 01	BBCH 76	Fruit	0.04[d]	30	Method AG-575A LOQ=0.01 mg/kg Analysis 08 – 11 Jan 02
			--	--	--	--	--	--	Fruit	<0.01	30	
Kühne-Thu H. (2002b) 2005/01 2214 Saxon/Valais, Switzerland (Northern EU)	Pear/ Williams	1. 1976 2. -- 3. 13 Aug 00	Foliar spray	6.5 6.5 6.5 6.5	864 864 864 864	56.25 56.25 56.25 56.25	12 June 01 22 June 01 02 July 01 13 July 01	BBCH 77	Fruit Fruit Fruit Fruit	0.06 0.04 0.04 0.01[a]	0 7 14 31	Method AG-575A LOQ=0.01 mg/kg Analysis 08 – 11 Jan 02
			--	--	--	--	--	--	Fruit Fruit Fruit	<0.01 <0.01 <0.01	0 14 31	
Pointurier R. (2002a) 00110501 2233 Clery St André, France (Northern EU)	Apple/ Golden	1. 1992 2. -- 3. 03 Oct 01	Foliar spray	3.75 3.75 3.75 3.75	1564 1518 1491 1503	58.66 56.91 55.92 56.36	08 Aug 01 17 Aug 01 27 Aug 01 06 Sep 01	BBCH 80-81	Fruit Fruit Fruit Fruit Fruit	0.14 0.17 0.07[a] 0.08 0.03[b]	0 7 14 21 27	Method AG-575A LOQ=0.01 mg/kg Analysis 08 March – 18 Apr 02
			--	--	--	--	--	--	Fruit Fruit Fruit	<0.01 <0.01 <0.01	0 14 27	

Reference Report No Trial No Location (a)	Commodity / Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment: g a.i. /hl	water l/ha	g a.i./ha	Dates of treatment(s) or number of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed	Residues (mg/kg)	PHI (days) (f)	Remarks: (g)
Krainz A. (2003e) 847423 2386 St Denis en Val, France (Northern EU)	Apple/ Golden Delicious	1. 1991 2. -- 3. 13 Sept 02	Foliar spray	3.75 3.75 3.75 3.75	1471 1444 1431 1614	55.16 54.15 53.66 60.53	17 July 02 30 July 02 06 Aug 02 14 Aug 02	BBCH 78	Fruit	0.05[a]	30	Method AG-575A LOQ=0.01 mg/kg Analysis 06 – 13 Aug 03
			--	--	--	--	--	--	Fruit	<0.01	30	
Krainz A. (2003f) 847422 2387 La Chapelle, France (Northern EU)	Apple/ Red Star	1. 1988 2. -- 3. 19 Sept 02	Foliar spray	3.75 3.75 3.75 3.75	1500 1500 1500 1500	56.25 56.25 56.25 56.25	23 July 02 02 Aug 02 13 Aug 02 22 Aug 02	BBCH 85	Fruit	0.06[a]	28	Method AG-575A LOQ=0.01 mg/kg Analysis 23 – 27 May 03
			--	--	--	--	--	--	Fruit	<0.01	28	
Krainz A. (2003g) 847424 2385 La Chapelle, France (Northern EU)	Apple/ Golden Delicious	1. Feb 1998 2. -- 3. 19 Sept 02	Foliar spray	3.75 3.75 3.75 3.75	1500 1500 1500 1500	56.25 56.25 56.25 56.25	23 July 02 02 Aug 02 13 Aug 02 22 Aug 02	BBCH 84	Fruit	0.07[a]	28	Method AG-575A LOQ=0.01 mg/kg Analysis 27 – 29 May 03
			--	--	--	--	--	--	Fruit	<0.01	28	
Pointurier R. (2001b) 0012201 2130 Roquecourbe, France (Southern EU)	Apple/ Royal Gala	1. 1994 2. -- 3. 14 Aug 00	Foliar spray	7.5 7.5 7.5 7.5	1025 950 1000 875	77 71 75 66	30 June 00 08 July 00 20 July 00 31 July 00	BBCH 77	Fruit	0.11[a]	14	Method AG-575A LOQ=0.01 mg/kg Analysis 05 – 09 Jan 01
			--	--	--	--	--	--	Fruit	<0.01	14	

Reference Report No Trial No Location (a)	Commodity / Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment: g a.i. /hl	water l/ha	g a.i./ha	Dates of treatment(s) or number of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed	Residues (mg/kg)	PHI (days) (f)	Remarks: (g)
Kühne-Thu H. (2001c) 2042/00 2175 Tyrnavos, Larissa, Greece	Apple/ Granny Smith	1. 1992 2. -- 3. 01 Nov 00	Foliar spray	5.4 5.3 5.3 5.3 5.3	1356 1354 1357 1356 1353	72.6 71.8 71.9 72.0 71.8	30 Aug 00 09 Sept 00 19 Sept 00 29 Sept 00 11 Oct 00	BBCH 81	Fruit Fruit Fruit Fruit	0.12 0.13 0.13[a] 0.08	0 7 14 21	Method AG-575A LOQ=0.01 mg/kg Analysis 09 – 16 March 01
(Southern EU)			--	--	--	--	--	--	Fruit Fruit Fruit	<0.01 <0.01 <0.01	0 14 21	
Kühne-Thu H. (2001d) 2036/00 2176 Valeggio sul Mincio (VR), Italy	Apple/ Golden Delicious Smoothee	1. 1983 2. -- 3. 27 Sept 00	Foliar spray	7.5 7.5 7.5 7.5	1014 1014 1033 1014	76.1 76.1 77.5 76.1	26 July 00 09 Aug 00 23 Aug 00 06 Sept 00	BBCH 85	Fruit Fruit Fruit	0.15 0.04[a] 0.06	0 14 21	Method AG-575A LOQ=0.01 mg/kg Analysis 14 – 21 Aug 01
(Southern EU)			--	--	--	--	--	--	Fruit Fruit Fruit	<0.01 <0.01 <0.01	0 14 21	
Kühne-Thu H. (2001e) 2025/00 2192 Barbens (Lérida), Spain	Apple/ Golden Delicious	1. 1991 2. -- 3. 19 Sept 00	Foliar spray	4.925 4.925 4.925 5.077 5.077	1511 1511 1511 1477 1466	74.4 74.4 74.4 75 74.4	01 Aug 00 08 Aug 00 15 Aug 00 22 Aug 00 29 Aug 00	BBCH 78-79	Fruit Fruit Fruit Fruit Fruit	0.21 0.29 0.25 0.14[a] 0.14	0 3 7 14 21	Method AG-575A LOQ=0.01 mg/kg Analysis 08 – 16 March 01
(Southern EU)			--	--	--	--	--	--	Fruit Fruit Fruit	<0.01 <0.01 <0.01	0 14 21	

Reference Report No Trial No Location (a)	Commodity / Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment: g a.i. /hl	water l/ha	g a.i./ha	Dates of treatment(s) or number of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed	Residues (mg/kg)	PHI (days) (f)	Remarks: (g)
Kühne-Thu H. (2001f) 2026/00 2193 Castellsera (Lérida), Spain	Apple/ World Gala	1. 1992 2. -- 3. 21 Aug 00	Foliar spray	5.316 4.969 5.081 5.031 5.031	1452 1493 1476 1476 1491	77.2 74.2 75 74.3 75	03 July 00 10 July 00 17 July 00 24 July 00 31 July 00	BBCH 78-79	Fruit Fruit Fruit Fruit Fruit	0.26 0.21 0.16 0.15[a] 0.10	0 3 7 14 21	Method AG-575A LOQ=0.01 mg/kg Analysis 08 – 16 March 01
(Southern EU)			--	--	--	--	--	--	Fruit Fruit Fruit	<0.01 <0.01 <0.01	0 14 21	
Solé C. (2002a) 2070/01 2229 Fossalta di Portogruaro (Venezia), Italy	Apple/ Fuji	1. March 1998 2. -- 3. 10 Oct 01	Foliar spray	5.2 5.2 5.2 5.2	1200 1200 1167 1174	62.5 62.5 60.5 60.9	24 Aug 01 04 Sept 01 12 Sept 01 19 Sept 01	BBCH 81 – 83	Fruit Fruit Fruit Fruit	0.19 0.12 0.08[a] 0.05	0 7 14 21	Method AG-575A LOQ=0.01 mg/kg Analysis 15 – 20 March 02
(Southern EU)			--	--	--	--	--	--	Fruit Fruit Fruit	<0.01 <0.01 <0.01	0 14 21	
Solé C. (2002b) 2019/01 2230 Tymavos, Greece	Apple/ Granny Smith	1. 1993 2. -- 3. 17 Oct 01	Foliar spray	6.257 6.253 6.255 6.24	1157 1161 1159 1179	72.39 72.60 72.50 73.57	03 Sept 01 13 Sept 01 23 Sept 01 03 Oct 01	BBCH 79	Fruit	0.05[a]	14	Method AG-575A LOQ=0.01 mg/kg Analysis 18 – 20 March 02
(Southern EU)			--	--	--	--	--	--	Fruit	<0.01	14	

Reference Report No Trial No Location (a)	Commodity / Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment: g a.i. /hl	water l/ha	g a.i./ha	Dates of treatment(s) or number of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed	Residues (mg/kg)	PHI (days) (f)	Remarks: (g)
Solé C. (2002c) 2020/01 2231 Abelonas, Greece	Pear/ Highland	1. 1996 2. -- 3. 23 Aug 01	Foliar spray	6.828 6.858 6.830 6.833	1058 1056 1057 1059	72.24 72.42 72.19 72.36	10 July 01 20 July 01 30 July 01 09 Aug 01	BBCH 81	Fruit	0.16[a]	14	Method AG-575A LOQ=0.01 mg/kg Analysis 18 – 21 March 02
(Southern EU)			--	--	--	--	--	--	Fruit	<0.01	14	
Pointurier R. (2002b) 00110601 2234 Roquecourbe, France	Apple/ Royal Gala/Pajam	1. 1995 2. -- 3. 20 Aug 01	Foliar spray	15.0 15.0 15.0 15.0	494 488 506 500	74.1 73.1 75.9 75.0	06 July 01 16 July 01 27 July 01 06 Aug 01	BBCH 81	Fruit Fruit Fruit Fruit Fruit	0.29[a] 0.24[a] 0.35[a] 0.23[a] 0.28[b]	0 3 7 10 14	Method AG-575A LOQ=0.01 mg/kg Analysis 12 March – 18 Apr 02
(Southern EU)			--	--	--	--	--	--	Fruit Fruit Fruit	<0.01 <0.01 <0.01	0 7 14	
Solé C. (2002d) 2096/01 2246 Alfamen Zaragoza, Spain	Apple/ Reineta	1. 1983 2. -- 3. 23 Aug 01	Foliar spray	4.9 4.9 4.9 1533	1489 1500 1624	73.56 74.08 80.34 75.73	28 June 01 12 July 01 26 July 01 09 Aug 01	BBCH 81	Fruit Fruit	0.19 0.10[a]	0 14	Method AG-575A LOQ=0.01 mg/kg Analysis 13 – 16 March 02
(Southern EU)			--	--	--	--	--	--	Fruit Fruit	<0.01 <0.01	0 14	

Reference Report No Trial No Location (a)	Commodity / Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment: g a.i. /hl	water l/ha	g a.i./ha	Dates of treatment(s) or number of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed	Residues (mg/kg)	PHI (days) (f)	Remarks: (g)
Krainz A. (2003h) 847421 2388 Bonnes (Vienne), France	Pear/ Conference	1. 1992 2. -- 3. 02 Sept 02	Foliar spray	5.0 5.0 5.0 5.0	1500 1500 1500 1500	75 75 75 75	19 July 02 29 July 02 09 Aug 02 19 Aug 02	BBCH 79	Fruit	0.07[a]	14	Method AG-575A LOQ=0.01 mg/kg Analysis 17 – 20 June 03
(Southern EU)			--	--	--	--	--	--	Fruit Fruit	<0.01 <0.01	14	

(a) According to the EU and Codex class classification (both) should be used
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc; overall broadcast, aerial spraying, row, individual plant, between plants - type of equipment used must be indicated.
(d) Year must be indicated
(e) BBCH monograph, growth stages of plants
(f) Minimum days after last treatment
(g) Remarks may include: Climatic conditions; reference to analytical method; information concerning metabolites included
[a] Two samples taken and analysed separately; mean result presented
[b] Two samples taken and analysed in duplicate; mean result (4 analyses) presented
[1] Method AG-575A involves extraction using methanol/ammonium hydroxide, partitioning with hexane and back partition with acetonitrile. Clean-up is performed on Sep-Pak silica, phenyl bond-elute and finally by partition into hexane ether. Difenoconazole is determined by GC using electron capture detection or mass spectrometry detection.
Recoveries: 72-118%. The methods are acceptable.

Table B.7.6-5. Results of residue trials performed with difenoconazole on carrot (data used for calculations of proposed MRLs are underlined, i.e. those corresponding or approximating to critical GAP).

Reference Report No Trial No Location (a)	Commodity / Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment: g a.i. /hl	water l/ha	g a.i./ha	Dates of treatment(s) or number of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed	Residues (mg/kg)	PHI (days) (f)	Remarks: (g)
Kühne-Thu H. (1988) 2005/87 0096 Les Barges Voury, Switzerland (Northern EU)	Carrot/ Nantaise Express	1. -- 2. -- 3. 03 Aug 87	Foliar spray	20.8	600	125	10 June 87	33% of crop mature	Root	0.06	0- (before)	Method AG-537[1]
				20.8	600	125	23 June 87		Root	0.07	0+ (after)	LOQ=0.03 mg/kg
				20.8	600	125	02 July 87		Root	0.13	7	
				20.8	600	125	13 July 87		Root	<u>0.07</u>	<u>14</u>	Analysis
									Root	0.07	21	Apr 88
			--	--	--	--	--	--	Root	<0.01	0	
									Root	<0.01	21	
Kühne-Thu H. (1989) 2006/87 0096 Les Barges Voury, Switzerland (Northern EU)	Carrot/ Tip-Top	1. -- 2. -- 3. 10 Sept 87	Foliar spray	20.8	600	125	21 July 87	Plants 1.5 cm in diameter	Root	0.14[a]	0- (before)	Method AG-514[1]
				20.8	600	125	04 Aug 87		Root	<0.02[b]	0+ (after)	LOQ=0.02 mg/kg
				20.8	600	125	12 Aug 87		Root	0.07	7	
				20.8	600	125	20 Aug 87		Root	<u>0.12</u>	<u>14</u>	Analysis
									Root	0.07	21	Nov 88
			--	--	--	--	--	--	Root	<0.01	0	
									Root	<0.01	21	
Maffezoni M. (1993a) OF91059 R039 Liesse, France (Northern EU)	Carrot/ Anglia	1. 12 June 91 2. -- 3. 25 Sept 91	Foliar spray	31.25	400	125	13 Aug 91	Plants 7 – 8 leaves	Root	<u>0.05</u>	<u>13</u>	Method RES 10/93[2]
				31.25	400	125	28 Aug 91					LOQ=0.02 mg/kg
				31.25	400	125	12 Sept 91					Analysis
			--	--	--	--	--	--	Root	<0.02	13	06 – 13 May 93
Maffezoni M. (1993b) OF91089 R051 Montaigu,	Carrot/ Luxor	1. 01 July 91 2. -- 3. 25 Sept 91	Foliar spray	31.25	400	125	13 Aug 91	Plants 7 – 8 leaves	Root	<u>0.03</u>	<u>13</u>	Method RES 10/93
				31.25	400	125	28 Aug 91					LOQ=0.02 mg/kg
				31.25	400	125	12 Sept 91					Analysis

Reference Report No Trial No Location (a)	Commodity / Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment: g a.i. /hl	water l/ha	g a.i./ha	Dates of treatment(s) or number of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed	Residues (mg/kg)	PHI (days) (f)	Remarks: (g)
France (Northern EU)			--	--	--	--	--	--	Root	<0.02	13	06 – 13 May 93
Maffezoni M. (1993c) OF92025 Y60 Tierce, France	Carrot/ Nantaise	1. 26 May 92 2. -- 3. 23 Sept 92	Foliar spray	25.0 12.5 12.5	500 1000 1000	125 125 125	28 Aug 92 11 Sept 92 25 Sept 92	Plants 18 – 22 cm	Root Root	<u>0.02</u> 0.02	<u>14</u> 28	Method RES 10/93 LOQ=0.02 mg/kg Analysis 27 Apr – 05 May 93
(Northern EU)			--	--	--	--	--	--	Root	<0.02	14	
Maffezoni M. (1995) OF93153 SJ88 Tierce, France	Carrot/ Nantaise	1. 01 July 93 2. -- 3. Nov 93	Foliar spray	12.5 12.5 12.5	1000 1000 1000	125 125 125	28 Sept 93 13 Oct 93 27 Oct 93	Plants 8 – 10 cm	Root Root	<u>0.02</u> 0.02	<u>14</u> 33	Method RES 10/93 LOQ=0.02 mg/kg Analysis 08 – 16 Aug 95
(Northern EU)			--	--	--	--	--	--	Root Root	0.03 <0.02	14 33	
Pointurier R. (2001c) 00110901 2124 St Martin le Beau, France	Carrot/ Nanda	1. 07 Nov 99 2. -- 3. 29 May 00	Foliar spray	31.3 31.3 31.3	413 391 413	129 122 129	25 Apr 00 05 May 00 15 May 00	BBCH 46	Root	<u>0.11[b]</u>	<u>14</u>	Method AG-575A LOQ=0.01 mg/kg Analysis 13 – 21 Dec 00
(Northern EU)			--	--	--	--	--	--	Root	<0.01	14	

Reference Report No Trial No Location (a)	Commodity / Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment: g a.i. /hl	water l/ha	g a.i./ha	Dates of treatment(s) or number of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed	Residues (mg/kg)	PHI (days) (f)	Remarks: (g)
Pointurier R. (2001d) 0012001 2127 Le Fresne, France (Northern EU)	Carrot/ Carotan	1. 23 Apr 00 2. -- 3. 09 Oct 00	Foliar spray	31.3 31.3 31.3	400 383 390	125 120 122	28 Aug 00 11 Sept 00 25 Sept 00	BBCH 46-47	Root Root Root Root Root	0.02 0.03 0.01 0.03 <u>0.04</u>[b]	0 3 7 10 <u>14</u>	Method AG-575A[3] LOQ=0.01 mg/kg Analysis 04 – 06 Jan 01
			--	--	--	--	--	--	Root Root Root	<0.01 <0.01 <0.01	0 7 14	
Maffezoni M. (1993) OF92025 G 45, 0864 Mauguio, France (Southern EU)	Carrot/ Nandrin	1. 15 July 92 2. -- 3. 30 Oct 92	Foliar spray	12.5 12.5 12.5	1000 1000 1000	125 125 125	02 Sept 92 17 Sept 92 02 Oct 92	Plants 25 – 40 cm	Root Root	<u>0.07</u> 0.02	<u>14</u> 28	Method RES 10/93 LOQ=0.02 mg/kg Analysis 27 Apr – 05 May 93
			--	--	--	--	--	--	Root	<0.02	14	
Maffezoni M. (1995) OF93153 AC78, 1138 Montfrin, France (Southern EU)	Carrot/ Valor	1. 14 July 93 2. -- 3. Oct/Nov 93	Foliar spray	12.5 12.5 12.5	1000 1000 1000	125 125 125	06 Sept 93 20 Sept 93 04 Oct 93	Plants 2 – 3 cm	Root Root	<u>0.11</u> 0.04	<u>14</u> 29	Method RES 10/93 LOQ=0.02 mg/kg Analysis 08 – 16 Aug 95
			--	--	--	--	--	--	Root Root	0.04 0.06	14 29	
Maffezoni M. (1999a) OF96134 LD 77, 1826 Seilh, France (Southern EU)	Carrot/ Boléro	1. 25 June 96 2. -- 3. 02 Oct 96	Foliar spray	31.25 31.25 31.25	400 400 400	125 125 125	21 Aug 96 04 Sept 96 18 Sept 96	BBCH 47	Root	<u>0.02</u>[b]	<u>14</u>	Method AGR/MOA/169374-1[4] LOQ=0.02 mg/kg Analysis 03 – 07 May 99
			--	--	--	--	--	--	Root	<0.02	14	

Reference Report No Trial No Location (a)	Commodity / Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment: g a.i. /hl	water l/ha	g a.i./ha	Dates of treatment(s) or number of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed	Residues (mg/kg)	PHI (days) (f)	Remarks: (g)
Maffezoni M. (1999b) OF96134 BY 18, 1827 Venejean, France (Southern EU)	Carrot/ Boléro	1. 12 July 96 2. -- 3. 18 Oct 96	Foliar spray	31.25 31.25 31.25	400 400 400	125 125 125	09 Sept 96 20 Sept 96 04 Oct 96	BBCH 45	Root	0.02[a]	14	Method AGR/MOA/169374-1 LOQ=0.02 mg/kg
			--	--	--	--	--	--	Root	<0.02	14	Analysis 12 – 14 May 99
Maffezoni M. (1999c) OF96134 AC 20, 1828 Mauguio, France (Southern EU)	Carrot/ Tourino	1. 20 Apr 96 2. -- 3. 29 July 96	Foliar spray	31.25 31.25 31.25	400 400 400	125 125 125	18 June 96 01 July 96 15 July 96	BBCH 49	Root	<0.02[a]	14	Method AGR/MOA/169374-1 LOQ=0.02 mg/kg
			--	--	--	--	--	--	Root	<0.02	14	Analysis 10 – 12 May 99
Pointurier R. (2001e) 0011902 2125 Roquecourbe, France (Southern EU)	Carrot/ Colmar Coeur Rouge	1. 03 Febr 00 2. -- 3. 19 June 00	Foliar spray	31.3 31.3 31.3	367 400 400	115 125 125	11 May 00 24 May 00 15 June 00	BBCH 47	Root	0.13[b]	14	Method AG-575A LOQ=0.01 mg/kg
			--	--	--	--	--	--	Root	<0.01	14	Analysis 13 – 21 Dec 00
Pointurier R. (2001f) 0011903 2126 Aimargues, France (Southern EU)	Carrot/ Presto	1. 10 Febr 00 2. -- 3. 30 May 00	Foliar spray	31.3 31.3 31.3	415 405 395	130 127 123	18 Apr 00 02 May 00 16 May 00	BBCH 42 – 43	Root	0.03[b]	14	Method AG-575A LOQ=0.01 mg/kg
			--	--	--	--	--	--	Root	<0.01	14	Analysis 13 – 21 Dec 00

Reference Report No Trial No Location (a)	Commodity / Variety (a)	Date of 1. Sowing or Planting 2. Flowering 3. Harvest (b)	Method of treatment (c)	Application rate per treatment: g a.i. /hl	water l/ha	g a.i./ha	Dates of treatment(s) or number of treatment(s) and last date (d)	Growth stage at last treatment or date (e)	Portion analysed	Residues (mg/kg)	PHI (days) (f)	Remarks: (g)
Pointurier R. (2001g) 0012002 2128 Marsillargues, France	Carrot/ Presto	1. 28 Febr 00 2. -- 3. 31 May 00	Foliar spray	31.3 31.3 31.3	380 390 407	119 122 127	18 Apr 00 02 May 00 16 May 00	BBCH 42 – 43	Root Root Root Root Root	0.01 0.01 0.03 0.02 0.02[b]	0 3 7 10 15	Method AG-575A LOQ=0.01 mg/kg Analysis 20 – 24 Dec 00
(Southern EU)			--	--	--	--	--	--	Root Root Root	<0.01 <0.01 <0.01	0 7 15	

(a) According to the EU and Codex class classification (both) should be used
(b) Only if relevant
(c) High or low volume spraying, spreading, dusting etc; overall broadcast, aerial spraying, row, individual plant, between plants - type of equipment used must be indicated.
(d) Year must be indicated
(e) BBCH monograph, growth stages of plants
(f) Minimum days after last treatment
(g) Remarks may include: Climatic conditions; reference to analytical method; information concerning metabolites included
[a] One sample taken and analysed in duplicate; mean result presented
[b] Two samples taken and analysed separately; mean result presented
[1] Method AG-537 and AG 514 involves extraction by boiling a sub sample under reflux in methanol/ammonium hydroxide. 1st clean-up by reextraction from methanol/water into hexane. 2nd clean up by reextraction from hexane into acetonitrile. 3rd clean up by chromatography on a silica Sep-Pack cartridge.
[2] Method RES 10/93 involves extraction using methanol, partition with dichloromethane, purification on an aluminia column. Quantification by GC with an electron capture detector. Determination by injection of specimen extracts and analytical standards.
[3] Method AG-575A involves extraction using methanol/ammonium hydroxide, partitioning with hexane and back partition with acetonitrile. Clean-up is performed on Sep-Pak silica, phenyl bond-elut and finally by partition into hexane ether. Difenoconazole is determined by GC using electron capture detection or mass spectrometry detection.
[4] Method AGR/MOA/169374-1 is based on method AG-575A.
Recoveries: 70-129%. The methods are acceptable.

Table B.7.6-6. Residues used for MRL determination.

Crop	Northern or Mediterranean Region	Trials results relevant to the critical GAP	Comments	MRL		STMR
				MRL – 1 (R_{max})	MRL – 2 (R_{ber})	
Pome fruit	Northern	9 trials: 0.01; 0.02; 0.03; 0.04; 2x0.05; 0.06; 2x0.07 mg/kg		0.11	0.13	0.05
	Southern	11 trials: 0.04; 0.05; 0.07; 0.08; 0.10; 0.11; 0.13; 0.14; 0.15; 0.16; 0.28 mg/kg		0.31	0.30	0.11
Carrot	Northern	8 trials: 2x0.02; 0.03; 0.04; 0.05; 0.07; 0.11; 0.12 mg/kg	In two trials 4 applications were made instead of the maximum of 3 applications.	0.18	0.20	0.05
	Southern	8 trials: <0.02; 3x0.02; 0.03; 0.07; 0.11; 0.13 mg/kg		0.20	0.20	0.03
Wheat grain	Northern	8 trials: 2x<0.01; 6x<0.02 mg/kg		0.03	0.04	0.02
	Southern	6 trials: 2x<0.01; 4x<0.02 mg/kg		0.04	0.04	0.02

MRL - 1: MRL calculated according to Method I (Doc. 7039/VI/95 EN 22/7/1997, Appendix I, Calculation of maximum residue levels and safety intervals).
MRL - 2: MRL calculated according to Method II (Doc. 7039/VI/95 EN 22/7/1997, Appendix I, Calculation of maximum residue levels and safety intervals).

Crop	Northern or Mediterranean Region	Trials results relevant to the critical GAP	Comments	MRL		STMR
				MRL – 1 (R_{max})	MRL – 2 (R_{ber})	
Wheat straw	Northern	8 trials: 2x<0.01; 2x<0.02; 2x<0.04; 0.04; 0.05 mg/kg		0.08	0.08	0.03
	Southern	6 trials: 2x<0.01; 3x<0.04; 0.05 mg/kg		0.10	0.085	0.04

MRL - 1: MRL calculated according to Method I (Doc. 7039/VI/95 EN 22/7/1997, Appendix I, Calculation of maximum residue levels and safety intervals).
MRL - 2: MRL calculated according to Method II (Doc. 7039/VI/95 EN 22/7/1997, Appendix I, Calculation of maximum residue levels and safety intervals).

Residues resulting from supervised trials – summary and conclusions

A total of 25 individual trials in countries of the Northern EU region and 25 individual trials in countries of the Southern EU region, were conducted in spring wheat, pome fruit and carrot over eight seasons.

The results of trials where applications were made corresponding or approximating to the GAP are presented together with the proposed MRL and STMR values in Table B.7.6-6.

There were 14 trials in spring wheat; eight were conducted in Northern EU and six in Southern EU over a period of three seasons. Eight of these trials were decline trials. Difenoconazole was applied once to seeds of spring wheat as a FS formulation containing 12.5 to 30g difenoconazole/L (alone or containing other active ingredients; see Table B.7.6-1) at application rates within ± 25% of the maximum GAP rate of 6 g a.i./100 kg seeds.

In the decline studies, difenoconazole residues in the whole plants 36 to 101 days after sowing were below the limit of quantification (LOQ) of the analytical method (<0.01 or <0.04 mg/kg).

In spring wheat in Northern EU, following one application to seeds at 4.64 to 6.1g difenoconazole/100 kg seed, residues of difenoconazole 126 to 156 days after sowing were below the LOQ (<0.01 to <0.02 mg/kg) in wheat grain and <0.01 to 0.05 mg/kg in wheat straw. Based on these values, a MRL value of 0.02 mg/kg is proposed for cereal grain (highest LOQ value). The maximum residue in cereal straw is calculated to be 0.08 mg/kg.

In spring wheat in Southern EU, following one application to seeds at 4.64 to 6.3 g difenoconazole/100 kg seed, residues of difenoconazole 99 to 147 days after sowing were below the LOQ (< 0.01 to <0.02 mg/kg) in wheat grain and <0.01 to 0.05 mg/kg in wheat straw. Based on these values, a MRL value of 0.02 mg/kg is proposed for cereal grain (highest LOQ value). The maximum residue in cereal straw is calculated to be 0.1 mg/kg. In Southern EU, there were six trials conducted in spring wheat rather than the eight specified by the document 7525/VI/95 rev. 7, 12/6/2001. However, as no residues above the LOQ were detected, additional trials in Southern EU are not required.

There were 20 trials in pome fruit (apple and pear); nine were conducted in Northern EU and eleven in Southern EU over three seasons. Ten of these trials were decline trials. Difenoconazole was applied four times as an EC formulation containing 250 g difenoconazole/L at application rates within ± 25% of the maximum GAP rate of 56.25 g a.i./ha in Northern and 75 g a.i./ha in Southern EU.

In three decline studies conducted in Northern EU, residues of difenoconazole in fruit were 0.06 to 0.16 mg/kg immediately after the final application, declining slowly to 0.04 to 0.17 mg/kg after 7 days, 0.04 to 0.10 mg/kg after 14 days and 0.01 to 0.07 mg/kg after 27 to 31 days. In Southern EU (7 trials), residues of difenoconazole in fruit were 0.12 to 0.29 mg/kg immediately after the final application. At some sites residues did not significantly decline and at others only declined very slowly. In three of the seven trials conducted in Southern EU five applications (instead of 4) were applied at rates within ± 25% of the dosage of 75 g a.i./ha (72 to 77 g a.i./ha) .

In pome fruit in Northern EU, residues of difenoconazole at harvest 27 to 31 days after application (i.e. within ± 25% of the PHI of 28 days) were 0.01 to 0.07 mg/kg. In Southern EU, residues of difenoconazole at harvest 14 days after application (i.e. ± 25% of the PHI of 14 days) were 0.04 to 0.28 mg/kg. Based on these values, a MRL value of 0.3 mg/kg is proposed for pome fruit. The STMR is 0.05 mg/kg for the Northern EU results and 0.11 mg/kg for the Southern EU results.

There were 16 trials in carrots; eight were conducted in Northern EU and eight in Southern EU over six seasons. Eight of these trials were decline trials. Difenoconazole was applied three times (in two trials four times) as an EC formulation containing 250 g difenoconazole/L at application rates of 115 to 130 g a.i./ha (i.e. within ± 25% of the maximum GAP rate of 125 g a.i./ha).

In carrot in Northern EU, residues of difenoconazole 13 or 14 days after application (i.e. within ± 25% of the PHI of 14 days) were 0.02 to 0.12 mg/kg. In Southern EU, residues of difenoconazole 14 or 15 days after application (i.e. ± 25% of the PHI of 14 days) were <0.02 to 0.13 mg/kg. Based on these values, a MRL value of 0.2 mg/kg is proposed for carrot. The STMR is 0.05 mg/kg for the Northern EU results and 0.03 mg/kg for the Southern EU results.

In five decline studies conducted in Northern EU, residues of difenoconazole in roots were 0.02 to 0.07 mg/kg immediately after the final application (2 observations). There was little or no decline in residue levels after application. After 3 days (1 observation) residues of difenoconazole were 0.03 mg/kg, 0.01 to 0.13 mg/kg after 7 days (3 observations), 0.03 mg/kg after 10 days (1 observation), 0.02 to 0.12 mg/kg after 14 days (5 observations) and 0.02 to 0.07 mg/kg after 21 to 33 days (4 observations). In Southern EU (3 trials), residues of difenoconazole in roots were 0.01 mg/kg immediately after the final application (1 observation), 0.02 to 0.11 mg/kg after 14 or 15 days (3 observations) and 0.02 to 0.04 mg/kg (2 observations) after 28 or 29 days.

B.7.6.1 Storage stability of residues prior to analysis

The storage stability of difenoconazole has been investigated in wheat, potato, lettuce, banana, cotton, animal tissues (cattle, poultry), milk and eggs.

B.7.6.1.1 Residue stability in potatoes

Reference:	**Beidler W.T. (1991a).** Stability of CGA 169374 residues in potatoes under freezer storage conditions for two years. Ciba-Geigy Corp., Greensboro, United States. Unpublished Report No. ABR-90070, SAM No. 0453.
Test Material:	1-[(2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole, lot number S85-0812, chemical purity 95.6%.
Guideline:	In house study, meets the requirements of Commission 7032/VI/95 rev 5 dated 22 July 1997.
GLP:	Yes (in part). The analytical and biological phases of this study were conducted prior to the effective date of the applicable GLP regulations (October 16, 1989).

Material and methods:

Test concentration:	0.5 mg a.i./kg
Test system:	Storage stability data have been generated for difenoconazole in potatoes. Potato tuber samples were fortified with difenoconazole at a level of 0.5 mg/kg and stored frozen at *ca* -20°C. The stored samples were re-analysed in duplicate using Analytical Method AG-514 (with minor modifications for the 24 month samples) after storage for approximately 0, 1, 3, 6, 14, 18 and 24 months and the results compared to initial values.
Duration:	Two years
Method of analysis:	Analytical method AG-514 for interval 0 day and intervals 1, 3, 6, 14 and 18 months. Minor modified AG-514 for interval 24 months. The procedure for AG-514 was extraction by refluxing for 2 hours with methanol and concentrated ammonium hydroxide (8:2, v/v), cleaned up by a silica Sep-Pak. Difenoconazole was determined by gas chromatography (using a packed 3% OV-17 column) equipped with a nitrogen-phosphorus detector (NPD).
Date of experiment:	15 April 1987 to 01 May 1989

Findings: There was no decrease in the levels of difenoconazole in potatoes. The residue levels measured following storage are summarised in Table B.7.6.1.1-1.

Table B.7.6.1.1-1. Residues of difenoconazole in potatoes following freezer storage.

Storage period (days)	Mean residues (mg difenoconazole/kg)[a]	Mean stored residue as a percentage of initial value
0	0.52	104
28	0.55	110
91	0.64	128
182	0.56	112
419	0.56	112
530	0.51	102
735	0.51	102

[a] Corrected for procedural recovery < 100%. Results are not corrected for control values.

Conclusions: Residues of difenoconazole in potatoes will be stable for at least 24 months when stored at *ca* <-20°C.

Comments by RMS: The study was well performed and reported.

B.7.6.1.2 Residue stability in tomatoes

Reference:	**Beidler W.T. (1991b).** Stability of CGA-169374 residues in tomatoes under freezer storage conditions for two years. Ciba-Geigy Corp., Greensboro, United States. Unpublished Report No. ABR-90069, SAM No. 0452.
Test Material:	1-[(2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole, lot number S85-0812, chemical purity 95.6%.
Guideline:	In house study, meets the requirements of Commission 7032/VI/95 rev 5 dated 22 July 1997.
GLP:	Yes (in part). The analytical and biological phases of this study were conducted prior to the effective date of the applicable GLP regulations (October 16, 1989).

Material and methods:

Test concentration:	0.5 mg/L
Test system:	Storage stability data have been generated for difenoconazole in tomatoes. Tomato fruit samples were fortified with difenoconazole at a level of 0.5 mg/kg and stored frozen at *ca* -20°C. The stored samples were re-analysed in duplicate after storage for approximately 0, 1, 3, 6, 14, 18 and 24 months using Analytical Method AG-514 (with minor modifications for the 24 month samples) and the results compared to initial values.
Duration:	Two years
Method of analysis:	Analytical method AG-514 for interval 0 day and intervals 1, 3, 6, 14 and 18 months. Minor modified AG-514 for interval 24 months. The procedure for AG-514 was extraction by refluxing for 2 hours with methanol and concentrated ammonium hydroxide (8:2, v/v), cleaned up by a silica Sep-Pak. Difenoconazole was determined by gas chromatography (using a packed 3% OV-17 column) equipped with a nitrogen-phosphorus detector (NPD).
Date of experiment:	08 April 1987 to May 1989

Findings: There was no decrease in the levels of difenoconazole in tomatoes. The residue levels measured following storage are summarised in Table B.7.6.1.2-1.

Table B.7.6.1.2-1. Residues of difenoconazole in tomato fruit following freezer storage.

Storage period (days)	Mean residues (mg difenoconazole/kg)[a]	Mean stored residue as a percentage of initial value
0	0.46	92
28	0.59	118
88	0.57	114
182	0.52	104
419	0.56	112
530	0.53	106
735	0.53	106

[a] Corrected for procedural recovery < 100%. Results are not corrected for control values.

Conclusions: Residues of difenoconazole in tomato fruit will be stable for at least 24 months when stored at *ca* <-20°C.

Comments by RMS: The study was well performed and reported.

B.7.6.1.3 Residue stability in lettuce, soybeans and wheat forage

Reference:	**Beidler W.T. (1992).** Stability of CGA-169374 residues in lettuce, soybeans and wheat forage under freezer storage conditions for one year. Ciba-Geigy Corp., Greensboro, United States. Unpublished Report No. ABR-91024, SAM No. 0617.
Test Material:	1-[(2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole, lot number S85-0812, chemical purity 95.6%.
Guideline:	In house study, meets the requirements of Commission 7032/VI/95 rev 5 dated 22 July 1997.
GLP:	Yes (in part). The analytical and biological phases of this study were conducted prior to the effective date of the applicable GLP regulations (October 16, 1989).

Material and methods:

Test concentration:	0.2 mg/kg and 0.5 mg/kg
Test system:	Storage stability data have been generated for difenoconazole in lettuce (head), soybean (beans) and wheat (forage). Samples were fortified at a level of 0.2 mg/kg (lettuce and soybean) and 0.5 mg/kg (wheat) and stored frozen at *ca* -20°C. The stored samples were re-analysed in duplicate following storage for approximately 0, 1, 3, 6 and 12 months using Analytical Method AG-514 (with minor modifications) and the results compared to initial values.
Duration:	One year
Method of analysis:	Analytical method AG-514 with minor modifications. The procedure was extraction by refluxing for 2 hours with methanol and concentrated ammonium hydroxide (8:2, v/v), cleaned up by a silica Sep-Pak. Difenoconazole was

determined by gas chromatography (using a packed 3% OV-17 column) equipped with a nitrogen-phosphorus detector (NPD). The limit of determination for residues of difenoconazole with this method, as established by procedural recoveries, is 0.05 ppm.

Date of experiment: 05 August 1988 to 10 October 1989

Findings: There was no decrease in the levels of difenoconazole in lettuce, soybean and wheat. The residue levels measured following storage are summarised in Table B.7.6.1.3-1.

Table B.7.6.1.3-1. Residues of difenoconazole in lettuce, soybean and wheat following freezer storage.

Commodity	Storage period (days)	Mean residues (mg difenoconazole/kg)[a]	Mean stored residue as a percentage of initial value
Lettuce (head)	0	0.15	75
	35	0.20	100
	91	0.22	110
	199	0.20	100
	371	0.24	120
Soybean (beans)	0	0.18	90
	35	0.24	120
	91	0.22	110
	199	0.19	95
	371	0.25	125
Wheat (forage)	0	0.47	94
	35	0.60	120
	91	0.55	110
	199	0.44	88
	371	0.47	94

[a] Corrected for procedural recovery < 100%. Results are not corrected for control values.

Conclusions: Residues of difenoconazole in lettuce (head), soybean (beans) and wheat (forage) will be stable for at least 12 months when stored at *ca* <-20°C.

Comments by RMS: The study was well performed and reported.

B.7.6.1.4 Residue stability in banana

Reference: **Kühne-Thu H. (1994).** Residue Stability of CGA-169374 (difenoconazole) in banana (whole fruit) under freezer storage conditions. Ciba-Geigy Ltd., Basel, Switzerland. Unpublished Report No. 125/93, SAM No. 0934.

Test Material: 1-[(2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole, batch number AMS 255/102, chemical purity 99.0%.

Guideline: Pesticide Assessment Guidelines, Residue Chemistry, Storage Stability Study, Subdivision O, Series 171-4.

GLP: Yes

Material and methods:

Test concentration: 0.2 mg/kg

Test system:	Storage stability data have been generated for difenoconazole in bananas. Homogenised whole banana (including skins) samples were fortified at a level of 0.2 mg/kg and stored frozen at *ca* -20°C. The stored samples were re-analysed in triplicate following storage for approximately 0, 1, 3, 6 and 12 months using Analytical Method AG-575B (with minor modifications) and the results compared to initial values.
Duration:	One year
Method of analysis:	Analytical method AG-575B with minor modifications: charcoal column cleanup omitted; difenoconazole was determined by gas chromatography (using wide bore instead of packed column) equipped with a electron capture instead of nitrogen-phosphorus detector (NPD). The chromatographic system was calibrated with external standards. They were injected intermediately with specimens.
Date of experiment:	04 May 1993 to 04 May 1994

Findings: There was no decrease in the levels of difenoconazole in bananas. The residue levels measured following storage are summarised in Table B.7.6.1.4-1.

Table B.7.6.1.4-1. Residues of difenoconazole in whole banana fruit following freezer storage.

Storage period (days)	Mean residues (mg difenoconazole/kg)[a]	Mean stored residue as a percentage of initial value
0	0.18	88
28	0.20	101
84	0.17	86
168	0.18	92
364	0.19	96

[a] Corrected for procedural recovery < 100%.

Conclusions: Residues of difenoconazole in whole banana will be stable for at least 12 months when stored at *ca* <-20°C.

Comments by RMS: The study was well performed and reported.

B.7.6.1.5 Residue stability in wheat and cotton

Reference:	**Hayworth C.G. (1998).** Stability of CGA-169374 fortified into wheat and cotton substrates under freezer storage conditions. Novartis Crop Protection Inc., Greensboro, United States. Unpublished Report No. ABR-98061, SAM No. 1644.
Test Material:	1-[(2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole, lot number S93-1669, chemical purity 96%.
Guideline:	EPA Guideline No. 860-1380.
GLP:	Yes

Material and methods:

Test concentration:	0.2 mg/kg, 0.4 mg/kg, 0.5 mg/kg and 1.0 mg/kg
Test system:	Storage stability data have been generated for difenoconazole in cotton (seed, oil and meal) and wheat (straw, forage and grain). Samples were fortified at levels of 0.2 mg/kg (wheat grain), 0.4 mg/kg (cottonseed and oil), 0.5 mg/kg (cottonseed meal) and 1.0 mg/kg (wheat straw and forage) and stored frozen at *ca* -20°C. The stored samples were re-analysed in duplicate after storage for periods up to approximately 24 months using Analytical Method AG-575B (with minor modifications) and the results compared to initial values.
Duration:	Two years
Method of analysis:	Analytical method AG-575B with minor modifications. The procedure was extraction by refluxing for 2 hours with methanol and concentrated ammonium hydroxide (8:2, v/v), cleaned up by a silica Sep-Pak. Difenoconazole was determined by gas chromatography using a DB-17 megabore column with nitrogen-phosphorus detector (NPD).
Date of experiment:	31 January 1996 to May 1998

Findings: There was no decrease in the levels of difenoconazole in cotton or wheat commodities. The residue levels measured following storage are summarised in Table B.7.6.1.5-1.

Table B.7.6.1.5-1. Residues of difenoconazole in lettuce, soybean and wheat following freezer storage.

Commodity	Storage period (days)	Mean residues (mg difenoconazole/kg)[a]	Mean residue as a percentage of initial value
Cottonseed Seed	0	0.44	90
	88	0.42	110
	214	0.44	105
	487	0.44	110
	735	0.53	133
Cottonseed Oil	0	0.40	100
	71	0.40	100
	209	0.38	95
	450	0.38	95
	715	0.41	103
Cottonseed Meal	0	0.44	88
	91	0.47	94
	216	0.52	104
	464	0.61	122
	729	0.59	118
Wheat Straw	0	1.1	110
	150	0.99	99
	290	0.98	98
	523	1.2	120
	808	1.2	120
Wheat Forage	0	1.0	100
	139	1.1	110
	264	0.88	88
	518	1.1	110
	777	1.2	120
Wheat Grain	0	0.21	105
	140	0.22	110
	258	0.19	95
	546	0.22[b]	110
	778	0.24[b]	120

[a] Residues are corrected for control values if detected and procedural recoveries <100%.
[b] Single samples.

Conclusions: Residues of difenoconazole in cotton (cottonseed oil) and wheat (straw, forage and grain) commodities will be stable for at least 24 months when stored at *ca* <-20°C.

Comments by RMS: The study was well performed and reported.

B.7.6.1.6 Residue stability in animal products

Reference:	**Wurz R.E.M. (1993).** Storage stability study of CGA-169374 in dairy and poultry tissues, eggs and milk under freezer storage conditions. Ciba-Geigy Corp., Greensboro, United States. Unpublished Report ABR-93012, SAM 0795.
Test Material:	1-[(2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole, lot number S85-0812, chemical purity 94.5%.
Guideline:	In house study, meets the requirements of Commission 7032/VI/95 rev 5 dated 22 July 1997.
GLP:	Yes (In part). The Study Director was not able to verify the conduct of all parts of

the study due to personnel changes or missing or conflicting raw data.
The study was terminated after 1 year due to incorrect disposal of samples.

Material and methods:

Test concentration:	0.1 mg/kg (milk), 0.2 mg/kg (eggs) and 0.5 mg/kg (tissues)
Test system:	Storage stability data have been generated for difenoconazole in milk, eggs, beef liver and poultry breast meat. Samples were fortified at levels of 0.1 mg/kg (milk), 0.2 mg/kg (eggs) and 0.5 mg/kg (tissues) and stored frozen at *ca* -20°C. The stored samples were re-analysed in duplicate using Analytical Method AG-544 (with minor modifications) after storage for 0, 1, 3, 6 and 12 months and the results compared to initial values.
Duration:	One year
Method of analysis:	Milk, tissues and eggs were extracted and analysed for difenoconazole using method AG-544 with minor modifications.
Date of experiment:	27 July 1988 to 28 August 1989

Findings: There was no decrease in the levels of difenoconazole in animal commodities. The residue levels measured following storage are summarised in Table B.7.6.1.6-1.

Table B.7.6.1.6-1. Residues of difenoconazole in animal commodities following freezer storage.

Animal commodity	Storage period (days)	Fortification level (mg difenoconazole/kg)	Mean residues (mg difenoconazole/kg)[a]	Mean stored residue as a percentage of the fortified value[a]
Eggs	0	0.2	0.21	105
	31	0.2	0.17	85
	87	0.2	0.22	110
	187	0.2	0.23	115
	367	0.2	0.20	100
Milk	0	0.1	0.10	100
	31	0.1	0.10	100
	87	0.1	0.11	110
	187	0.1	0.11	110
	367	0.1	0.12	120
Poultry breast	0	0.5	0.50	100
	31	0.5	0.56	112
	87	0.5	0.59	98
	187	0.5	0.47	94
	367	0.5	0.54	108
Beef liver	0	0.5	0.49	100
	31	0.5	0.48	98
	87	0.5	0.65	110
	187	0.5	0.42	86
	367	0.5	0.44	90

[a] Corrected for procedural recoveries.

Conclusions: Residues of difenoconazole in animal commodities (eggs, milk, beef liver and poultry breast) will be stable for at least 12 months when stored at *ca* <-20°C.

Comments by RMS: The study was well performed and reported.

B.7.6.1.7 Residue stability of difenoconazole and its metabolite CGA-205375 in animal products

Reference:	**Tribolet R. (2000).** Residue of difenoconazole (CGA-169374) and its metabolite CGA-205375 in milk, blood and tissues (muscle, fat, liver, kidney) of dairy cattle resulting from feeding of difenoconazole at three dose levels. Novartis Crop Protection AG, Basel, Switzerland. Unpublished Report 202/99, SAM No. 2039.
Test Material:	1-[(2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]-1H-1,2,4-triazole, batch number 804156A, chemical purity 94.4%.
Guideline:	Commission document 7031/VI/95 rev 4, dated 22 July 1996; Appendix G, Livestock Feeding Studies, does not meets the requirements of Commission 7032/VI/95 rev 5 dated 22 July 1997, but provides useful supporting data.
GLP:	Yes (Certified Laboratory).

Material and methods:

Test concentration:	0.10 mg/kg (muscle, liver, kidney and fat); 0.05 mg/kg (milk and blood)
Test system:	Storage stability data have been obtained for difenoconazole and CGA 205375 in milk, blood, fat, kidney, liver and muscle from dairy cattle. These data were generated as part of a livestock feeding study with difenoconazole. Five samples of each matrix were fortified at levels of 50 µg/L (milk), 100 µg/L (blood) and 0.2 mg/kg (tissues) and stored frozen at -18°C for periods of *ca* 10 months (depending on the matrix).
Duration:	10 months
Method of analysis:	The stored samples were re-analysed using Analytical Method AG-544A (with minor modifications) and the results compared to the nominal level of fortification.
Date of experiment:	10 March 1999 to 29 May 2000

Findings: The mean recovery of difenoconazole residues in milk, blood and tissues following storage for up to *ca* 10 months at -18°C ranged from 80 to 101% of the fortified values. The mean recovery of CGA 205375 residues in milk, blood and tissues following storage for up to *ca* 10 months at -18°C ranged from 89 to 108% of the fortified values. Initial values prior to storage were not determined.

The residue levels measured following storage are summarised in Table B.7.6.1.7-1.

Table B.7.6.1.7-1. Residues of difenoconazole and metabolite CGA-205375 in animal tissues following freezer storage.

Animal commodity	Storage period (days)	Fortification level	Mean stored residues[a]		Mean procedural recovery		Mean stored residue as a % of the fortification level[a]	
			A	B	A	B	A	B
Milk	305	50 µg/L	40 µg/L	38 µg/L	87[b]	80[b]	92	94
Blood	276	100 µg/L	83 µg/L	87 µg/L	82	83	101	105
Fat	303	0.2 mg/kg	0.16 mg/kg	0.16 mg/kg	78	76	101	108
Kidney	301	0.2 mg/kg	0.13 mg/kg	0.15 mg/kg	79	78	80	95
Liver	296	0.2 mg/kg	0.13 mg/kg	0.18 mg/kg	79	89[c]	83	100[c]
Muscle	312	0.2 mg/kg	0.17 mg/kg	0.17 mg/kg	103	95	84	89

[a] Corrected for procedural recovery.
[b] Single sample
[c] Repeat analysis.
A=Difenoconazole
B=CGA-205375

Conclusions: Residues of difenoconazole and CGA-205375 in products of animal origin will be stable for at least 10 months when stored at <-18°C.

Comments by RMS: The study was well performed and reported.

B.7.7 Effects of industrial processing and/or household preparation (Annex IIA 6.5; Annex IIIA 8.4)

B.7.7.1 Effects on the nature of the residue

A high temperature hydrolysis study was conducted to determine whether difenoconazole hydrolyses, and to identify any major degradates formed, under conditions representing the extremes of pH and temperature that could occur during the processes involved in pasteurisation, baking, brewing, boiling and sterilisation.

Reference:	**Muir G.T. (2003).** Difenoconazole. Aqueous hydrolysis at 90, 100 and 120ºC. Syngenta, Jealott's Hill International, Bracknell, UK. Unpublished Report No. RJ3360B, SAM No. 2312.
Test Material:	[Triazole-^{14}C] difenoconazole, batch number ILA-50.2-2, radiochemical purity 99.5%, specific activity 29.6 mCi/mmol. The structure and labelling position are given in Figure B.7.1-1.
Guideline:	Annex II part A, section 6 and Annex III, part A, section 8 of Directive 91/414/EEC; Appendix E, Processing studies; 7035/VI/95, rev 5, 22 July 1997.
GLP:	Yes (Certified Laboratory)

Material and methods:

Duplicate sterilised aqueous solutions of the test substance in acetate buffer (1.9 mg/L) were incubated at 90°C for 20 minutes (pH 4), 100°C for 60 minutes (pH 5) and 120°C for 20 minutes (pH 6). Control samples were set up in the same way but kept at room temperature. Samples were taken after incubation and analysed for radioactivity by liquid scintillation counting (LSC). Quantification of

difenoconazole and degradation products was carried out by TLC.

Date of experiment: 24 October 2002 to 09 January 2003

Findings: The mean total applied radioactivity recovered after incubation and neutralization was 103.9 to 108.9% for the three processing conditions (Table B.7.7.1-1). The majority of the applied radioactivity consisted of parent difenoconazole (95.6 to 98.6%). There was less than 5% degradation of difenoconazole following hydrolysis. One minor degradation product was observed at low levels (mean ≤ 1.0% radioactivity) at pH 5 and 6, and no other degradation products were quantifiable.

Table B.7.7.1-1. Radioactivity recovery following incubation under different processing conditions.

Simulated process	Incubation			Radioactivity recovery (% of applied)[a]		
	pH	Time (minutes)	Temperature (°C)	Total	Difenoconazole	Unknown degradates
Pasteurisation	4	20	90	103.9	95.6	0.0
Baking/Brewing/Boiling	5	60	100	108.9	98.1	1.0
Sterilisation	6	20	120	104.9	98.6	0.9

[a] after incubation and neutralisation. Mean of two samples.

Conclusions: There was no significant hydrolysis of difenoconazole following incubation at different pH values and temperatures. Difenoconazole is therefore considered stable under conditions representative of pasteurisation, baking, brewing and boiling, and sterilisation.

Comments by RMS: The study was well performed and reported.

B.7.7.2 Effects on the residue levels

The Commission Directive 96/68/EC states that processing studies are not normally required if no significant residues, i.e. residues less than 0.1 mg/kg, or no analytically determinable residues occur in the plant or plant product which would be processed, or if the total theoretical maximum daily intake (TMDI) is less than 10% of the ADI. Calculations of the TMDI according to the UK PSD consumer exposure model indicate that the predicted intake of difenoconazole for toddlers and infants was 0.0053 and 0.0038 mg/kg bw/day, respectively, which is equivalent to 53% and 38% of the ADI**,** respectively (see B.7.15.1, Table B.7.15.1-5 and Table B.7.15.1-6).

The effect of processing on difenoconazole residues in pome fruit has been investigated.

Distribution of the residues in peel/pulp

Balance studies on a core set of representative processes

Reference:	**Simon P. (2002c).** Determination of residues of difenoconazole after application of Score in apples and processed commodities in Germany. Syngenta Agro GmbH, D-63477 Maintal, Germany. Unpublished Report No. gap82901, SAM No. 2282.
Test Material:	SYD 21310/A-7402 G (EC formulation containing 250 g difenoconazole/L), batch number P.706093.
Guideline:	Guideline for the Generation of Residue Data (Commission of the European Communities, doc 1607/VI/97), BBA Guidelines Teil IV, 3-3 (1990) and IVA Guidelines, Residue Studies, Industrieverband Agrar, Frankfurt/Main, Germany, (May 1994).
GLP:	Yes

Material and methods:

Test concentration:	337.25 g difenoconazole/ha
Test system:	The study was conducted outside in a field plot (103 m^2) in Germany. Difenoconazole (formulated as an EC) was applied using a Kubota-tractor with LIPCO-application equipment and HOLDER-blower (OVS 25). At harvest, apples (variety *Mondial Gala*) were manually taken from more than 12 threes. Fruits were taken from each side and all parts of the threes, high and low, exposed and protected by foliage. Samples of treated fruit at harvest were taken for processing into juice and puree. For juice processing, residues of difenoconazole were determined in final and intermediate products including washed fruit, wet pomace, dry pomace, raw (pre-pasteurisation) juice and pasteurised juice. For puree processing, residues of difenoconazole were determined in final and waste products including washed fruit, the residue remaining after filtration and puree.
Stage of application:	BBCH 74-75, 75-77, 81-85, 85

No. of applications:	Four
Sampling time points:	Mature apple fruit (BBCH 87).
Method of analysis:	AG 575-A. LOQ: 0.02 mg/kg for apple fruits, 0.01 mg/kg for processed commodities including washed fruits.
Date of experiment:	19 July 2001 to 27 May 2002

Findings: Mean residues of difenoconazole in unprocessed apple fruit after the forth application were 0.42 to 0.56 mg/kg (Table B.7.7.2-1). Difenoconazole residues in processed commodities were 0.30 to 0.47 mg/kg in washed fruit, 1.5 mg/kg in wet pomace, 6.6 mg/kg in dry pomace, < 0.01 mg/kg in raw and pasteurised apple juice, 1.5 mg/kg in residue remaining after filtration and 0.08 mg/kg in puree (Table B.7.7.2-2). Transfer factors from apple fruit to processed commodities were 0.71 and 0.84 in washed fruit, 3.6 in wet pomace, 15.7 in dry pomace, less than 0.02 in raw and pasteurised juice and 0.14 in puree (Table B.7.7.2-2).

Table B.7.7.2-1. Residues of difenoconazole in apple.

Commodity/ Variety	Application			No. of appl.	Growth stage at last treatment	Portion analysed	Residues (mg/kg)[a]	PHI (days)
	Form. type and content	g a.i. /ha	water l/ha					
Apple/ Mondial Gala	EC 250 g/L	337.25	1.349	4	BBCH 85	Juice processing Apple fruits	0.36, 0.47	18
						Marmalade processing Apple fruits	0.52, 0.60	18

[a] Corrections of results are not made for recoveries

Table B.7.7.2-2. Effect of processing on residues of difenoconazole in apple.

Processing	Residue of difenoconazole in unprocessed apple (mg/kg)	Washed fruits		Wet pomace		Dry pomace		Before/after pasteurisation	
		Residue (mg/kg)	TF	Residue (mg/kg)	TF	Residue (mg/kg)	TF	Residue (mg/kg)	TF
Juice	0.42[a]	0.30	0.71	1.48	3.5	6.55	15.6	<0.01 / <0.01	0.02 / 0.02
Processing	Residue of difenoconazole in unprocessed apple (mg/kg)	Washed fruits		Residue after filtration		Puree			
		Residue (mg/kg)	TF	Residue (mg/kg)	TF	Residue (mg/kg)	TF		
Marmalade	0.56[a]	0.47	0.84	1.47	2.6	0.08	0.14		

[a] Mean of two samples (see Table B.7.7.2-1).
TF= Transfer factor

Conclusion: Residues of difenoconazole in apple fruit were reduced slightly by washing, and decreased significantly during processing into juice and puree. Residues of difenoconazole concentrated in wet and dry pomace.

Comments by RMS: The application rate in the present study was 4 to 6 times higher than the critical GAP proposed for pome fruit in Southern and Northern Europe (75 and 56.2 g a.i./ha, respectively). The first application was made at the growth stage BBCH 74 – 75 (fruit about half final size), which was later than the proposed GAP (BBCH 61 – beginning of flowering: about 10% of flowers open). Thus, lower residues in the unprocessed apple would be expected.

Follow-up studies to determine concentration or dilution factors

Reference:	**Kühne-Thu H. (1997a).** Magnitude of residues of difenoconazole applied as EC 250 (double rate) on apple trees in Chile. Determination of difenoconazole and CGA 205375 (metabolite) in apples and processed fractions. Novartis Crop Protection, CH-4002 Basel, Switzerland. Unpublished Report No. 2205/94, SAM No. 1424.
Test Material:	A-7402 G (EC formulation containing 250 g difenoconazole/L), batch number 305008.
Guideline:	FAO Guidelines on Producing Pesticide Residues Data from Supervised Trials (Rome, 1990). Study meets the requirements of Annex II part A, section 6 and Annex III, part A section 8 of Directive 91/414/EEC and Commission document 7035/VI/95 - rev 5 of 22 July 1997.
GLP:	Yes

Material and methods:

Test concentration:	91.3 to 97.2 g a.i./ha (totally 574.6 g a.i./ha)
Test system:	The study was conducted outside in a field plot (0.5 ha per plot) in Chile (Los Niches/Curicó, Región VII). Difenoconazole (formulated as a EC 250) was applied using a orchard boom sprayer. Apples (variety *Golden Delicious*) were manually taken at six intervals after application. Samples of treated fruit at harvest were taken for processing into juice and pomace. The fruit were processed at the Field Station of Residue Analysis, Switzerland (CH-1896 Vouvry). The fruit were allowed to thaw 18 hours before being processed to apple juice and pomace. Two half liter bottles of juice and 2 kg of pomace were collected per specimen (treated and control) and deep frozen for analysis.
Stage of application:	BBCH and (diameter in mm): 65, 71, 72(18), 72(22-26), 74(32-38), 74-75(36-45)
No. of applications:	Six
Sampling time points:	0, 14, 27, 41, 55, 91 days after the last application
Method of analysis:	For parent difenoconazole: Method AG-575-A modified: - charcoal column omitted, GC with electron capture instead of P/N-detector. For metabolite CGA 205375: Method REM 147.03
Date of experiment:	10 October 1994 to 14 November 1996

Findings: Residues of difenoconazole in apple fruit immediately after the final application were 0.24 mg/kg. The residues declined to 0.02 mg/kg after 91 days. Difenoconazole residues in processed commodities were <0.02 mg/kg in apple juice and 0.13 mg/kg in wet pomace (see Table B.7.7.2-3). Residues of the metabolite CGA 205375 were only found at 14 and 27 days the last application, at the LOQ of 0.02 mg/kg.

Transfer factor from unprocessed apple fruit to juice was less than 1, indicating a reduction of residues on processing while the transfer factor from apple fruit to wet pomace was 6.5 (Table B.7.7.2-4). No residues above the LOQ were found in untreated fruit.

Conclusion: Residues of difenoconazole in apple fruit were low, at the LOQ, and below the LOQ in apple juice. Residues of difenoconazole were concentrated in wet pomace.

Comments by RMS: The application rate in the present study was 1.6 times higher than the critical GAP proposed for pome fruit in Northern Europe (56.2 g a.i./ha) and within ± 25% of the rate of 75 g a.i./ha in Southern Europe. Six applications were made instead of the maximum of 4 applications.

Reference:	**Kühne-Thu H. (1997b).** Magnitude of residues of difenoconazole applied as EC 250 on apple trees in Chile. Determination of difenoconazole and CGA 205375 (metabolite) in apples and processed fractions. Novartis Crop Protection, CH-4002 Basel, Switzerland. Unpublished Report No. 2204/94, SAM No. 1426.
Test Material:	A-7402 G (EC formulation containing 250 g difenoconazole/L), batch number 305008.
Guideline:	FAO Guidelines on Producing Pesticide Residues Data from Supervised Trials (Rome, 1990). Study meets the requirements of Annex II part A, section 6 and Annex III, part A section 8 of Directive 91/414/EEC and Commission document 7035/VI/95 - rev 5 of 22 July 1997.
GLP:	Yes

Material and methods:

Test concentration:	45.7 to 49.6 g a.i./ha (totally 290 g a.i./ha)
Test system:	The study was conducted outside in a field plot (1 ha per plot) in Chile (Los Niches/Curicó, Región VII). Difenoconazole (formulated as a EC 250) was applied using a orchard boom sprayer. Apples (variety *Golden Delicious*) were manually taken at six intervals after application. Samples of treated fruit at harvest were taken for processing into juice and pomace. The fruit were processed at the Field Station of Residue Analysis, Switzerland (CH-1896 Vouvry). The fruit were allowed to thaw 18 hours before being processed to apple juice and pomace. Two half liter bottles of juice and 2 kg of pomace were collected per specimen (treated and control) and deep frozen for analysis.
Stage of application:	BBCH and (diameter in mm): 65, 71, 72(18), 72(22-26), 74(32-38), 74-75(36-45)
No. of applications:	Six

Sampling time points:	0, 14, 27, 41, 55, 91 days after the last application
Method of analysis:	For parent difenoconazole: Method AG-575-A modified: - charcoal column omitted, GC with electron capture instead of P/N-detector. For metabolite CGA 205375: Method REM 147.03
Date of experiment:	10 October 1994 to 13 November 1996

Findings: Residues of difenoconazole in apple fruit immediately after the final application were 0.11 mg/kg. The residues declined to 0.02 mg/kg after 91 days. Difenoconazole residues in processed commodities were <0.02 mg/kg in apple juice and 0.08 mg/kg in wet pomace (see Table B.7.7.2-3). Residues of the metabolite CGA 205375 were all <0.02 mg/kg.

Transfer factor from unprocessed apple fruit to juice was less than 1, indicating a reduction of residues on processing while the transfer factor from apple fruit to wet pomace was 4.0 (Table B.7.7.2-4). No residues above the LOQ were found in untreated fruit.

Conclusion: Residues of difenoconazole in apple fruit were low, at the LOQ, and below the LOQ in apple juice. Residues of difenoconazole were concentrated in wet pomace.

Comments by RMS: The application rate in the present study was within ± 25% of the critical GAP proposed for pome fruit in Northern Europe of 56.25 g a.i./ha but lower the critical GAP proposed for pome fruit in Southern Europe (75 g a.i./ha). Six applications were made instead of the maximum of 4 applications.

Reference:	**Kühne-Thu H. (1997c).** Magnitude of residues of difenoconazole applied as EC 250 (double rate) on apple trees in Brazil. Determination of difenoconazole and CGA 205375 (metabolite) in apples and processed fractions. Novartis Crop Protection, CH-4002 Basel, Switzerland. Unpublished Report No. 2195/94, SAM No. 1431.
Test Material:	A-7402/780 A (EC formulation containing 250 g difenoconazole/L), batch number 206 (Brazilian).
Guideline:	FAO Guidelines on Producing Pesticide Residues Data from Supervised Trials (Rome, 1990). Study meets the requirements of Annex II part A, section 6 and Annex III, part A section 8 of Directive 91/414/EEC and Commission document 7035/VI/95 - rev 5 of 22 July 1997.
GLP:	Yes

Material and methods:

Test concentration:	67.9 to 104.7 g a.i./ha (totally 774 g a.i./ha)
Test system:	The study was conducted outside in a field plot (360 m^2) in Brazil (Sao Joaquim). Difenoconazole (formulated as a EC 250) was applied using a motorized mistblower. Apples (variety *Fuji*) were manually taken at eight intervals after

	application. Samples of treated fruit at harvest were taken for processing into juice and pomace. The fruit were processed at the Field Station of Residue Analysis, Switzerland (CH-1896 Vouvry). The fruit were allowed to thaw 18 hours before being processed to apple juice and pomace. Two half liter bottles of juice and 2 kg of pomace were collected per specimen (treated and control) and deep frozen for analysis.
Stage of application:	BBCH: 51, 65, 67, 69, 70, 70 – 71, 71 – 72, 72 - 74
No. of applications:	Eight
Sampling time points:	0, 14, 28, 42, 102 days after the last application
Method of analysis:	For parent difenoconazole: Method AG-575-A modified: charcoal column omitted, GC with electron capture instead of P/N-detector. For metabolite CGA 205375: Method REM 147.03
Date of experiment:	29 September 1994 to 30 October 1996

Findings: Residues of difenoconazole in apple fruit immediately after the final application were 0.85 mg/kg. The residues declined to 0.03 mg/kg after 102 days. Difenoconazole residues in processed commodities were <0.02 mg/kg in apple juice and 0.09 mg/kg in wet pomace (see Table B.7.7.2-3). Residues of the metabolite CGA 205375 decreased from 0.06 (interval 0) to 0.03 mg/kg, (after 102 days).

Transfer factor from unprocessed apple fruit to juice was less than 1, indicating a reduction of residues on processing while the transfer factor from apple fruit to wet pomace was 3.0 (Table B.7.7.2-4). No residues above the LOQ were found in untreated fruit.

Mean transfer factors for residues from apple fruit were 4.5 in wet pomace and <0.9 in raw juice (see Table B.7.7.2-4).

Conclusion: Residues of difenoconazole in apple fruit were low, at the LOQ, and below the LOQ in apple juice. Residues of difenoconazole were concentrated in wet pomace.

Table B.7.7.2-3. Residues of difenoconazole in processed apple commodities.

Reference Report No Trial No Location	Commodity/ Variety	Application			No. of appl.	Growth stage at last treatment BBCH	Portion analysed	Residues (mg/kg)	PHI (days)
		Form. type and content	g a.i. /ha	kg a.i. /hl					
Kühne-Thu H. (1997a) 2205/94 1424 Los Niches, Curicó, Región VII, Chile	Apples/ Golden Delicious	EC 250 g/L	91.3 - 97.2	6.5 – 7.1	6	74 – 75	Fruit	0.24	0[b]
							Fruit	0.17	14
							Fruit	0.16	27
							Fruit	0.06	41
							Fruit	0.05	55
							Fruit	0.02	91
							Juice	<0.02	91
							Wet pomace	0.13[a]	91
Kühne-Thu H. (1997b) 2204/94 1426 Los Niches, Curicó, Región VII, Chile	Apples/ Golden Delicious	EC 250 g/L	45.7 – 49.6	3.3 – 3.6	6	74 - 75	Fruit	0.11	0[b]
							Fruit	0.10	14
							Fruit	0.07	27
							Fruit	0.03	41
							Fruit	0.02	55
							Fruit	0.02	91
							Juice	<0.02	91
							Wet pomace	0.08[a]	91
Kühne-Thu H. (1997c) 2195/94 1431 Sao Joaquim, Sta. Catarina, Brazil	Apples/ Fuji	EC 250 g/L	68 - 105	7.0	8	72 - 74	Fruit	0.85	0[b]
							Fruit	0.35	14
							Fruit	0.21	28
							Fruit	0.12	42
							Fruit	0.03	102
							Juice	<0.02	102
							Wet pomace	0.09[a]	102

[a] Mean of two samples
[b] immediately after the final application

Table B.7.7.2-4. Effect of processing on residues of difenoconazole in apple.

Reference Report No Trial No Location	Residue of difenoconazole in unprocessed fruit (mg/kg)	Wet pomace		Raw juice[a]	
		Residue (mg/kg)	Transfer factor	Residue (mg/kg)	Transfer factor
Kühne-Thu H. (1997a) 2205/94 1424 Los Niches, Curicó, Región VII, Chile	0.02	0.13	6.5	<0.02	<1.0
Kühne-Thu H. (1997b) 2204/94 1426 Los Niches, Curicó, Región VII, Chile	0.02	0.08	4.0	<0.02	<1.0
Kühne-Thu H. (1997c) 2195/94 1431 Sao Joaquim, Sta. Catarina, Brazil	0.03	0.09	3.0	<0.02	<0.7
Mean			**4.5**		**<0.9**

[a] Raw juice is pre-pasteurisation

Comments by RMS: The application rate in the present study was within ± 25% of the critical GAP proposed for pome fruit in Northern Europe and Southern Europe (56.25 and 75 g a.i./ha, respectively). The first application was made at the growth stage BBCH 51 (stage 5: inflorescence emergence), which was prior the flowering stage (BBCH 61). Eight applications were made instead of the maximum of 4 applications.

Even though the application rate in one of the four available studies was six times higher than the critical GAP in Northern Europe (56.25 kg a.i./ha), difenoconazole residues were not concentrated in juice.
The intake of difenoconazole residues through apple juice was estimated to comprise 0.6% and 3.8% of the acute reference dose (ARfD) for adults and toddlers, respectively (see B.7.15.4-1). Difenoconazole was concentrated in dry and wet pomace.

Since the maximum residue detected in carrots was 0.13 mg/kg (see Table B.7.6-5), the notifier has also been investigated the effect of processing on difenoconazole residues in carrot. The samples have been analysed and the final report is being written. The estimated completion is mid-2006. The RMS suggest that this study could be included in an Addendum.

B.7.8 Livestock feeding studies (Annex IIA 6.4; Annex IIIA 8.3)

B.7.8.1 Dairy cattle

The transfer of residues of difenoconazole from cattle into tissues and milk has been investigated. Residue data was supported by fortified procedural recovery.

Reference:	**Tribolet R. (2000).** Residue of difenoconazole (CGA 169374) and its metabolite CGA 205375 in milk, blood and tissues (muscle, fat, liver, kidney) of dairy cattle resulting from feeding of difenoconazole at three dose levels. Novartis Crop Protection AG, Basel, Switzerland. Unpublished Report 202/99, SAM No. 2039.
Test Material:	Difenoconazole (CGA 169374), batch number 804156A, chemical purity 94.4%.
Guideline:	Commission document 7031/VI/95 rev 4, dated 22 July 1996; Appendix G, Livestock Feeding Studies.
GLP:	Yes

Material and methods:

Test concentration:	0, 20, 60 and 200 mg difenoconazole/day (equivalent to 0, 1, 3 and 10 mg/kg in the diet dried material assuming 20 kg dry matter daily intake per day).
Test system:	A total of 11 lactating cows of Holstein were used in the study (9 cows for three treatment groups and two control animals). They were selected out of 18 animals 16 days before the first treatment on the basis of milk production, general health and body weight. Cows were dosed with the test substance in gelatine capsules via a balling gun daily for 29 to 30 days. One cow from each group was sacrificed on dose day 29 and two cows from the 1X (20 mg), 3X (60 mg) and 10X (200 mg) groups on day 30.
Duration:	30 days
Sampling time points:	Milk samples were collected on days 0 (pre-dose), 2, 5, 8, 12, 15, 19, 22, and 28. Muscle (tenderloin, round steak, and diaphragma muscle), fat (omental and perirenal), liver, kidney and blood were sampled at sacrifice on day 29 and 30 (day 29: one cow in each treatment group; day 30: two cows from each treatment group, excluding the control group).
Method of analysis:	Milk and tissues were extracted and analysed for difenoconazole and metabolite CGA 205375 using method AG-544A with minor modifications. The LOQ for milk was 0.005 mg/L and for blood 0.010 mg/L. The LOQ for animal tissues was 0.01mg/kg.
Number of animals:	11
Date of experiment:	10 March 1999 to 29 May 2000

Findings: No effects upon the general health of the cows were observed during the course of the study.

Validation data: results of the recovery analyses are summarised in Table B.7.8.1-1.

There was no significant degradation of difenoconazole or CGA 205375 in milk, blood or tissues stored at ≤-20°C for periods equivalent to the maximum period of storage of the samples in the study (Table B.7.8.1-2).

Residues of difenoconazole were below 5 µg/L in the milk samples taken on all days during the feeding period from all treatment groups (Table B.7.8.1-3). Residues of difenoconazole were less than 0.01 mg/kg in all tissues samples, with the exception of liver, which contained a residue of 0.01 mg/kg following dosing at the highest rate (10 mg/kg (10X). Residues of difenoconazole in blood were less than 10 µg/L.

Residues of CGA 205375 were below 5 µg/L in the milk samples taken on all days during the feeding period following difenoconazole 1 mg/kg (1X) and 3 mg/kg (3X). Following 10 mg/kg (10X), the mean residue of CGA 205375 was 7 µg/L. Residues of CGA 205375 in milk reached a plateau after two days of dosing and concentrations were maintained until the end of the study (Table B.7.8.1-4).

Following 3 and 10 mg/kg, maximum residues of CGA 205375 in muscle were 0.022 mg/kg and 0.028 mg/kg, respectively. In liver, residues of CGA 205375 were 0.04, 0.13 and 0.35 mg/kg following doses of 1, 3 and 10 mg/kg, respectively. In kidney, residues of CGA 205375 were <0.01, 0.02 and 0.05 mg/kg following doses of 1, 3 and 10 mg/kg, respectively. In fat, residues of CGA 205375 were 0.01, 0.03 and 0.09 mg/kg following doses of 1, 3 and 10 mg/kg, respectively. In blood, residues of CGA 205375 were <10, <10 and 19 µg/L following doses of 1, 3 and 10 mg/kg, respectively.

Residue levels in the liver, kidney, muscle, fat, blood and milk are presented in Table B.7.8.1-3.

Table B.7.8.1-1. Procedural recoveries for the analysis of Difenoconazole and CGA 205375 in tissues, blood and milk.

Commodity	Compound added	Fortification (mg/kg)	Mean recovery (%)[a]	RSD (%)[b]	n
Muscle	Difenoconazole	0.01 / 0.10	93 / 78	26 / 8.7	3 / 3
	CGA 205375	0.01 / 0.10	99 / 90	3.5 / 15	3 / 3
Liver	Difenoconazole	0.01 / 0.10 / 0.2	68, 78 / 80 / 72	--	2 / 1 / 1
	CGA 205375	0.01 / 0.10 / 0.2	84, 117 / 85, 89 / 88	--	2 / 2 / 1
Kidney	Difenoconazole	0.01 / 010	83, 76 / 81	--	2 / 1
	CGA 205375	0.01 / 0.10	113, 73 / 83	--	2 / 1
Fat	Difenoconazole	0.01 / 0.10	86, 77 / 79, 78	--	2 / 2
	CGA 205375	0.01 / 0.10	83 / 83, 79	12 / --	3 / 2
Blood	Difenoconazole	0.01 / 0.10	84, 84 / 79, 80	--	2 / 2
	CGA 205375	0.01 / 0.10	83, 84 / 80, 81	--	2 / 2
Milk	Difenoconazole	0.005 / 0.050	87 / 84	8.1 / 5.6	9 / 9
	CGA 205375	0.005 / 0.050	91 / 88	10 / 11	10 / 10

[a] for sample numbers less than 3, the individual values are mentioned instead of the mean.
[b] RSD: relative standard deviation; for sample numbers less than 3, the calculation was not performed.

Table B.7.8.1-2. Storage stability of difenoconazole and CGA 205375 in tissues, blood and milk.

Animal Commodity	Compound	Storage period (days)	Fortification (mg/kg or µg/L)	Mean procedural recovery (%)	Average (%)[a]
Muscle	Difenoconazole	312 days	0.2	103	84
	CGA 205375	312 days	0.2	95	89
Liver[b]	Difenoconazole	296 days	0.2	79	83
	CGA 205375	296 days	0.2	101	59
	CGA 205375	389 days	0.2	89[c]	100
Kidney	Difenoconazole	301 days	0.2	79	80
	CGA 205375	301 days	0.2	78	95
Fat	Difenoconazole	303 days	0.2	78	101
	CGA 205375	303 days	0.2	76	108
Blood	Difenoconazole	276 days	0.10	82	101
	CGA 205375	276 days	0.10	83	105
Milk	Difenoconazole	305 days	0.05	87[c]	92
	CGA 205375	305 days	0.05	80[c]	94

[a] Average recovery expressed as percentage of nominal value. The averages are corrected for individual procedural recovery.
[b] As the first analysis revealed a reduced stability for CGA 205375, the reserve set of specimens was also analysed for CGA 205375.
[c] Single sample

Table B.7.8.1-3. Maximum residues of difenoconazole and CGA 205375 in tissues, blood and milk.

Sample	Mean Residues (mg/kg)			
	0 mg/kg (Control)	1 mg/kg (1 X)	3 mg/kg (3 X)	10 mg/kg (10 X)
Difenoconazole				
Muscle - tenderloin	< 0.01	< 0.01	< 0.01	< 0.01
Muscle - round steak	< 0.01	< 0.01	< 0.01	< 0.01
Muscle - diaphragm	< 0.01	< 0.01	< 0.01	< 0.01
Liver	< 0.01	< 0.01	< 0.01	0.01
Kidney	< 0.01	< 0.01	< 0.01	< 0.01
Fat - perirenal	< 0.01	< 0.01	< 0.01	< 0.01
Fat - omental	< 0.01	< 0.01	< 0.01	< 0.01
Blood (µg/L)	< 10	< 10	< 10	< 10
Milk (0 to 28 days, µg/L)[a]	< 5	< 5	< 5	< 5
CGA 205375				
Muscle - tenderloin	< 0.01	< 0.01	0.01	0.02
Muscle - round steak	< 0.01	< 0.01	0.01	0.02
Muscle - diaphragm	< 0.01	< 0.01	0.01	0.02
Liver	< 0.01	0.04	0.12	0.30
Kidney	< 0.01	< 0.01	0.02	0.04
Fat - perirenal	< 0.01	0.01	0.03	0.07
Fat - omental	< 0.01	0.01	0.03	0.08
Blood (µg/L)	< 10	< 10	< 10	16
Milk (0 to 28 days, µg/L)[a]	< 5	< 5	< 5	7[b]

[a] Milk samples were taken on Day 2, 5, 8, 12, 15, 19, 22 and 28 during dosing (see Table B.7.8.1-4).
[b] A mean level of 7 µg/L was recorded at each interval, with the exception of Day 22 where a level of 6 µg/L was recorded (see Table B.7.8.1-4).

Table B.7.8.1-4. Mean residues of difenoconazole and CGA 205375 in milk (µg/L).

Day of test	Difenoconazole			CGA 205375		
	1 mg/kg (1 X)	3 mg/kg (3 X)	10 mg/kg (10 X)	1 mg/kg (1 X)	3 mg/kg (3 X)	10 mg/kg (10 X)
0 AM+PM	<5	<5	<5	<5	<5	<5
2 AM+PM	<5	<5	<5	<5	<5	7
5 AM+ PM	<5	<5	<5	<5	<5	7
8 AM+PM	<5	<5	<5	<5	<5	7
12 AM+PM	<5	<5	<5	<5	<5	7
15 AM+PM	<5	<5	<5	<5	<5	7
19 AM+PM	<5	<5	<5	<5	<5	7
22 AM+PM	<5	<5	<5	<5	<5	6
28 AM+PM	<5	<5	<5	<5	<5	7

Day of test = day after the first administration of difenoconazole in gelatine capsules.
Day O = before treatment.
AM+PM = morning milk and evening milk has been pooled (1 vol. + 1 vol.) and this mixture has been analysed.
The results were not corrected for control value nor for recoveries.

Conclusions: Residues of difenoconazole and CGA-205375 did not accumulate in milk, fat, liver, kidney or muscle following doses of 1 to 10 mg difenoconazole/kg in the diet. Residues of difenoconazole and CGA-205375 in the liver were 0.01 mg/kg and 0.04 mg/kg following administration of 10 mg/kg diet and of 1 mg/kg diet, respectively (i.e., 43 and 4.3 times higher than the realistic estimated feeding rate of 0.23 mg difenoconazole/kg diet calculated in B.7.8.1.1-3). Hence, following the use of difenoconazole according to the proposed GAP, residues of difenoconazole in liver are not expected to exceed 0.01 mg/kg.

Comments by RMS: The supporting procedural recoveries and storage stability data were satisfactory. The study was well performed and reported. A full discussion of the expected dietary burden and the relevant residue will be addressed in the EU MRL submission by the notifier. However, a new feeding study has recently being conducted. The samples have being analysed for parent difenoconazole and the metabolite CGA-205375 using updated analytical methods. Additionally, the level of 1,2,4-triazole in the samples has also being determined. The estimated completion of the final report for the study is mid-2006. The RMS suggest that this study could be included in an Addendum.

B.7.8.1.1 Intake calculations for dairy and beef cattle

Intake of residues by dairy and beef cattle may be expected from cereal grain, cereal straw and fruit pomace. The potential dietary exposure of dairy and beef cattle to difenoconazole is calculated according to the document 7031/VI/95 rev. 4, 22/7/1996. Residue intakes were calculated using the proposed maximum residue level (MRL) in cereal grain, cereal straw and in pome fruit (0.02, 0.1 and 0.3 mg/kg, respectively) as well as the supervised trials median residue (STMR) for cereal grain, cereal straw and pome fruit (0.02, 0.04 and 0.11mg/kg, respectively, see Table B.7.6-6). The MRL and STMR value in pome fruit was multiplied by the mean transfer factor (TF) for difenoconazole residues from apple fruit to wet pomace (TF=4.5; Table B.7.7.2-4).

Calculation using MRL

Taking the proposed MRL in cereal grain of 0.02 mg/kg, in cereal straw of 0.1 mg/kg and in pome fruit of 0.3 mg/kg (multiplied by mean TF of 4.5, i.e. 1.35 mg/kg), the dietary exposure level for dairy and beef cattle is 12.4 and 27.61mg difenoconazole/animal/day, respectively (Table B.7.8.1.1-1 and B.7.8.1.1-2). This gives an estimated worst-case daily feeding rate of 0.62 mg difenoconazole/kg diet (12.4/20), assuming 20 kg as the daily intake of dry matter (DM) for dairy cattle of 550 kg body weight or 0.022 mg/kg bw/day (12.4/550) and of 1.84 mg difenoconazole/kg diet (27.61/15), assuming 15 kg as the daily intake of DM for beef cattle of 350 kg body weight or 0.079 mg/kg bw/day (27.61/350).

Table B.7.8.1.1-1. Calculation of difenoconazole dietary exposure level in dairy cattle.

Crop	% Dry matter	% Diet contribution (dry weight)	Intake of dry matter (kg/animal/day)	Intake of fresh material (kg/animal/day)	MRL (mg/kg) fresh weight	Difenoconazole intake (mg/animal/day)
Grain	86	40	8	9.3	0.02	0.186
Straw	86	20	4	4.7	0.1	0.47
Fruit pomace	23	10	2	8.7	1.35[a]	11.75
Total intake			14	22.7		12.40

[a] MRL in pome fruit (0.3 mg/kg) multiplied by mean transfer factor of 4.5 from fruit to pomace (Table B.7.7.2-4).

Table B.7.8.1.1-2. Calculation of difenoconazole dietary exposure level in beef cattle.

Crop	% Dry matter	% Diet contribution (dry weight)	Intake of dry matter (kg/animal/day)	Intake of fresh material (kg/animal/day)	MRL (mg/kg) fresh weight	Difenoconazole intake (mg/animal/day)
Grain	86	80	12	13.95	0.02	0.279
Straw	86	50	7.5	8.7	0.1	0.87
Fruit pomace	23	30	4.5	19.6	1.35[a]	26.46
Total intake			24	42.2		27.61

[a] MRL in pome fruit (0.3 mg/kg) multiplied by mean transfer factor of 4.5 from fruit to pomace (Table B.7.7.2-4).

Calculation using STMR

Taking the maximum STMR for cereal grain of 0.02 mg/kg, for cereal straw of 0.04 mg/kg and for pome fruit of 0.11 mg/kg (multiplied by mean transfer factor of 4.5, i.e. 0.495 mg/kg), the dietary exposure level for dairy and beef cattle is 4.68 and 10.33 mg difenoconazole/animal/day, respectively (Table B.7.8.1.1-3 and B.7.8.1.1-4). This gives an estimated worst-case daily feeding rate of 0.23 mg difenoconazole/kg diet (4.68/20), assuming 20 kg as the daily intake of DM for dairy cattle of 550 kg body weight or 0.008 mg/kg bw/day (4.68/550) and of 0.69 mg difenoconazole/kg diet (10.33/15), assuming 15 kg as the daily intake of DM for beef cattle of 350 kg body weight or 0.029 mg/kg bw/day (10.33/350).

Table B.7.8.1.1-3. Calculation of difenoconazole dietary exposure level in dairy cattle.

Crop	% Dry matter	% Diet contribution (dry weight)	Intake of dry matter (kg/animal/day)	Intake of fresh material (kg/animal/day)	STMR (mg/kg) fresh weight	Difenoconazole intake (mg/animal/day)
Grain	86	40	8	9.3	0.02	0.186
Straw	86	20	4	4.7	0.04	0.188
Fruit pomace	23	10	2	8.7	0.495[a]	4.31
Total intake			14	22.7		4.68

[a] STMR in pome fruit (0.11 mg/kg) multiplied by mean transfer factor of 4.5 from fruit to pomace (Table B.7.7.2-4).

Table B.7.8.1.1-4. Calculation of difenoconazole dietary exposure level in beef cattle.

Crop	% Dry matter	% Diet contribution (dry weight)	Intake of dry matter (kg/animal/day)	Intake of fresh material (kg/animal/day)	STMR (mg/kg) fresh weight	Difenoconazole intake (mg/animal/day)
Grain	86	80	12	13.95	0.02	0.279
Straw	86	50	7.5	8.7	0.04	0.348
Fruit pomace	23	30	4.5	19.6	0.495[a]	9.70
Total intake			24	42.2		10.33

[a] STMR in pome fruit (0.11 mg/kg) multiplied by mean transfer factor of 4.5 from fruit to pomace (Table B.7.7.2-4).

B.7.8.2 Intake calculations for laying hens

Intake of residues by hens may be expected from grain (straw is not considered as feed commodity for hens). The potential dietary exposure of poultry to difenoconazole is calculated according to the document 7031/VI/95 rev. 4, 22/7/1996. Residue intakes were calculated using the proposed MRL or STMR of 0.02 mg/kg for cereal grain (see Table B.7.6-6).

Calculation using MRL or STMR

Taking the MRL or STMR in cereal grain of 0.02 mg/kg, the dietary exposure level for a hen is 0.0019 mg difenoconazole/hen/day (Table B.7.8.2-1). This gives an estimated worst-case daily feeding rate of 0.016 mg difenoconazole/kg diet (0.0019/0.12), assuming 0.12 kg as the daily intake of DM for a hen of 1.9 kg body weight or 0.001 mg/kg bw/day (0.0019/1.9).

Table B.7.8.2-1. Calculation of difenoconazole dietary exposure level in poultry.

Crop	% Dry matter	% Diet contribution (dry weight)	Intake of dry matter (kg/hen/day)	Intake of fresh material (kg/hen/day)	MRL or STMR (mg/kg) fresh weight	Difenoconazole intake (mg/hen/day)
Cereal Grain	86	70	0.084	0.098	0.02	0.00196

In the metabolism studies with [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole (see B.7.2-4, B.7.2-5 and B.7.2-6), hens were dosed at rates equivalent to 5, 68 and 121 mg/kg diet for 14, 3 and 4 consecutive days, respectively. These concentrations are approximately 312, 4250 and 7562 times the more realistic estimated feeding rate of 0.016 mg difenoconazole/kg diet calculated above. Total radioactive residues in the available studies were up to 0.137, 0.248 and 2.72 mg difenoconazole equivalents/kg in egg white in hens dosed with 5, 68 and 121 mg difenoconazole/kg diet, respectively. In egg yolks the TRR were up to 0.240, 0.139 and up to 2.72 mg difenoconazole equivalents/kg, respectively, while in egg yolks and in tissues the TRR were up to 0.795, 7.76 and 40.86 mg difenoconazole equivalents/kg, respectively.

Parent difenoconazole residues in egg white in hens dosed with 68 mg/kg diet were up to 0.013 mg/kg. No parent difenoconazole residues were detected in hens dosed with 121 mg/kg diet. In egg yolks, parent difenoconazole residues were up to 0.001 and 0.236 mg/kg, respectively, and in tissues up to 0.207 and 2.80 mg/kg, respectively (see Table B.7.2.5-3, B.7.2.5-4 and B.7.2.6-4).

In practice following realistic exposure levels of 0.016 mg difenoconazole/kg diet, residues of difenoconazole will not exceed 0.01 mg/kg in eggs or any edible tissue. However, the transfer of residues of difenoconazole from poultry into tissues and eggs has been investigated in a new study by the notifier. The level of 1,2,4-triazole

in the tissues and eggs after feeding of difenoconazole has also being determined. The samples have been analysed and the final report is currently being written. The estimated completion of the final report for the study is mid-2006. The RMS suggest that this study could be included in an Addendum.

B.7.9 Residues in succeeding or rotational crops (Annex IIA 6.6, Annex III 8.5)

Residues in rotational crops were investigated in three studies conducted outdoors. Two radiolabelled forms of difenoconazole, [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole, were used (see Figure B.7.1-1).

B.7.9.1 Outdoor crop rotation study with [phenyl-^{14}C] difenoconazole

Reference:	**Walser M. (1994a).** Outdoor confined accumulation study on rotational crops after bare ground soil application of [^{14}C-Phenyl]-CGA-169374. Ciba-Geigy Limited, Divisional Unit R&D, Division Plant Protection, Basel, Switzerland. Unpublished Report 8/94, SAM No. 0924.
Test Material:	[^{14}C-phenyl] CGA 169374, JAK-5-79, radiochemical purity >95%, specific activity 0.862 MBq/mg (23.32 µCi/mg).
Guideline:	Pesticide Assessment Guidelines, Subdivision N, Chemistry, Environmental Fate, Series 165-1, U.S. Environmental Protection Agency, Washington, D.C., October 1982. Pesticide Assessment Guidelines, Subdivision N, Chemistry, Environmental Fate, Series 165-1, Addendum 7 on Data Reporting, U.S. Environmental Agency, Washington, D.C., October 1988. Agricultural chemicals Laws and Regulations, Japan, Metabolism in Plants, society of Agricultural Chemical Industry. (1985).
GLP:	Yes

Material and methods:

Test concentration:	125 g a.i./ha
Test system:	The study was conducted outside in a field plot (4 m^2, 2x2 m) in Switzerland in sandy loam soil. The soil characteristics were: pH (7.3), organic carbon (2.26%), sand (24.8%), silt (47.1%), clay (27%), CEC (29.6 mmol/z//100g). Difenoconazole, formulated as an EC 250 was applied once to bare soil at 125 g a.i./ha, using a spraying device equipped with four TeeJet flat-fan nozzles. The rate applied consisted with the GAP for Northern and Southern Europe on carrots**.** Four rotational crops were planted/seeded according to procedures simulating normal agricultural practices, i.e. lettuce (variety *Soraya*), winter wheat (variety *Sardona*), maize (variety *DK 250*), and sugar beet (variety *Regina*), 98 days, 126 days, 342 days, and 369 days after the soil treatment. Immature and mature samples of lettuce, wheat, maize and sugar beet commodities were taken at different maturity stages after application. Four soil cores (0 – 5 cm, 5 – 10 cm, 10 – 20 cm and 20 - 30 cm) were taken at planting and each harvest for the four crops.
No. of applications:	One
Method of analysis:	The TRR in all samples was determined by combustion/LSC. Soil and plant samples were sequentially extracted with methanol/water (8:2 v/v) and n-

propanol/water (8:2 v/v, with microwave assistance). Non-extracted residue levels were confirmed by combustion/LSC. Identification of the extracted residues from soil was carried out by co-chromatography against standard reference compounds using TLC.

Date of experiment: 04 June 1992 to 30 November 1993

Findings: The TRR in rotational crops following soil application of [phenyl-^{14}C] difenoconazole at a rate of 125 g a.i./ha were very low. Lettuce heads harvested 126 and 151 days after application contained 0.003 and 0.002 mg difenoconazole equivalents/kg, respectively (Table B.7.9.1-1). Immature winter wheat harvested 167, 342 and 369 days after application contained 0.003, 0.002 and 0.001 mg difenoconazole equivalents/kg, respectively, with mature straw, husks and grain harvested 418 days after application containing 0.009, 0.002 and 0.003 mg difenoconazole equivalents/kg, respectively. Sugar beet tops harvested 427, 473 and 488 days after application contained < 0.001, < 0.001 and 0.001 mg difenoconazole equivalents/kg, respectively, with the corresponding roots containing 0.001 mg difenoconazole equivalents/kg at each interval. Residues of 0.001 mg difenoconazole equivalents/kg were detected in immature and mature maize commodities harvested at intervals of 398, 427 and 488 days after application.

Plant samples were not extracted for characterisation because of the very low levels of TRR (< 0.01 mg/kg) present in all samples.

In soil (0 to 5 cm layer), the TRR decreased from 0.197 mg difenoconazole equivalents/kg one hour after application to between 0.038 and 0.055 mg difenoconazole equivalents/kg after 488 days (Table B.7.9.1-2). The amount of difenoconazole in the soil declined from 0.152 mg difenoconazole equivalents/kg (77.2% TRR) immediately after application to 0.008 mg difenoconazole equivalents/kg (15.5% TRR) after 488 days. Non-extracted residues increased to *ca* 70% of the TRR from 400 days onwards, with the remainder of the residue at each interval made up of low levels of the metabolites CGA-205375 (3.0 to 10.5%), CGA-205374 (0.2 to 1.1%) and CGA-189138 (0.3 to 1.0%; Table B.7.9.1-3).

Table B.7.9.1-1. Summary of radioactive residues in rotational crops.

Crop	Planting /seeding Interval[a]	Harvest Date[a]	Growth stage	Crop part	TRR (mg/kg)[b]	E1 (% TRR)	E2 (%TRR)	Non-extracted (% TRR)	Total (% TRR)
Lettuce	98	126	50% maturity	head	0.003	n.a.	n.a.	n.a.	
		151	maturity	head	0.002	n.a.	n.a.	n.a.	
Winter wheat	126	167	emergence	whole tops	0.003	n.a.	n.a.	n.a.	
		342	25% maturity	whole tops	0.002	n.a.	n.a.	n.a.	
		369	50% maturity	whole tops	0.001	n.a.	n.a.	n.a.	
		418	maturity	straw husks grain	0.009 0.002 0.003	n.a.	n.a.	n.a.	
Sugar beet	369	427	25% maturity	tops roots	<LD 0.001	n.a.	n.a.	n.a.	
		473	50% maturity	tops roots	<LD 0.001	n.a.	n.a.	n.a.	
		488	maturity	tops roots	0.001 0.001	n.a.	n.a.	n.a.	
Maize	342	398	25% maturity	whole tops	0.001	n.a.	n.a.	n.a.	
		427	50% maturity	whole tops	0.001	n.a.	n.a.	n.a.	
		488	maturity	stalks cobs grain	0.001 0.001 0.001	n.a.	n.a.	n.a.	

[a] Days after application.
[b] mg difenoconazole equivalents/kg.
E1 = Extract 1, cold extraction methanol/water (8:2).
E2 = Extract 2, MLS with n-propanol/water (8:2); MLS: Microwave assisted extraction.
% TRR results based on figure determined by combustion.
n.a. = Not applicable (contains TRR < 0.01 mg/kg).
LD for combustion: 0.001 ppm.

Table B.7.9.1-2. Summary of radioactive residues in soil sampled at different times after treatment with [phenyl-^{14}C] difenoconazole.

Crop	Days after treatment	Soil Layers (cm)	TRR (mg/kg)[a]	TRR (%)[b]	Difenoconazole (mg/kg)[a]	E1 (% TRR)[c]	E2 (% TRR)[c]	Non-extracted (% TRR)[c]	Total (% TRR)[c]
Lettuce	98	0-5	0.128	89.1	0.044	51.2	6.5	35.9	93.6
		5-10	0.014	9.6	n.a.	n.a.	n.a.	n.a.	
		10-20	0.001	1.1	n.a.	n.a.	n.a.	n.a.	
		20-30	<LD	0.3	n.a.	n.a.	n.a.	n.a.	
	151	0-5	0.076	60.9	0.026	51.0	6.0	38.7	95.7
		5-10	0.038	31.7	n.a.	n.a.	n.a.	n.a.	
		10-20	0.003	5.7	n.a.	n.a.	n.a.	n.a.	
		20-30	0.001	1.7	n.a.	n.a.	n.a.	n.a.	
Winter wheat	126	0-5	0.100	71.8	0.033	51.3	1.6	44.1	97.0
		5-10	0.016	9.5	n.a.	n.a.	n.a.	n.a.	
		10-20	0.011	18.1	n.a.	n.a.	n.a.	n.a.	
		20-30	<LD	0.7	n.a.	n.a.	n.a.	n.a.	
	418	0-5	0.059	55.9	0.010	28.4	n.a.	73.6	102.0
		5-10	0.026	20.9	n.a.	n.a.	n.a.	n.a.	
		10-20	0.008	20.1	n.a.	n.a.	n.a.	n.a.	
		20-30	0.001	3.1	n.a.	n.a.	n.a.	n.a.	
Sugar beet	314	0-5	0.072	63.0	0.018	39.5	n.a.	56.4	95.9
		5-10	0.023	20.4	n.a.	n.a.	n.a.	n.a.	
		10-20	0.010	12.7	n.a.	n.a.	n.a.	n.a.	
		20-30	0.002	3.9	n.a.	n.a.	n.a.	n.a.	
	488	0-5	0.038	53.5	n.a.	23.1	n.a.	64.4	87.5
		5-10	0.015	22.1	n.a.	n.a.	n.a.	n.a.	
		10-20	0.007	22.0	n.a.	n.a.	n.a.	n.a.	
		20-30	0.001	2.4	n.a.	n.a.	n.a.	n.a.	
Maize	342	0-5	0.049	59.1	0.013	41.0	n.a.	58.1	99.1
		5-10	0.020	20.7	n.a.	n.a.	n.a.	n.a.	
		10-20	0.008	17.6	n.a.	n.a.	n.a.	n.a.	
		20-30	0.001	2.6	n.a.	n.a.	n.a.	n.a.	
	488	0-5	0.055	59.0	0.008	19.8	n.a.	71.6	91.4
		5-10	0.021	24.3	n.a.	n.a.	n.a.	n.a.	
		10-20	0.006	13.8	n.a.	n.a.	n.a.	n.a.	
		20-30	0.001	2.9	n.a.	n.a.	n.a.	n.a.	

[a] mg difenoconazole equivalents/kg.
[b] in % of the radioactivity found in the sub-balanced plant parts/total soil system.
[c] in % of the radioactivity found in the plant part / soil layer, determined by combustion.
E1 = Extract 1, cold extraction methanol/water (8:2).
E2 = Extract 2, MLS with n-propanol/water (8:2); MLS: Microwave assisted extraction.
n.a. = Not analysed.
LD for combustion: 0.001ppm

Table B.7.9.1-3. Identification of metabolites in soil extracts (0 – 5 cm).

	Total residue ppm	CGA 169374 %	CGA 205375 %	CGA 205374 %	CGA 189138 %	Un-resolved %	MLS %	Non-extracted %	Total %
Soil lettuce 0%	0.128	34.5	9.7	1.1	0.8	3.4	6.5	35.9	91.9
Soil lettuce 100%	0.076	34.5	10.2	1.0	1.0	3.3	6.0	38.7	94.7
Soil wheat 0%	0.100	33.4	10.5	1.1	1.0	2.1	1.6	44.1	93.8
Soil wheat 100%	0.059	17.4	6.5	0.5	0.3	1.4	n.a.	73.6	99.7
Soil Sugar beet 0% [a]	0.072	24.7	9.6	0.9	1.0	2.4	n.a.	56.4	95.0
Soil corn 0%	0.049	26.1	9.8	0.6	0.8	2.7	n.a.	58.1	98.1
Soil corn 100%	0.055	15.0	3.0	0.2	0.4	0.9	n.a.	71.6	91.1

[a] The 80% MeOH extract of the soil sample sugar beet (100%) contained TRR <0.01 and was not further analysed.
n.a. = Not analysed.
MLS=microwave extraction.

Conclusion: TRR in rotational crops planted 98 to 488 days after one application of difenoconazole applied at a rate of 125 g a.i./ha to bare ground (i.e. in accordance with the proposed GAP for carrots within the EU region), was very low. The level of TRR in all harvested commodities from lettuce, winter wheat, sugar beet and maize

planted as rotational crops were less than 0.01 mg/kg ranging from <LD to 0.009 mg difenoconazole equivalents/kg.

Comments by RMS: The study was well performed and reported.

B.7.9.2 Outdoor crop rotation study with [triazole-^{14}C] difenoconazole

Reference:	**Walser M. (1994b).** Outdoor confined accumulation study on rotational crops after bare ground soil application of [^{14}C-Triazole]-CGA-169374. Ciba-Geigy Limited, Divisional Unit R&D, Division Plant Protection, Basel, Switzerland. Unpublished Report 4/94, SAM No. 2395.
Test Material:	[^{14}C-triazole] CGA 169374, JAK-5-80, radiochemical purity >95%, specific activity 1.04 MBq/mg (28.1 µCi/mg).
Guideline:	Pesticide Assessment Guidelines, Subdivision N, Chemistry, Environmental Fate, Series 165-1, U.S. Environmental Protection Agency, Washington, D.C., October 1982. Pesticide Assessment Guidelines, Subdivision N, Chemistry, Environmental Fate, Series 165-1, Addendum 7 on Data Reporting, U.S. Environmental Agency, Washington, D.C., October 1988. Agricultural chemicals Laws and Regulations, Japan, Metabolism in Plants, society of Agricultural Chemical Industry. (1985).
GLP:	Yes

Material and methods:

Test concentration:	125 g a.i./ha
Test system:	The study was conducted outside in a field plot (4 m^2, 2x2 m) in Switzerland in sandy loam soil. The soil characteristics were: pH (7.3), organic carbon (2.26%), sand (24.8%), silt (47.1%), clay (27%), CEC (29.6 mmol/z//100g). Difenoconazole, formulated as an EC 250 was applied once to bare soil at 125 g a.i./ha, using a spraying device equipped with four TeeJet flat-fan nozzles. The rate applied consisted with the GAP for Northern and Southern Europe on carrots**.** Four rotational crops were planted/seeded according to procedures simulating normal agricultural practices, i.e. lettuce (variety *Soraya*), winter wheat (variety *Sardona*), maize (variety *DK 250*), and sugar beet (variety *Regina*), 98 days, 126 days, 342 days, and 369 days after the soil treatment. Immature and mature samples of lettuce, wheat, maize and sugar beet commodities were taken at different maturity stages after application. Four soil cores (0 – 5 cm, 5 – 10 cm, 10 – 20 cm and 20 - 30 cm) were taken at planting and each harvest for the four crops.
No. of applications:	One
Method of analysis:	The TRR in all samples was determined by combustion/LSC. Soil and plant samples were sequentially extracted with methanol/water (8:2 v/v) and n-propanol/water (8:2 v/v, with microwave assistance). Non-extracted residue levels

were confirmed by combustion/LSC. Identification of the extracted residues from soil was carried out by co-chromatography against standard reference compounds using TLC.

Date of experiment: 04 June 1992 to 30 November 199

Findings: The TRR in rotational crops following soil application of [triazole-^{14}C] difenoconazole at a rate of 125 g a.i./ha were low. Lettuce heads harvested 126 and 151 days after application contained 0.021 and 0.017 mg difenoconazole equivalents/kg, respectively (Table B.7.9.2-1). Immature winter wheat harvested 167, 342 and 369 days after application contained 0.028, 0.045 and 0.072 mg difenoconazole equivalents/kg, respectively, with mature straw, husks and grain harvested 418 days after application containing 0.112, 0.154 and 0.341 mg difenoconazole equivalents/kg, respectively. Sugar beet tops harvested 427, 473 and 488 days after application contained 0.019, 0.034 and 0.029 mg difenoconazole equivalents/kg, respectively, with the corresponding roots containing 0.011, 0.007 and 0.005 mg difenoconazole equivalents/kg at each interval. Sugar beet tops harvested 427, 473 and 488 days after application contained < 0.001, < 0.001 and 0.001 mg difenoconazole equivalents/kg, respectively, with the corresponding roots containing 0.001mg difenoconazole equivalents/kg at each interval. Residues of 0.071, 0.057 and 0.027 mg difenoconazole equivalents/kg were detected in immature and mature maize harvested at intervals of 398, 427 and 488 days after application, with mature cobs and grain containing 0.040 and 0.211 mg difenoconazole equivalents/kg, respectively at harvest 488 days after application. .

Extraction of plant samples with aqueous methanol released > 80% of the TRR. Non-extracted residues contained < 0.05 mg/kg in each sample (Table B.7.9.2-1). Extractable residues in mature lettuce, wheat, sugar beet and corn commodities were predominantly aqueous soluble (79.5 to 90.4% TRR) and were composed of the metabolites CGA-131013 (10.4 to 66.2% TRR), CGA-205369 (9.7 to 54.3% TRR) and CGA-142856 (2.7 to 39.4% TRR; Table B.7.9.2-2).

In soil (0 to 5 cm layer), the TRR decreased from 0.143 mg difenoconazole equivalents/kg to between 0.039 and 0.051 mg difenoconazole equivalents/kg after 488 days (harvest of corn; Table B.7.9.2-3). The amount of difenoconazole in the soil declined from 0.057 mg difenoconazole equivalents/kg to 0.005 mg difenoconazole equivalents/kg after 488 days. Unextracted residues increased from 31.9% to 72% of the TRR over the same period, with the remainder of the residue at each interval made up of low levels of the metabolites CGA-205375 (5.9 to 12.0%), CGA-205374 (0.3 to 1.6%) and CGA-189138 (0.2 to 1.2%; Table B.7.9.2-4).

Table B.7.9.2-1. Summary of radioactive residues in rotational crops.

Crop	Planting /seeding Interval[a]	Harvest Date[a]	Growth stage	Crop part	TRR (mg/kg)[b]	E1 (% TRR)	E2 (% TRR)	Non-extracted (% TRR)	Total (% TRR)
Lettuce	98	126	50% maturity	head	0.021	95.8	n.a.	5.0	100.8
		151	maturity	head	0.017	94.2	n.a.	4.0	98.2
Winter wheat	126	167	emergence	whole tops	0.028	n.a.	n.a.	n.a.	
		342	25% maturity	whole tops	0.045	88.9	n.a.	4.7	93.6
		369	50% maturity	whole tops	0.072	101.2	n.a.	5.8	107.0
		418	maturity	straw husks grain	0.112 0.154 0.341	82.4 88.0 87.6	n.a. n.a. 8.9	20.9 14.5 9.2	103.3 102.5 105.7
Sugar beet	369	427	25% maturity	tops roots	0.019 0.011	90.2 83.4	n.a.	7.9 24.0	98.1 107.4
		473	50% maturity	tops roots	0.034 0.007	94.5 n.a.	n.a.	3.3 n.a	97.8
		488	maturity	tops roots	0.029 0.005	96.2 n.a.	n.a.	3.8 n.a.	100.0
Maize	342	398	25% maturity	whole tops	0.071	100.3	n.a.	4.1	104.4
		427	50% maturity	whole tops	0.057	89.4	n.a.	5.3	95.7
		488	maturity	stalks cobs grain	0.027 0.040 0.211	76.9 66.3 85.6	n.a.	22.4 31.2 20.5	99.3 97.5 106.1

[a] Days after application.
[b] mg difenoconazole equivalents/kg.
E1 = Extract 1, cold extraction methanol/water (8:2).
E2 = Extract 2, MLS with n-propanol/water (8:2); MLS: Microwave assisted extraction.
% TRR results based on figure determined by combustion.
n.a. = Not analysed (contains TRR < 0.01 mg/kg).
LD for combustion: 0.001 ppm.

Table B.7.9.2-2. Identification of metabolites in rotational crops at maturity.

Crop	Crop Part	TRR (mg/kg)	% of TRR							
			Organic phase	Aqueous phase				MLS	N.E.	Total
				CGA-131013	CGA-142856	CGA-205369	Unresolved	Total			
Maize	Grain	0.211	1.2	66.2	--	9.7	3.6	79.5	n.a.	20.5	101.2
Wheat	Grain	0.341	1.9	44.0	25.9	--	11.3	81.2	8.9	9.2	101.2
Wheat	Stalks	0.112	2.3	10.4	36.2	21.2	5.6	73.4	n.a.	20.9	96.6
Wheat	Husks	0.154	0.2	19.0	39.4	12.1	7.8	78.3	n.a.	14.5	93.0
Lettuce	Head	0.017	0.3	30.5	3.3	42.8	5.8	82.4	n.a.	4.0	86.7
Sugar beet	Tops	0.029	0.3	25.3	2.7	54.3	8.1	90.4	n.a.	3.8	94.5

MLS = microwave extraction.
N.E. = non-extractable.
n.a. = Not applicable (contains TRR < 0.01 mg/kg).

Table B.7.9.2-3. Summary of radioactive residues in soil sampled at different times after treatment with [triazole-^{14}C] difenoconazole.

Crop	Days after treatment	Soil Layers (cm)	TRR (mg/kg)[a]	TRR (%)[b]	Difenoconazole (mg/kg)[a]	E1 (% TRR)[c]	E2 (% TRR)[c]	Non-extracted (% TRR)[c]	Total (% TRR)[c]
Lettuce	98	0-5	0.143	76.9	0.057	71.8	n.a.	31.9	103.7
		5-10	0.036	19.7	n.a.	n.a.	n.a.	n.a.	
		10-20	0.002	2.7	n.a.	n.a.	n.a.	n.a.	
		20-30	0.001	0.7	n.a.	n.a.	n.a.	n.a.	
	151	0-5	0.097	52.9	0.029	63.2	n.a.	42.7	105.7
		5-10	0.056	39.8	n.a.	n.a.	n.a.	n.a.	
		10-20	0.005	6.5	n.a.	n.a.	n.a.	n.a.	
		20-30	<0.001	0.7	n.a.	n.a.	n.a.	n.a.	
Winter wheat	126	0-5	0.108	40.8	0.035	62.1	n.a.	43.2	105.3
		5-10	0.113	48.5	n.a.	n.a.	n.a.	n.a.	
		10-20	0.011	9.9	n.a.	n.a.	n.a.	n.a.	
		20-30	0.001	0.8	n.a.	n.a.	n.a.	n.a.	
	418	0-5	0.072	56.2	0.012	33.0	n.a.	64.8	97.8
		5-10	0.028	20.3	n.a.	n.a.	n.a.	n.a.	
		10-20	0.010	18.1	n.a.	n.a.	n.a.	n.a.	
		20-30	0.002	5.4	n.a.	n.a.	n.a.	n.a.	
Sugar beet	314	0-5	0.098	70.5	0.022	45.4	n.a.	51.8	97.2
		5-10	0.028	18.8	n.a.	n.a.	n.a.	n.a.	
		10-20	0.007	8.7	n.a.	n.a.	n.a.	n.a.	
		20-30	0.002	2.0	n.a.	n.a.	n.a.	n.a.	
	488	0-5	0.039	48.7	n.a.	24.3	n.a.	73.3	97.6
		5-10	0.024	24.2	n.a.	n.a.	n.a.	n.a.	
		10-20	0.002	4.0	n.a.	n.a.	n.a.	n.a.	
		20-30	0.008	23.1	n.a.	n.a.	n.a.	n.a.	
Maize	342	0-5	0.085	55.1	0.014	34.6	n.a.	60.7	95.3
		5-10	0.044	32.1	n.a.	n.a.	n.a.	n.a.	
		10-20	0.007	10.6	n.a.	n.a.	n.a.	n.a.	
		20-30	0.001	2.2	n.a.	n.a.	n.a.	n.a.	
	488	0-5	0.051	39.2	0.005	22.9	n.a.	72.0	94.9
		5-10	0.035	28.2	n.a.	n.a.	n.a.	n.a.	
		10-20	0.014	23.0	n.a.	n.a.	n.a.	n.a.	
		20-30	0.004	9.7	n.a.	n.a.	n.a.	n.a.	

[a] mg difenoconazole equivalents/kg.
[b] in % of the radioactivity found in the sub-balanced plant parts/total soil system.
[c] in % of the radioactivity found in the plant part / soil layer, determined by combustion.
E1 = Extract 1, cold extraction methanol/water (8:2).
E2 = Extract 2, MLS with n-propanol/water (8:2); MLS: Microwave assisted extraction.
n.a. = Not analysed.
LD for combustion: 0.001ppm

Table B.7.9.2-4. Identification of metabolites in soil extracts (0 – 5 cm).

	TRR (mg/kg)	% of TRR								
		Aqueous phase	Organic phase					MLS	N.E.	Total
			CGA-169374	CGA-205375	CGA-205374	CGA-189138	Un-res.	Total			
Soil lettuce 0%	0.143	7.8	39.7	11.2	1.2	1.2	2.1	55.4	n.a.	31.9	95.1
Soil lettuce 100%	0.097	9.9	29.4	11.3	0.8	0.9	2.2	45.4	n.a.	42.5	97.8
Soil wheat 0%	0.108	5.3	31.9	12.0	1.6	1.1	1.7	48.3	n.a.	43.2	96.8
Soil wheat 100%	0.072	5.3	16.1	8.3	0.7	0.2	2.6	27.9	n.a.	64.8	98.0
Soil Sugar beet 0% [a]	0.098	3.5	22.4	10.6	0.8	0.9	1.8	36.5	n.a.	51.8	91.8
Soil corn 0%	0.085	1.5	16.8	8.3	0.7	0.7	1.7	28.2	n.a.	60.7	90.4
Soil corn 100%	0.051	4.2	9.3	5.9	0.3	0.4	1.4	17.3	n.a.	72.0	93.5

[a] The 80% MeOH extract of the soil sample sugar beet (100%) contained TRR <0.01 and was not further analysed.
n.a. = Not analysed.; MLS=microwave extraction.
N.E. = non-extractable.

Conclusion: TRR levels in lettuce, sugar beet (tops and roots), immature wheat, and immature maize following [triazole-14C] difenoconazole application at a rate of 125 g a.i./ha (i.e. in accordance with the proposed GAP for carrots within the EU region), were ≦ 0.072 mg/kg. Residues in mature wheat grain and in mature maize grain were 0.341 and 0.211 mg/kg, respectively. These residues occurred by selective transport of triazole derivatives from the soil to the grain. Analysis showed that these grain residues contained CGA-131013 (triazole alanine), CGA-142856 (triazole acetic acid) and CGA-205369 (triazole lactic acid).

Comments by RMS: Following the intended difenoconazole application on cereal seed at a rate of 12 g a.i./ha (i.e. 10 times lower than in the present study), negligible residues in soil and in succeeding crops will be expected.

B.7.9.3 Outdoor crop rotation study with [phenyl-^{14}C] difenoconazole

Reference:	**Close C. (1995).** ^{14}C-CGA-169374: Uptake and distribution of residues in confined rotational crops. Ciba-Geigy Corporation, Ciba Crop Protection, Biochemistry Department, Greensboro, North Carolina, USA. Unpublished Report ABR-95057, SAM No. 1118.
Test Material:	[^{14}C-phenyl] CGA 169374, synthesis reference CL-XXXV-41, radiochemical purity 98.6%, specific activity 30.3 µCi/mg.
Guideline:	Nature of the Residue in Plants: 40 CFR 158 Pesticide Assessment Guidelines, Subdivision N, Chemistry, Environmental Fate, Series 165-1, U.S. Environmental Protection Agency, Washington, D.C., October 1982. Pesticide Assessment Guidelines, Subdivision N, Chemistry, Environmental Fate, Series 165-1, Addendum 7 on Data Reporting, U.S. Environmental Agency, Washington, D.C., October 1988.
GLP:	Yes

Material and methods:

Test concentration:	32.4 g a.i./ha (applied in methanol solution)
Test system:	The study was conducted outside in 4 field plots in Sanger, California in sandy loam soil. Uptake, distribution, and degradation of [phenyl-^{14}C] difenoconazole after overall spray application to bare soil at a rate of 32.4 g a.i./ha (applied in methanol solution) were investigated in spring wheat (variety *Aldura*), mustard (variety *Florida broadleaf*) and turnip (variety *Purple top white globe*) rotational crops, planted 30 days (Trial 1) and 33 days (Trial 2) after application. The second trial was initiated after high background levels were observed in Day 0 soil cores. Spring wheat, mustard, and turnips seeds were sowed directly by hand into the treated soil. Immature and mature samples of wheat, mustard and turnip commodities were taken at different intervals after application. Soil cores were taken before and after treatment, and at planting for both trials. The soil

characteristics in Trial 1 were: pH (5.5), organic carbon (0.54%), sand (60.8%), silt (30%), clay (9.2%), CEC (4.40 meg/100g) and Trial 2: pH (7.2), organic carbon (0.49%), sand (64.8%), silt (28%), clay (7.2%), CEC (4.07 meg/100g).

No. of applications: One

Method of analysis: TRR in all samples was determined by combustion/LSC.

Date of experiment: 27 October 1994 to 08 June 1995

Findings: The TRR in rotational crops following soil application of [phenyl-^{14}C] difenoconazole at a rate of 32.4 g/ha were very low. Mature mustard, turnip tops and roots, harvested 137 and 129 days after treatment in Trial 1 and Trial 2, respectively contained up to 0.002 mg/kg (Table B.7.9.3-1). Mature wheat forage harvested up to 179 days after treatment contained up to 0.004 mg/kg. Mature straw and grain harvested 218 and 175 days after treatment in Trial 1 and Trial 2, respectively contained up to 0.007 mg/kg. TRR levels in soil were 0.007 to 0.011 mg/kg after application in the two trials and 0.010 mg/kg, 30 to 33 days later (Table B.7.9.3-2).

Plant and soil samples were not extracted for characterisation because of the very low levels of TRR present in all samples.

Table B.7.9.3-1. Summary of radioactive residues in rotational crops.

Crop	Growth stage	Harvest interval after application (days)	TRR (mg/g)[a]
Trial 1			
Turnip	Mature tops	137	0.001
	Mature roots	137	0.002
Mustard	Mature greens	137	0.002
Spring wheat	25% mature tops	137	0.001
	50% mature tops	179	0.004
	Mature straw	218	0.006
	Mature grain	218	0.007
Trial 2			
Turnip	Mature tops	129	<0.0001
	Mature roots	129	0.0001
Mustard	Mature greens	129	<0.0001
Spring wheat	25% mature tops	109	0.002
	Mature straw	175	0.004
	Mature grain	175	0.001

[a] mg difenoconazole equivalents/kg.

Table B.7.9.3-2. Summary of radioactive residues in soil sampled at different times after treatment with [phenyl-^{14}C] difenoconazole.

Soil		Harvest interval after application (days)	TRR (mg/g)[a]
Trial 1			
0 –15 cm layer	Post-application	0	0.007
	Day of planting	30	0.010
Trial 2			
0 –15 cm layer	Post-application	0	0.011
	Day of planting	33	0.010

[a] mg difenoconazole equivalents/kg.

Conclusion: The uptake of radioactivity into succeeding crops, following bare soil application of [phenyl-^{14}C] difenoconazole at 32.4 g a.i./ha, was minimal. The levels of TRR in all harvested commodities from turnip, mustard and wheat planted as rotational crops were less than 0.01 mg/kg.

Comments by RMS: The study was well performed and reported.

B.7.9.4 Determination of difenoconazole and triazolylalanine in field soil and rotational crop (carrot)

Reference:	**Heyer R. (1995a).** Determination of difenoconazole and triazolylalanine in field soil and rotational crop (carrot). RCC Umweltchemie GmbH, D-64380 Rossdorf, Germany. Unpublished Report 488002, SAM No. 1215.
Test Material:	Difenoconazole (CGA 169374), batch number AMS 255/102, purity 99.0%±0.3%. Triazolyalanine derivate (for quantification), batch number CGA 276164 (L43/97), purity 97.2%.
Guideline:	Richtlinie für die amtliche Prüfung von Pflanzenschutzmitteln Part IV, 3-10, Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA), Federal Republic of Germany, 1986.
GLP:	Yes

Material and methods:

Test concentration:	750 g a.i./ha
Test system:	The study was conducted outside in a field plot in Kötschau, Saxony, Germany. The uptake of difenoconazole and the metabolite triazole alanine (CGA-131013) after overall spray application of difenoconazole to bare soil at a rate of 750 g a.i./ha (applied as an EC formulation) were investigated in a carrot rotational crop, planted 30 days after application. Prior to sowing carrot seed, the test plot was cultivated to a depth of 10 to 20 cm. Immature and mature carrot roots were taken at different intervals after application. Soil cores were taken before and after treatment, and at crop harvest intervals.
No. of applications:	One
Method of analysis:	Residues of difenoconazole in carrot were extracted with methanol/ammonia (8:2 v/v), purified by hexane partition and silica SPE and analysed by GC with electron capture detection (ECD). Residues of triazole alanine in carrot were extracted with methanol/1M HCL (7:3 v/v), purified by ion exchange chromatography and derivatised prior to analysis by GC with mass selective detection (MSD).
Date of experiment:	13 May 1994 to 26 July 1995

Findings: Residues of difenoconazole and triazole alanine in rotational carrot roots following soil application of difenoconazole at a rate of 750 g a.i./ha, were less than the LOD of 0.02 mg/kg and 0.05 mg/kg, respectively, at each harvest interval (Table B.7.9.4-1).

Table B.7.9.4-1. Residues of difenoconazole and triazole alanine in rotational carrot and soil samples following bare soil application of difenoconazole.

Sample	Harvest interval after application (days)	Residue (mg/g)
Difenoconazole		
Soil	0	0.26
	30	0.12
	97	0.19
	114	0.20
	136	0.18
Carrot	97	<0.02
	114	<0.02
	136	<0.02
Triazole alanine		
Carrot	97	<0.05
	114	<0.05
	136	<0.05

Conclusion: Residues of difenoconazole and the metabolite triazole alanine (CGA-131013) in roots of carrot planted 30 days following application of difenoconazole to bare soil at 750 g a.i./ha were less than the LOD, although the application rate was six times higher than the intended application rate of 125 g a.i./ha on carrots.

Comments by RMS: The study was well performed and reported.

B.7.9.5 Determination of difenoconazole and triazolylalanine in field soil and rotational crop (spinach)

Reference:	**Heyer R. (1995b).** Determination of difenoconazole and triazolylalanine in field soil and rotational crop (spinach). RCC Umweltchemie GmbH, D-64380 Rossdorf, Germany. Unpublished Report 488001, SAM No. 1216.
Test Material:	Difenoconazole (CGA 169374), batch number AMS 255/102, purity 99.0%±0.3%. Triazolyalanine derivate (for quantification), reference substance number 930122ELB01, purity 97.2%.
Guideline:	Richtlinie für die amtliche Prüfung von Pflanzenschutzmitteln Part IV, 3-10, Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA), Federal Republic of Germany, 1986.
GLP:	Yes

Material and methods:

Test concentration:	750 g a.i./ha
Test system:	The study was conducted outside in a field plot in Motterwitz, Saxony, Germany.

The uptake of difenoconazole and the metabolite triazole alanine (CGA-131013) after overall spray application of difenoconazole to bare soil at a rate of 750 g a.i./ha (applied as an EC formulation) were investigated in a spinach rotational crop, planted 31 days after application. Prior to sowing spinach seed, the test plot was cultivated to a depth of 10 to 20 cm. Immature and mature spinach samples were taken at different intervals after application. Soil cores were taken before and after treatment, and at crop harvest intervals.

No. of applications: One

Method of analysis: Residues of difenoconazole in spinach were extracted with methanol/ammonia (8:2 v/v), purified by hexane partition and silica SPE and analysed by GC with electron capture detection (ECD). Residues of triazole alanine in spinach were extracted with methanol/1M HCL (7:3 v/v), purified by ion exchange chromatography and derivatised prior to analysis by GC with mass selective detection (MSD).

Date of experiment: 13 May 1994 to 11 October 1995

Findings: Residues of difenoconazole and triazole alanine in rotational spinach following soil application of difenoconazole at a rate of 750 g a.i./ha, were less than the LOD of 0.02 mg/kg and 0.05 mg/kg, respectively, at each harvest interval (Table B.7.9.5-1).

Table B.7.9.5-1. Residues of difenoconazole and triazole alanine in rotational spinach and soil samples following bare soil application of difenoconazole.

Sample	Harvest interval after application (days)	Residue (mg/g)
Difenoconazole		
Soil	0	0.39
	31	0.10
	62	0.16
	70	0.23
	77	0.15
Spinach	62	<0.02
	70	<0.02
	77	<0.02
Triazole alanine		
Spinach	62	<0.05
	70	<0.05
	77	<0.05

Conclusion: Residues of difenoconazole and the metabolite triazole alanine in leaves of spinach planted 31 days following application of difenoconazole to bare soil at 750 g a.i./ha were less than the LOD.

Comments by RMS: The study was well performed and reported.

Overall conclusions for rotational crops

In five available studies, total radioactive residues in rotational crops (wheat, sugar beet, maize, lettuce, turnips and mustard) planted 62 to 488 days after one application of difenoconazole applied to bare ground at a rates of 32.4, 125 and 750 g a.i./ha ranged from <0.0001 to 0.34 mg difenoconazole equivalents/kg.

Following application equivalent to twice the maximum recommended rate for carrots in Northern and Southern Europe (3 x 125 g a.i./ha), residues of difenoconazole were below the LOD (<0.02 and <0.05 mg/kg). Although the PHI was not within 25% of the critical GAP in Northern and Southern Europe (14 vs. 30), the exaggerated application rate of 750 g a.i./ha represents a worst-case for residues of difenoconazole in rotational crops and in commercial practice residues of difenoconazole will not be expected in succeeding crops.

The uptake of radioactive residues in succeeding crops following [triazole-^{14}C] difenoconazole application at 125 g a.i./ha to bare soil was higher than observed in the corresponding [phenyl-^{14}C] difenoconazole study. The TRR levels in lettuce, sugar beet (tops and roots), immature wheat, and immature maize were equal to or less than 0.072 mg/kg. Residues in wheat grain and maize grain at harvest were 0.341 and 0.211 mg/kg, respectively. The magnitude of these residues was due to the selective transport of triazole derivatives (CGA-131013, CGA-142856 and CGA-205369) from the soil. Based on these findings it is concluded that the application of difenoconazole to seeds of cereals will lead to negligible residues in succeeding crops.

The metabolite CGA-205369 (triazole lactic acid) was neither detected in wheat grain nor in the plant metabolism studies (see Figure B.7.1.13-1).

B.7.10 Proposed pre-harvest intervals for envisaged uses, or withholding periods, in the case of post-harvest uses (Annex IIA 6.8, Annex III 8.7)

The proposed pre-harvest interval (PHI) in pome fruit is 28 days in Northern EU regions and 14 days in Southern EU regions. In carrots, the PHI is 14 days in Northern and Southern EU. Difenoconazole is applied as seed treatment to cereals pre-sowing and a PHI for cereals is not applicable. There is no requirement for any re-entry, withholding or waiting periods.

B.7.11 Community MRLs and MRLs in EU Member States (Annex IIA 12.2)

Difenoconazole is registered in 20 EU Member States. No MRLs have been established on EU level but MRLs exist at MS level (Table B.7.11-1).

Table B.7.11-1. MRLs in EU Member States.

Crop	Member State	MRL (mg/kg)
Apple	Belgium	0.2
	France	0.2
	Italy	0.5
	Luxembourg	0.2
	Netherlands	0.5
	Portugal	0.5
	Spain	0.5
Apricot	France	0.2
Artichoke, Jerusalem	Germany	0.1
Asparagus	France	0.02
	Italy	0.05
	Spain	0.02
Banana	France	0.1
	Germany	0.1
	Italy	0.1
	Netherlands	0.1
Barley group/seed	Austria	0.05
Bean group	Portugal	1.0
Beet group	Belgium	0.02
Beetroot	Germany	0.1
Brassica, head	Austria	0.2
	Germany	0.2
Brussels sprout	Belgium	0.2
Cabbage, Chinese	Austria	0.5
	Germany	0.2
Cabbage, head	Belgium	0.2
Carrot	Austria	0.1
	Belgium	0.2
	France	0.2
	Germany	0.2
	Italy	0.2
	Portugal	0.3
Cauliflower	France	0.05
	Italy	0.1
	Portugal	0.2
Celeriac	Austria	2.0
	Belgium	0.3
	Germany	0.5
Celery	Austria	0.5
	France	3.0
	Germany	0.5
	Italy	2.0
	Spain	2.0
Cereal group	France (grain)	0.02
	France (straw)	0.5
Chicory	Belgium	0.05
	France	0.05
	Germany	0.1
Corn, sweet	France	0.05
Cucumber	Italy	0.1
Fresh herbs	Germany	2.0
Garlic	Spain	0.02
Grape	France (fruit)	0.5
	France (wine)	0.01
Horseradish	Germany	0.2
Leek	Germany	0.5
	Portugal	0.1
Lettuce	Spain	2.0
Maize	France	0.05
Medlar	Spain	0.5
Oat group/seed	Austria	0.05
Olive	Spain	0.02
Parsley, root	Germany	0.2
Parsnip	Belgium	0.2

Crop	Member State	MRL (mg/kg)
	Germany	0.2
Pea	Portugal	1.0
Pea group	France	0.05
Pea, field	France	0.05
Pea, garden	France	0.05
Peach	France	0.2
Pear	Belgium	0.2
	France	0.2
	Italy	0.5
	Luxembourg	0.2
	Portugal	0.5
	Spain	0.5
Plum	France	0.2
Pome fruit group	Belgium	0.2
	Germany	0.2
	Spain	0.5
Potato	Italy	0.1
	Spain	0.02
Quince	Luxembourg	0.2
Rape, oilseed	Belgium	0.05
	France	0.1
	Germany	0.2
Rye	Germany	0.1
Salsify	France	0.2
	Germany	0.2
Sugar beet	Austria	0.1
	France	0.1
	Germany	0.1
	Greece	0.1
	Italy	0.2
	Spain	0.05
Sunflower	France	0.05
Swede	Germany	0.1
Tomato	France	0.5
	Italy	0.1
	Portugal	0.5
	Spain	0.5
Triticale group/seed	Austria	0.05
Turnip	Germany	0.1
Wheat group	Austria	0.05
	Belgium	0.05
	Netherlands	0.05
Wheat, winter	Austria	0.05
	Germany	0.1
Other products of plant origin	Austria	0.02
	Germany	0.05
	Netherlands	0.05

B.7.12 Proposed EU MRLs and justification for the acceptability of those MRLs (Annex IIA 6.7; Annex IIIA 8.6)

Proposed MRLs

The MRL proposed by the notifier is:

Commodity	Proposed MRL (mg/kg)
Pome fruit	0.3
Carrot	0.2
Wheat, Barley, Oat, Triticale, Rye - Grain**	0.02
Wheat, Barley, Oat, Triticale, Rye - Straw**	0.1*

* pseudo-MRL only proposed for dietary burden calculation

** in the original dossier submission MRL was only proposed for wheat. As a resulty of the EFSA quality check of the DAR the GAP for Dividend was clarified, and as a consequence a MRL for the cereal group was proposed (this was actually the intented use in the original dossier though it was not entirely consistent over all sections).

Justification of proposed MRLs

Pome fruit

There were nine trials in Northern EU and eleven in Southern EU conducted according to the proposed GAP. Residue levels were higher in Southern EU than in Northern EU. Based on the residues in Southern EU (which represents the worst-case), a MRL of 0.3 mg/kg is proposed for difenoconazole in pome fruit

Carrot

There were eight trials in Northern EU and eight in Southern EU conducted according to the proposed GAP. Residue levels were similar in both regions. No single residue value measured in the supervised trials was above 0.2 mg/kg. A MRL of 0.2 mg/kg is proposed for difenoconazole in carrot.

Cereals

There were eight trials in Northern EU and six trials in Southern EU conducted according to the proposed GAP. Residue levels in wheat grain were similar in both regions. No single residue value measured in the supervised trials was above the LOQ of 0.02 mg/kg . A MRL of 0.02 mg/kg is proposed for difenoconazole in cereal grain. In Southern EU there were six trials rather than the eight specified by the document 7525/VI/95 rev. 7, 12/6/2001. However, as no residues above the LOQ were detected, additional trials in Southern EU are not required. Since the application is a seed treatment, the residue trial data for wheat can be extrapolated to barley, oats, rye and triticale (Lundehn, Appendix D, Table 5, 7029/VI/95- rev. 5 22 Jul 97).

Table B.7.12-1. Residues used for MRL determination.

Pome fruit	Northern	0.01[a]	0.02[b]	0.03[b]	0.04[b]	0.05[a]	0.05[b]
		0.06[b]	0.07[b]	0.07[b]			
	Southern	0.04[b]	0.05[b]	0.07[a]	0.08[b]	0.10[b]	0.11[b]
		0.13[b]	0.14[b]	0.15[b]	0.16[a]	0.28[b]	
Carrot	Northern	0.02	0.02	0.03	0.04	0.05	0.07
		0.11	0.12				
	Southern	<0.02	0.02	0.02	0.02	0.03	0.07
		0.11	0.13				
Wheat grain	Northern	<0.01	<0.01	<0.02	<0.02	<0.02	<0.02
		<0.02	<0.02				
	Southern	<0.01	<0.01	<0.02	<0.02	<0.02	<0.02

[a] Pear; [b] Apple

Summary of proposed MRLs

The MRL and STMR proposed by the RMS based on the available residue data are shown in Table B.7.12-2.

Table B.7.12-2. Proposed MRL and STMR by the RMS.

Crops	MRL (mg/kg)	STMR (mg/kg)
Pome fruit	0.3	0.11
Carrot	0.2	0.05
Cereal grain (wheat, barley, oat, triticale, rye)	0.02	0.02

Comments by RMS: The MRL proposed by the notifier is confirmed (with regard to extrapolation from wheat to cereals reference to Appendix D, Doc. 7525/VI/95-rev. 7 12/6/2001).

Proposed MRLs for rotational crops

Maximum residues of difenoconazole in human edible commodities of succeeding crops grown in rotation after cereals (seed treatment) and carrot (foliar application) are not expected to exceed 0.01 mg/kg. Therefore, it is not considered necessary to set MRLs for succeeding crops.

Rotational crops are not planted after pome fruit and therefore succeeding crop residues following use of difenoconazole in pome fruit are not considered relevant.

Proposed MRLs for animal products

Since any calculation of the dietary burden based solely on representative crops, i.e. pome fruit, wheat and carrots would not reflect the actual exposure; no MRL/STMR proposal is made at this time for products of

animal origin. A full discussion of the expected dietary burden and the relevant residue will be addressed in the EU MRL submission.

B.7.13 Proposed EU Import tolerances and justification for the acceptability of those residues

Not applicable, since no non-EU applications are proposed in the current dossier.

B.7.14 Basis for differences, if any, in conclusions reached having regard to established or proposed Codex MRLs

Not applicable, since no Codex MRLs have been established or proposed yet.

B.7.15 Estimates of potential and actual dietary exposure through diet and other means (Annex IIA 6.9; Annex IIIA 8.8)

The Acceptable Daily Intake (ADI) has been proposed at 0.01 mg/kg bw/day. It is based upon a no observed adverse effect level (NOAEL) of 1.0 mg/kg bw/day from a 2-year oral toxicity study in rats. Safety factor 100.

B.7.15.1 Calculation of Theoretical Maximum Daily Intake (TMDI)

Estimation of chronic exposure through diet

WHO European model

The TMDI based upon the WHO European regional diet was calculated applying proposed MRLs. The results are shown in Table B.7.15.1-1. The total TMDI is 0.00040 mg/kg bw/day (adult, 60 kg bw). The result indicates that 4.0% of the ADI is accounted for.

Table B.7.15.1-1. Theoretical Maximum Daily Intake (TMDI) according to the WHO European model (adult, 60 kg).

Commodity	European intake (kg/person/day)	MRL (mg/kg)	TMDI (mg/day)
Carrots	0.022	0.2	0.0044
Pome fruits	0.0514	0.3	0.01542
Wheat	0.178	0.02	0.00356
Barley	0.0198	0.02	0.000396
Oats	0.002	0.02	0.00004
Rye flour	0.0015	0.02	0.00003

Total exposure (mg/person/day)	0.023846
Total exposure (mg/kg bw/day)	0.00040
Proposed ADI (mg/kg bw/day)	0.01
Total dietary exposure (% of ADI)	4.0%

BBA Guideline

The TMDIs for a 4 to 6 year old female child (13.5 kg bw) were calculated according to BBA Guideline Part IV, 3-7 (1993), applying proposed MRLs. The results are shown in Table B.7.15.1-2. The TMDI for a female child is 0.001357 mg/kg bw/day. The results indicate that 13.6% of the ADI is accounted for.

Table B.7.15.1-2. Theoretical Maximum Daily Intake (TMDI) according to German BBA guideline (4-6 year old girl).

Commodity	European intake (kg/child/day)	MRL (mg/kg)	TMDI (mg/day)
Carrots	0.0086	0.2	0.00172
Pome fruits	0.0486	0.3	0.01458
Flour	0.0917	0.02	0.001834
Pasta	0.0088	0.02	0.000176
Bran	0.0002	0.02	0.000004

Total exposure (mg/child/day)	0.018314
Total exposure (mg/kg bw/day)	0.001357
Proposed ADI (mg/kg bw/day)	0.01
Total dietary exposure (% of ADI)	13.6 %

UK Consumer Exposure Model (average of extreme consumers)

The TMDIs for 16 – 64+ year adults (70.1 kg bw), for 10/11 and 14/15 year schoolchildren (43.6 kg bw), for 1.5 – 4.5 year toddlers (14.5 kg bw) and for 6 – 12 month infants (8.7 kg bw) were calculated according to the PSD Guidance on the estimation of dietary intakes of pesticides residues, Part Three/A3/Appendix 1c (1999). Results of the calculations are shown in Tables B.7.15.1-3 – B.7.15.1-7.

Table B.7.15.1-3. Theoretical Maximum Daily Intake (TMDI) according to UK consumer exposure model (16-64+ year adults, 70.1 kg).

Commodity	European intake (kg/person/day)	MRL (mg/kg)	TMDI (mg/day)
Carrots	0.0569	0.2	0.01138
Pome fruits	0.1703	0.3	0.05109
Wheat	0.25	0.02	0.005
Barley	0.0236	0.02	0.000472
Oats	0.0409	0.02	0.000818
Bran	0.0156	0.02	0.000312

Total exposure (mg/person/day)	0.069072
Total exposure (mg/kg bw/day)	0.000985
Proposed ADI (mg/kg bw/day)	0.01
Total dietary exposure (% of ADI)	9.9 %

Table B.7.15.1-4. Theoretical Maximum Daily Intake (TMDI) according to UK consumer exposure model (10/11 and 14/15 year schoolchildren, 43.6 kg).

Commodity	European intake (kg/child/day)	MRL (mg/kg)	TMDI (mg/day)
Carrots	0.0336	0.2	0.00672
Pome fruits	0.1357	0.3	0.04071
Wheat	0.221	0.02	0.00442
Barley	0.0036	0.02	0.000072
Oats	0.0304	0.02	0.000608
Bran	0.0216	0.02	0.000432

Total exposure (mg/child/day)	0.052962
Total exposure (mg/kg bw/day)	0.0012147
Proposed ADI (mg/kg bw/day)	0.01
Total dietary exposure (% of ADI)	12.1%

Table B.7.15.1-5. Theoretical Maximum Daily Intake (TMDI) according to UK consumer exposure model (1.5-4.5 year toddlers, 14.5 kg).

Commodity	European intake (kg/toddler/day)	MRL (mg/kg)	TMDI (mg/day)
Carrots	0.0381	0.2	0.00762
Pome fruits	0.2232	0.3	0.06696
Wheat	0.1053	0.02	0.002106
Barley	L/C	0.02	0.000000
Oats	0.0197	0.02	0.000394

Total exposure (mg/toddler/day)	0.07708
Total exposure (mg/kg bw/day)	0.00532
Proposed ADI (mg/kg bw/day)	0.01
Total dietary exposure (% of ADI)	53.2 %

L/C = Low % consumers (less than 60 consumers in survey).

Table B.7.15.1-6. Theoretical Maximum Daily Intake (TMDI) according to UK consumer exposure model (6-12 month infants, 8.7 kg).

Commodity	European intake (kg/infant/day)	MRL (mg/kg)	TMDI (mg/day)
Carrots	0.0294	0.2	0.00588
Pome fruits	0.0874	0.3	0.02622
Wheat	0.0614	0.02	0.001228
Barley	N/C	0.02	0.000000
Oats	0.0182	0.02	0.000364
Bran	N/C	0.02	0.000000

Total exposure (mg/infant/day)	0.033692
Total exposure (mg/kg bw/day)	0.003873
Proposed ADI (mg/kg bw/day)	0.01
Total dietary exposure (% of ADI)	38.7%

N/C = No consumers in survey.

Table B.7.15.1-7. Estimation of the potential exposure through the diet.

Model	Consumer Group	Total TMDI (mg/kg bw/day)	ADI (mg/kg bw/day)	Total TMDI in % of ADI
WHO (1997)	Adult (60 kg bw)	0.00040	0.01	4.0
German BBA (1993)	Girl (13.5 kg bw)	0.00136	0.01	13.6
UK PSD (1999)[a]	Adult (70.1 kg bw)	0.00099	0.01	9.9
	Child (43.6 kg bw)	0.00121	0.01	12.1
	Toddler (14.5 kg bw)	0.00532	0.01	53.2
	Infant (8.7 kg bw)	0.00387	0.01	38.7

[a]average of extreme consumers

Comments by RMS: The WHO European model, the German BBA and the UK PSD consumer exposure models lead to low TMDI values and the contribution to the proposed ADI of 0.01 mg/kg bw/day is of maximum 10% for adults, 12% for schoolchildren, 53% for toddlers and 39% of the ADI for infants. The potential chronic dietary exposure poses no risk to the consumers. If however, the intended use of difenoconazole will be extended the conclusion may be re-evaluated.

B.7.15.2 Calculation of National Estimated Daily Intake (NEDI)

The calculations of the IEDI (international estimated daily intake) and NEDI are based on the long term consumption of wheat, pome fruits and carrots. These calculations give conservative estimates of intake because they assume that 100% of crops with proposed uses will be treated and contain residues with levels at the Supervised Trials Median Residue (STMR). The used STMR values in the present calculation were the higher of the values from either Northern or Southern Europe. In the case of pome fruit , the Southern Europe results were used. For carrot, the Northern Europe results were used (see Table B.7.6-6).

WHO European model

The IEDI based upon the WHO European regional diet was calculated applying STMRs. The results are shown in Table B.7.15.2-1. The total IEDI is 0.00018 mg/kg bw/day (adult, 60 kg bw). The result indicates that 1.8% of the ADI is accounted for.

Table B.7.15.2-1. International Estimated Daily Intake (IEDI) according to the WHO European model (adult, 60 kg).

Commodity	European intake (kg/person/day)	STMR (mg/kg)	IEDI (mg/day)
Carrots	0.022	0.05	0.0011
Pome fruits	0.0514	0.11	0.0056
Wheat	0.178	0.02	0.00356
Barley	0.0198	0.02	0.000396
Oats	0.002	0.02	0.00004
Rye flour	0.0015	0.02	0.00003

Total exposure (mg/person/day)	0.01073
Total exposure (mg/kg bw/day)	0.00018
Proposed ADI (mg/kg bw/day)	0.01
Total dietary exposure (% of ADI)	1.8%

BBA Guideline

The NEDIs for a 4 to 6 year old female child (13.5 kg bw) were calculated according to BBA Guideline Part IV, 3-7 (1993), applying STMRs. The results are shown in Table B.7.15.2-2. The NEDI for a female child is 0.0006 mg/kg bw/day. The results indicate that 5.8% of the ADI is accounted for.

Table B.7.15.2-2. National Estimated Daily Intake (NEDI) according to German BBA guideline (4-6 year old girl).

Commodity	European intake (kg/child/day)	STMR (mg/kg)	NEDI (mg/day)
Carrots	0.0086	0.05	0.00043
Pome fruits	0.0486	0.11	0.00534
Flour	0.0917	0.02	0.00183
Pasta	0.0088	0.02	0.00017
Bran	0.0002	0.02	0.000004

Total exposure (mg/child/day)	0.00781
Total exposure (mg/kg bw/day)	0.00058
Proposed ADI (mg/kg bw/day)	0.01
Total dietary exposure (% of ADI)	5.8%

UK Consumer Exposure Model

The NEDIs for 16 – 64+ year adults (70.1 kg bw), for 10/11 and 14/15 year schoolchildren (43.6 kg bw), for 1.5 – 4.5 year toddlers (14.5 kg bw) and for 6 – 12 month infants (8.7 kg bw) were calculated according to the PSD Guidance on the estimation of dietary intakes of pesticides residues, Part Three/A3/Appendix 1c (1999). Results of the calculations are shown in Tables B.7.15.2-3 – B.7.15.2-7.

Table B.7.15.2-3. National Estimated Daily Intake (NEDI) according to UK consumer exposure model (16-64+ year adults, 70.1 kg).

Commodity	European intake (kg/person/day)	STMR (mg/kg)	NEDI (mg/day)
Carrots	0.0569	0.05	0.0028
Pome fruits	0.1703	0.11	0.0187
Wheat	0.25	0.02	0.005
Barley	0.0236	0.02	0.000472
Oats	0.0409	0.02	0.000818
Bran	0.0156	0.02	0.000312

Total exposure (mg/person/day)	0.0281
Total exposure (mg/kg bw/day)	0.00040
Proposed ADI (mg/kg bw/day)	0.01
Total dietary exposure (% of ADI)	4.0%

Table B.7.15.2-4. National Estimated Daily Intake (NEDI) according to UK consumer exposure model (10/11 and 14/15 year schoolchildren, 43.6 kg).

Commodity	European intake (kg/child/day)	STMR (mg/kg)	NEDI (mg/day)
Carrots	0.0336	0.05	0.00168
Pome fruits	0.1357	0.11	0.01492
Wheat	0.221	0.02	0.00442
Barley	0.0036	0.02	0.000072
Oats	0.0304	0.02	0.000608
Bran	0.0216	0.02	0.000432

Total exposure (mg/child/day)	0.022132
Total exposure (mg/kg bw/day)	0.00051
Proposed ADI (mg/kg bw/day)	0.01
Total dietary exposure (% of ADI)	5.1%

Table B.7.15.2-5. National Estimated Daily Intake (NEDI) according to UK consumer exposure model (1.5-4.5 year toddlers, 14.5 kg).

Commodity	European intake (kg/toddler/day)	STMR (mg/kg)	NEDI (mg/day)
Carrots	0.0381	0.05	0.00190
Pome fruits	0.2232	0.11	0.02455
Wheat	0.1053	0.02	0.002106
Barley	L/C	0.02	0.000000
Oats	0.0197	0.02	0.000394

Total exposure (mg/toddler/day)	0.02895
Total exposure (mg/kg bw/day)	0.00200
Proposed ADI (mg/kg bw/day)	0.01
Total dietary exposure (% of ADI)	20%

L/C = Low % consumers (less than 60 consumers in survey).

Table B.7.15.2-6. National Estimated Daily Intake (NEDI) according to UK consumer exposure model (6-12 month infants, 8.7 kg).

Commodity	European intake (kg/infant/day)	STMR (mg/kg)	NEDI (mg/day)
Carrots	0.0294	0.05	0.00147
Pome fruits	0.0874	0.11	0.00961
Wheat	0.0614	0.02	0.001228
Barley	N/C	0.02	0.000000
Oats	0.0182	0.02	0.000364
Bran	N/C	0.02	0.000000

Total exposure (mg/infant/day)	0.012672
Total exposure (mg/kg bw/day)	0.001457
Proposed ADI (mg/kg bw/day)	0.01
Total dietary exposure (% of ADI)	14.6%

N/C = No consumers in survey.

Table B.7.15.2-7. Estimation of the potential exposure through the diet.

Model	Consumer Group	Total NEDI (mg/kg bw/day)	ADI (mg/kg bw/day)	Total NEDI in % of ADI
WHO (1997)	Adult (60 kg bw)	0.00018	0.01	1.8%
German BBA (1993)	Girl (13.5 kg bw)	0.0006	0.01	5.8 %
UK PSD (1999)[a]	Adult (70.1 kg bw)	0.00040	0.01	4.0 %
	Child (43.6 kg bw)	0.000514	0.01	5.1%
	Toddler (14.5 kg bw)	0.00200	0.01	20 %
	Infant (8.7 kg bw)	0.001457	0.01	14.6%

[a]average of extreme consumers

Comments by RMS: The calculated IEDI accounts for 1.8% of the ADI. The German BBA and the UK PSD consumer exposure models lead to low NEDI values and the contribution to the proposed ADI of 0.01 mg/kg bw/day is of maximum 4.0% for adults, 5.1% for schoolchildren, 20% for toddlers and 14.6% of the ADI for infants. The potential chronic dietary exposure poses no risk to the consumers. If however, the intended use of difenoconazole will be extended the conclusion may be re-evaluated.

The NEDI was approximately 2-fold lower than the TMDI value.

B.7.15.3 Acute Reference Dose (ARfD)

The acute reference dose (ARfD) represents the level at or below which dietary exposure over a short period (generally taken to be one day) will not pose appreciable risk to human health. The ARfD for difenoconazole has been proposed at 0.20 mg/kg bw/ day. It is based upon a no observed adverse effect level (NOAEL) of 20 mg/kg bw/day from a 90-day oral toxicity study in rats.

B.7.15.4 Calculation of National Estimate of Short-Term Intake (NESTI)

Estimation of acute exposure through diet

The calculation of the NESTI is based on the short term consumption of cereals, pome fruit, carrot and processed commodities. The highest residue (HR) values used were the higher of the values from either Northern or Southern Europe (Table B.7.6-6). The calculation is based on the UK model using the UK acute consumption data for the relevant commodities.

Table B.7.15.4-1. NESTI according to UK consumer exposure model for adults (70.1 kg bw) and toddlers (14.5 kg bw).

Commodity	HR (mg/kg)	Unit weights (kg)[a]	V Factor[b]	Adults (70,1 kg bw)			Toddlers (14,5 kg bw)		
				Intake (kg/day)	NESTI (mg/kg bw/day)	% of ARfD	Intake (kg/day)	NESTI (mg/kg bw/day)	% of ARfD
* Pears	0.16	0.150	7	0.274	0.0027	1.3	0.279	0.0130	6.5
* Apples – fruit	0.28	0.112	7	0.308	0.0039	2.0	0.199	0.0168	8.4
** Apples – juice	0.20[c]		1	0.452	0.0013	0.6	0.559	0.0077	3.8
* Carrots	0.13	0.080	7	0.226	0.0013	0.6	0.104	0.0052	2.6
** Wheat	0.02		1	0.301	0.00009	0.04	0.128	0.00018	0.09
** Barley	0.02		1	0.043	0.000012	0.006	0.006	0.000008	0.04
** Oats	0.02		1	0.061	0.000017	0.01	0.063	0.000087	0.04
** Bran (wheat)	0.02		1	0.080	0.00002	0.01	0.045	0.00006	0.03
** Bread (total)	0.02		1	0.343	0.00010	0.05	0.045	0.00006	0.03

* Case 2a (where composite residue data do not reflect residue levels in the food commodity as consumed); ** Case 1 (where composite residue data reflect residue levels in the food commodity as consumed).
[a] Unit weight of the edible portion (kg).
[b] Variability factor.
[c] Highest residue on pome fruit multiplied by the mean transfer factor from fruit till juice till (TF 0.71; Table 7.7.2-2).

Comments by RMS: The calculated NESTI values are all considerably lower than the ARfD, with the highest short-term consumption being 8.4% of the ARfD for apples in toddlers. It is therefore concluded that there is negligible acute dietary risk posed by the consumption of difenoconazole residues in treated wheat, pome fruits and carrots.

B.7.16 Summary and evaluation of residue behaviour (Annex IIA 6.10; Annex IIIA 8.9)

Plant metabolism

Plant metabolism studies with [phenyl-^{14}C] or [triazole-^{14}C] difenoconazole were carried out in four crops, representing four crop groupings – cereals (wheat), root vegetables (potato), pulses/oilseeds (oilseed rape) and fruits (grape and tomato). The application methods were seed and foliar treatment for wheat and foliar treatment for potato, grape, tomato and oilseed rape. The intended uses for difenoconazole in the Southern and Northern Europe are on cereals (seed treatment), pome fruit (foliar treatment) and carrots (foliar treatment). The available plant metabolism studies meet the requirements of the Commission Directive 96/68/EC.

Difenoconazole was extensively degraded in wheat, potato, tomato, grape and oilseed rape with very similar pathways of metabolism in all four crop types. The primary metabolic process in all four crop types involves hydrolysis of the dioxolane ring to form the ketone CGA-205374, which is then reduced to the corresponding alcohol CGA-205375. Oxidation of CGA-205375 occurred resulting in cleavage of the alkyl bridge to form CGA- 189138 and CGA-71019 (≤10% of the TRR). Hydroxylation of parent compound and the metabolites CGA-205374 and CGA-205375 was also observed. Sugar conjugation of parent compound and hydroxylated metabolites, and conjugation of 1,2,4-triazole were observed as a secondary metabolism process. Conjugation of 1,2,4-triazole resulted in the formation of triazole alanine (CGA-131013), which was further degraded to triazole acetic acid (CGA-142856).

Animal metabolism

Metabolism studies were carried out using [phenyl-^{14}C] and [triazole-^{14}C] difenoconazole in lactating goats and laying hens. The test compound was administered orally in the diet at a concentrations of 5 and 100 mg/kg to the lactating goats and 5, 68 and 121mg/kg to the laying hens.

Difenoconazole was rapidly metabolised, with the majority of the applied radioactivity (up to 96.8% in laying hens and >88% in the lactating goats) excreted in the urine and faeces. Maximum radioactive residue levels were present in the liver and kidney, at 9.790 and 2.731mg difenoconazole equivalents/kg, respectively, in lactating goats and up to 4.660 and 2.247mg difenoconazole equivalents/kg, respectively, in laying hens.

Maximum residues of parent difenoconazole were detected in the liver and fat of the lactating goats and laying hens, at concentrations up to 0.891mg/kg (9.1% of the TRR) and 1.912 mg/kg (18.4% of the TRR), respectively. In other edible tissues, residues of parent difenoconazole were ≤0.107 mg/kg (2.2% of the TRR). In milk, residues of parent difenoconazole were up to 0.028 mg/kg (8.8% of the TRR) and up to 0.236 mg/kg (5.3% of the TRR) in egg yolk.

CGA-205375 was the major metabolite in the goats and hens, occurring at levels up to 7.127 mg/kg (72.8% TRR) in liver, 1.180 mg/kg (43.2% TRR) in kidney, 0.949 mg/kg (91.7% TRR) in fat, 0.423 mg/kg (91.4% TRR) in muscle and up to 0.130 mg/kg (34.4% TRR) in milk, egg white and egg yolk. 1,2,4-triazole CGA-71019 was transported preferentially to eggs and milk, occurring at levels of 0.182 mg/kg (67.7% TRR) and 0.043 mg/kg (32.3% TRR) in egg white and yolk, respectively and levels up to 0.022 mg/kg (5.8% TRR) in milk. Ring hydroxylated difenoconazole, CGA-205374 and CGA-205375 were observed in the goats at levels up to 0.235 mg/kg (3.9% TRR) in liver and 0.021 mg/kg (15.2% TRR) in milk.

The primary metabolic processes in each animal involves hydrolysis of the dioxolane ring to form the ketone CGA-205374, with subsequent reduction of CGA-205374 to give the corresponding alcohol CGA-205375 as a major metabolite. Oxidation of CGA-205374 resulted in cleavage of the alkyl bridge, leading to the formation of the acid CGA-186138 and 1,2,4-triazole CGA-71019. A second pathway involves hydroxylation of difenoconazole to form the hydroxylated CGA-205374 and CGA-205375. Sulphate ester, glycine and glucuronide conjugation were observed as secondary metabolism processes in the lactating goats. Hydroxy acetic acid difenoconazole and amino acid (glutamic acid/threonine) conjugates of CGA-189138 were also observed as urine specific metabolites. Similar pathways of metabolism were observed in lactating goats and laying hens and consequently a study in pig is not required.

Definition of the residue

Proposed residue definition (plants, plant products):

Based on the results of the metabolism studies in cereals (wheat), root vegetables (potatoes), fruits (tomatoes, grapevine) and pulses/oilseeds (oilseed rape), the proposed residue definition in plants is difenoconazole alone for both monitoring and risk assessment purposes. If, however, the intended use within the EU is to expand the use pattern to include foliar application to cereals and/or for use in oilseed rape, this conclusion should be re-evaluated.

Proposed residue of definition (animals, products of animal origin):

Based on the results of metabolism studies in the lactating goats and laying hens, the proposed definition of the residue in animals and products of animal origin is parent difenoconazole for risk assessment purposes. For the purposes of monitoring, the proposed definition in animals and products of animal origin is parent difenoconazole plus metabolite CGA-205375.

Residues resulting from supervised trials

A total of 25 individual trials in countries of the Northern EU region and 25 individual trials in countries of the Southern EU region, were conducted in cereals (wheat), pome fruit and carrot over eight seasons.

There were 14 trials in wheat; eight were conducted in Northern EU and six in Southern EU over a period of three seasons. Eight of these trials were decline trials. Difenoconazole was applied once to seeds of wheat as a FS formulation containing 12.5 to 30 g difenoconazole/L at application rates within ± 25% of the maximum GAP rate of 6 g a.i./100 kg seeds. In wheat in Northern EU, following one application to seeds at 4.64 to 6.1 g difenoconazole/100 kg seed, residues of difenoconazole 126 to 156 days after sowing were below the LOQ (<0.01 to <0.02 mg/kg) in grain and <0.01 to 0.05 mg/kg in straw. In wheat in Southern EU, following one application to seeds at 4.64 to 6.3 g difenoconazole/100 kg seed, residues of difenoconazole 99 to 147 days after sowing were below the LOQ (<0.01 to <0.02 mg/kg) in grain and <0.01 to 0.05 mg/kg in straw.

There were 20 trials in pome fruit (apple and pear); nine were conducted in Northern EU and eleven in Southern EU over three seasons. Ten of these trials were decline trials. Difenoconazole was applied four times as an EC formulation containing 250 g difenoconazole/L at application rates within ± 25% of the maximum GAP rate of 56.25 g a.i./ha in Northern and 75 g a.i./ha in Southern EU. In three decline studies conducted in Northern EU, residues of difenoconazole in fruit were 0.06 to 0.16 mg/kg immediately after the final application, declining slowly to 0.04 to 0.17 mg/kg after 7 days, 0.04 to 0.10 mg/kg after 14 days and 0.01 to 0.07 mg/kg after 27 to 31 days. In Southern EU (7 trials), residues of difenoconazole in fruit were 0.12 to 0.29 mg/kg immediately after the final application. At some sites residues did not significantly decline and at others only declined very slowly. In three of the seven trials conducted in Southern EU five applications (instead of 4) were applied at rates within ± 25% of the dosage of 75 g a.i./ha (72 to 77 g a.i./ha). In pome fruit in Northern EU, residues of difenoconazole at harvest 27 to 31 days after application (i.e. within ± 25% of the PHI of 28 days) were 0.01 to 0.07 mg/kg. In

Southern EU, residues of difenoconazole at harvest 14 days after application (i.e. ± 25% of the PHI of 14 days) were 0.04 to 0.28 mg/kg.

There were 16 trials in carrots; eight were conducted in Northern EU and eight in Southern EU over six seasons. Eight of these trials were decline trials. Difenoconazole was applied three times (in two trials four times) as an EC formulation containing 250 g difenoconazole/L at application rates of 115 to 130 g a.i./ha (i.e. within ± 25% of the maximum GAP rate of 125 g a.i./ha). In carrot in Northern EU, residues of difenoconazole 13 or 14 days after application (i.e. within ± 25% of the PHI of 14 days) were 0.02 to 0.12 mg/kg. In Southern EU, residues of difenoconazole 14 or 15 days after application (i.e. ± 25% of the PHI of 14 days) were <0.02 to 0.13 mg/kg.

Storage stability of residues prior to analysis

Residues of difenoconazole in tomato fruit, potato tubers, cottonseed, cottonseed oil, cottonseed meal, wheat forage, wheat straw and wheat grain will be stable for at least 24 months, in lettuce head, soybeans, whole bananas, eggs, milk, poultry breast and beef liver for at least 12 months and at least 10 months in blood, fat, milk and tissues from dairy cattle when stored at <-18°C. CGA 205375 was shown to be stable in animal commodities for at least 10 months stored at <-18°C.

Effects of industrial processing and/or household preparation on the nature and magnitude of residues

There was no significant hydrolysis of difenoconazole following incubation at different pH values and temperatures. Difenoconazole is therefore stable under conditions representative of pasteurisation, baking, brewing and boiling, and sterilisation.

In studies to determine the effect of processing on residue levels, residues of difenoconazole in apple were reduced by washing (mean transfer factor 0.8) and were not concentrated in juice. Residues of difenoconazole were concentrated in wet pomace (mean transfer factor 4.5) and in dry pomace (mean transfer factor 15.7).

Since the maximum residue detected in carrots was 0.13 mg/kg, the notifier has also been investigated the effect of processing on difenoconazole residues in carrot. The samples have been analysed and the final report is being written. The estimated completion is mid-2006. The RMS suggest that this study could be included in an Addendum.

Residues in succeeding or rotational crops

Rotational crop studies were carried out in range of crops (wheat, sugar beet, maize, lettuce, turnips and mustard). The metabolism was determined to be the same as in tomato, wheat, potato, grapes and oilseed rape target crops, with the exception of the absence of parent difenoconazole and the presence of the metabolite CGA-205369 (triazole lactic acid) in the rotational crops.

The uptake of radioactive residues in succeeding crops following [triazole-^{14}C] difenoconazole application at 125 g a.i./ha was higher than observed in the corresponding [phenyl-^{14}C] difenoconazole study. The TRR levels in lettuce, sugar beet (tops and roots), immature wheat, and immature maize were equal to or less than 0.072 mg/kg. Residues in wheat grain and maize grain at harvest were 0.341 and 0.211 mg/kg, respectively.

The magnitude of these residues was due to the selective transport of triazole derivatives (CGA-131013, CGA-142856 and CGA-205369) from the soil.

Following application equivalent to twice the maximum recommended rate for carrots in Northern and Southern Europe (3 x 125 g a.i./ha), residues of difenoconazole were below the LOD (<0.02 and <0.05 mg/kg). Although the PHI was not within 25% of the critical GAP in Northern and Southern Europe (14 vs. 30), the exaggerated application rate of 750 g a.i./ha represents a worst-case for residues of difenoconazole in rotational crops and in commercial practice residues of difenoconazole will not be expected in succeeding crops.

Livestock feeding

A livestock feeding study was conducted in dairy cows using dose levels of 1, 3 and 10 mg/kg in the diet. Following dosing at exaggerated levels, residues of difenoconazole (the toxicologically significant residue in animal tissues) were below 5 µg/L in the milk samples taken on all days during the feeding period from all treatment groups. No measurable residues of difenoconazole (< 0.01 mg/kg) were found in all tissues samples with the exception of liver, which contained a residue of 0.01 mg/kg following administration of 10 mg/kg. Residues of difenoconazole in blood were less than 10 µg/L. Following the use of difenoconazole according to the proposed GAP, residues of difenoconazole in liver are not expected to exceed 0.01 mg/kg.
Residues of CGA-205375 in the 1 mg/kg feeding level were less than the LOQ in all tissues apart from liver (0.04 mg/kg) and fat (0.01 mg/kg).

A new feeding study in dairy cattle has recently being conducted. The samples have being analysed for parent difenoconazole and the metabolite CGA-205375 using updated analytical methods. Additionally, the level of 1,2,4-triazole in the samples has also being determined. The estimated completion of the final report for the study is mid-2006. The RMS suggest that this study could be included in an Addendum.

A livestock feeding study in poultry is not considered to be required, since the calculated dietary burden for poultry is much less than the trigger value of 0.1 mg/kg in the feed (0.016 mg/kg feed). However, the transfer of residues of difenoconazole from poultry into tissues and eggs has been investigated in a new study. The level of 1,2,4-triazole in the tissues and eggs after feeding of difenoconazole has also being determined. The samples have been analysed and the final report is currently being written. The estimated completion of the final report for the study is mid-2006. The RMS suggest that this study could be included in an Addendum.

A livestock feeding study in pigs is not considered to be required since the metabolism of difenoconazole in ruminants and poultry is considered to be substantially equivalent.

Pre-harvest intervals (PHI)

The proposed pre-harvest interval (PHI) in pome fruit is 28 days in Northern EU regions and 14 days in Southern EU regions. In carrots, the PHI is 14 days in Northern and Southern EU. Difenoconazole is applied as seed treatment to cereals pre-sowing and a PHI for wheat is not applicable.

Withholding periods

There is no requirement for any re-entry, withholding or waiting periods.

Proposed EU MRLs

In pome fruit, there were nine trials in Northern EU and eleven in Southern EU conducted according to the proposed GAP. Residue levels were higher in Southern EU than in Northern EU. Based on the residues in Southern EU (which represents the worst-case), a MRL of 0.3 mg/kg is proposed for difenoconazole in pome fruit

In carrot, there were eight trials in Northern EU and eight in Southern EU conducted according to the proposed GAP. Residue levels were similar in both regions. No single residue value measured in the supervised trials was above 0.2 mg/kg. A MRL of 0.2 mg/kg is proposed for difenoconazole in carrot.

In wheat, there were eight trials in Northern EU and six trials in Southern EU conducted according to the proposed GAP. Residue levels in wheat grain were similar in both regions. No single residue value measured in the supervised trials was above the LOQ of 0.02 mg/kg . A MRL of 0.02 mg/kg is proposed for difenoconazole in wheat grain. Since wheat treatment residue trial data extrapolates to barley, oats, rye and triticale, the trials summarised are suitable for the proposal of a MRL for cereals.

Crops	MRL (mg/kg)	STMR (mg/kg)
Pome fruit	0.3	0.11
Carrot	0.2	0.05
Wheat, Barley, Oat, Triticale, Rye - Grain	0.02	0.02

Maximum residues of difenoconazole in human edible commodities of succeeding crops grown in rotation after cereals (seed treatment) and carrot (foliar application) are not expected to exceed 0.01 mg/kg. Therefore, it is not considered necessary to set MRLs for succeeding crops.

No MRLs are being proposed for products of animal origin. Proposals for MRLs for products of animal origin will be made in the EU MRL submission.

Consumers risk assessment

The WHO European model, the German BBA and the UK PSD consumer exposure models lead to low TMDI values and the contribution to the proposed ADI of 0.01 mg/kg bw/day is of maximum 10% for adults, 12% for schoolchildren, 53% for toddlers and 39% of the ADI for infants. The potential chronic dietary exposure poses no risk to the consumers. If however, the intended use of difenoconazole will be extended the conclusion may be re-evaluated.

National Estimated Maximum Daily Intake (NEDI)

The calculated IEDI accounts for 1.8% of the ADI. The German BBA and the UK PSD consumer exposure models lead to low NEDI values and the contribution to the proposed ADI of 0.01 mg/kg bw/day is of maximum 4.0% for adults, 5.1% for schoolchildren, 20% for toddlers and 14.6% of the ADI for infants. The potential chronic dietary exposure poses no risk to the consumers. If however, the intended use of difenoconazole will be extended the conclusion may be re-evaluated.

Acute Reference Dose (ARfD)

The ARfD for difenoconazole has been proposed at 0.20 mg/kg bw/ day.

National Estimate of Short-Term Intake (NESTI)

The calculated NESTI values are all considerably lower than the ARfD, with the highest short-term consumption being 8.4% of the ARfD for apples in toddlers. It is therefore concluded that there is negligible acute dietary risk posed by the consumption of difenoconazole residues in treated cereals, pome fruit and carrot commodities.

B.7.17 References relied on

Annex point/ reference number	Author (s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N *	Owner **
IIA 6.0/01	Beidler WT.	1991a	Stability of CGA-169374 residues in potatoes under freezer storage conditions for 2 years. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Corp., Greensboro, United States Report No ABR-90070. GLP, Unpublished. Syngenta File No CGA 169374/0453	N	Syngenta
IIA 6.0/02	Beidler WT.	1991b	Stability of CGA-169374 residues in potatoes under freezer storage conditions for 2 years. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Corp., Greensboro, United States Report No ABR-90069. GLP, Unpublished. Syngenta File No CGA 169374/0452	N	Syngenta
IIA 6.0/03	Beidler WT.	1992	Stability of CGA-169374 residues in lettuce, soybeans and wheat forage under freezer storage conditions for one year. Syngenta Crop Protection AG, Basel, Switzerland. Ciba-Geigy Corp., Greensboro, United States Report No ABR-91024. GLP, Unpublished. Syngenta File No CGA 169374/0617	N	Syngenta
IIA 6.0/04	Kühne-Thu H.	1994	Residue stability of CGA-169374 (difenoconazole) in banana (whole fruit) under freezer storage conditions. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd. Basel, Switzerland. Report No 125/93. GLP, Unpublished. Syngenta File No CGA 169374/0934	N	Syngenta
IIA 6.0/05	Hayworth CG.	1998	Stability of CGA-169374 fortified into wheat and cotton substrates under freezer conditions. Novartis Crop Protection AG, Basel, Switzerland. Novartis Crop Protection Inc., Greensboro, United States Report No ABR-98061. GLP, Unpublished. Syngenta File No CGA 169374/1644	N	Syngenta
IIA 6.0/06	Wurz REM.	1993a	Storage stability study of CGA-169374 in dairy and poultry tissues, eggs and milk under freezer storage conditions. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Corp., Greensboro, United States Report No ABR-93012. GLP, Unpublished. Syngenta File No CGA 169374/0795	N	Syngenta

Annex point/ reference number	Author (s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N *	Owner **
IIA 6.0/07 IIA 6.4/01	Tribolet R.	2000	Residue of difenoconazole (CGA 169374) and its metabolite CGA 205375 in milk, blood, and tissues (muscle, fat, liver, kidney) of dairy cattle resulting from feeding of difenoconazole at three dose levels. Novartis Crop Protection AG, Basel, Switzerland. Report No 202/99. GLP, Unpublished. Syngenta File No CGA 169374/2039	Y	Syngenta
IIA 6.1/01	Madrid SO. Huber MK	1987a	The distribution and characterization of phenyl-14C vs. triazole 14C-CGA 169374 on spray treated tomatoes – a side by side comparison study in greenhouse. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Corp., Greensboro, United States Report No ABR-87025. Not GLP, Unpublished. Syngenta File No CGA 169374/0043	N	Syngenta
IIA 6.1/02	Madrid SO. Huber MK	1987b	The distribution and characterization of phenyl-14C vs. triazole 14C-CGA 169374 on their metabolites in field grown tomatoes. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Corp., Greensboro, United States Report No ABR-87033. Not GLP, Unpublished. Syngenta File No CGA 169374/0044	N	Syngenta
IIA 6.1/03	Velagaleti PR.	1990a	Metabolism of triazole-14C-CGA 169374 in spray-treated tomatoes. Novartis Crop Protection AG, Basel, Switzerland. Battelle, Columbus, United States Report No N-0964-0600. GLP, Unpublished. Syngenta File No CGA 169374/0355	N	Syngenta
IIA 6.1/04	Schweitzer MG	1990a	Metabolism of phenyl-14C-CGA 169374 in spray-treated tomatoes. Novartis Crop Protection AG, Basel, Switzerland. Battelle, Columbus, United States Report No N-0964-0700. GLP, Unpublished. Syngenta File No CGA 169374/0356	N	Syngenta
IIA 6.1/05	Hubbard L.	1991a	Uptake and metabolism of 14C-CGA 169374 by wheat resulting from seed treatment application under field conditions. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Corp., Greensboro, United States Report No ABR-90009. GLP, Unpublished. Syngenta File No CGA 169374/0415	N	Syngenta

Annex point/ reference number	Author (s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N *	Owner **
IIA 6.1/06	Hubbard L.	1991b	Uptake and metabolism of 14C-CGA 169374 by wheat resulting from seed treatment application under greenhouse conditions. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Corp., Greensboro, United States Report No ABR-90010. GLP, Unpublished. Syngenta File No CGA 169374/0416	N	Syngenta
IIA 6.1/07	Hubbard L.	1991c	Uptake and metabolism of 14C-CGA 169374 by wheat resulting from foliar spray application under greenhouse environment. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Corp., Greensboro, United States Report No ABR-90011. GLP, Unpublished. Syngenta File No CGA 169374/0417	N	Syngenta
IIA 6.1/08	Schweitzer MG	1990b	Metabolism of phenyl-14C-CGA 169374 in spray-treated potatoes. Novartis Crop Protection AG, Basel, Switzerland. Battelle, Columbus, United States Report No N-0964-0400. GLP, Unpublished. Syngenta File No CGA 169374/0357	N	Syngenta
IIA 6.1/09	Velagaleti PR.	1990b	Metabolism of triazole-14C-CGA 169374 in spray-treated potatoes. Novartis Crop Protection AG, Basel, Switzerland. Battelle, Columbus, United States Report No N-0964-0500. GLP, Unpublished. Syngenta File No CGA 169374/0489	N	Syngenta
IIA 6.1/10	Capps T.	1992	Uptake and metabolism of 14C-CGA 169374 by grapes from foliar spray treatment. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Corp., Greensboro, United States Report No ABR-92003. GLP, Unpublished. Syngenta File No CGA 169374/0537	N	Syngenta
IIA 6.1/11	Neumann Ch.	1993a	Metabolism of [Phenyl-^{14}C] CGA-169374 in field grown spring rape. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd. Basel, Switzerland. Report No 11/93. GLP, Unpublished. Syngenta File No CGA 169374/0809	N	Syngenta
IIA 6.1/12	Neumann Ch.	1993b	Metabolism of [Triazole-^{14}C] CGA-169374 in field grown spring rape. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd. Basel, Switzerland. Report No 12/93. GLP, Unpublished. Syngenta File No CGA 169374/0810	N	Syngenta

Annex point/ reference number	Author (s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N *	Owner **
IIA 6.2/01	Madrid SO.	1988	Metabolism of triazole and phenyl-14C-CGA 169374 in lactating goats dosed daily for ten consecutive days. Novartis Crop Protection AG, Basel, Switzerland. [redacted] Report No ABR-88087. Not GLP, Unpublished. Syngenta File No CGA 169374/0234	N	Syngenta
IIA 6.2/02	Maynard MS.	1990a	[^{14}C] CGA-169374 phenyl and triazole label distribution, elimination and metabolism in goats. Novartis Crop Protection AG, Basel, Switzerland. [redacted] Report No ABR-89100. GLP, Unpublished. Syngenta File No CGA 169374/0379	N	Syngenta
IIA 6.2/03	Ray WJ.	1996	Metabolism of phenyl-14C-CGA 169374 in lactating goats. Novartis Crop Protection AG, Basel, Switzerland. [redacted] No ABR-95099. GLP, Unpublished. Syngenta File No CGA 169374/1232	N	Syngenta
IIA 6.2/04	Madrid SO.	1989	Metabolism of triazole and phenyl-14C-CGA 169374 in laying hens dosed daily for fourteen consecutive days. Novartis Crop Protection AG, Basel, Switzerland. [redacted] Report No ABR-89051. GLP, Unpublished. Syngenta File No CGA 169374/0270	N	Syngenta
IIA 6.2/05	Maynard MS.	1990b	[^{14}C] CGA-169374 phenyl and triazole label distribution, elimination and metabolism in hens. Novartis Crop Protection AG, Basel, Switzerland. [redacted] Report No ABR-89101. GLP, Unpublished. Syngenta File No CGA 169374/0364	N	Syngenta
IIA 6.2/06	Ray WJ.	2004	[Triazole-14C] CGA-169374- nature of the residue in laying hens. Syngenta Crop Protection AG, Basel, Switzerland. [redacted] Report No 786-02. GLP, Unpublished. Syngenta File No CGA 169374/2441	Y	Syngenta

Annex point/ reference number	Author (s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N *	Owner **
IIA 6.3.1/01	Pointurier R.	2000a	Residue study with CGA 293343 + CGA 173506 + CGA 169374 + Tefluthrin in or on spring wheat in north of France. Novartis Crop Protection AG, Basel, Switzerland. ADME – Bioanalyses, Aigues – Vives, France. Report No 9840501. GLP, Unpublished. Syngenta File No CGA 169374/2012	Y	Syngenta
IIA 6.3.1/02	Pointurier R.	2000b	Residue study with CGA 293343 + CGA 173506 + CGA 169374 + Tefluthrin in or on spring wheat in north of France. Novartis Crop Protection AG, Basel, Switzerland. ADME – Bioanalyses, Aigues – Vives, France. Report No 9840502. GLP, Unpublished. Syngenta File No CGA 169374/2013	Y	Syngenta
IIA 6.3.1/03	Pointurier R.	2000c	Residue study with CGA 293343 + CGA 173506 + CGA 169374 in or on spring wheat in north of France. Novartis Crop Protection AG, Basel, Switzerland. ADME – Bioanalyses, Aigues – Vives, France. Report No 9840601. GLP, Unpublished. Syngenta File No CGA 169374/2016	Y	Syngenta
IIA 6.3.1/04	Pointurier R.	2000d	Residue study with CGA 293343 + CGA 173506 + CGA 169374 in or on spring wheat in north of France. Novartis Crop Protection AG, Basel, Switzerland. ADME – Bioanalyses, Aigues – Vives, France. Report No 9840602. GLP, Unpublished. Syngenta File No CGA 169374/2017	Y	Syngenta
IIA 6.3.1/05	Simon P.	2002a	Determination of residues of difenoconazole after seed treatment with Dividend in spring wheat in Germany. Syngenta Crop Protection AG, Basel, Switzerland. Syngenta Agro GmbH, Maintal, Germany. Report No gwh 14101 GLP, unpublished. Syngenta File No CGA 169374/2300	Y	Syngenta
IIA 6.3.1/06	Simon P.	2002b	Determination of residues of difenoconazole after seed treatment with Dividend in spring wheat in Germany. Syngenta Crop Protection AG, Basel, Switzerland. Syngenta Agro GmbH, Maintal, Germany. Report No gwh 94101 GLP, unpublished. Syngenta File No CGA 169374/2301	Y	Syngenta

Annex point/ reference number	Author (s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N *	Owner **
IIA 6.3.1/07	Krainz A.	2003a	Residue study with difenoconazole (CGA 169374) in or on wheat in the United Kingdom. Syngenta, Jealott´s Hill, United Kingdom RCC Ltd., Itingen, Switzerland. Report No02-4014 GLP, unpublished. Syngenta File No CGA 169374/2413	Y	Syngenta
IIA 6.3.1/08	Krainz A.	2003b	Residue study with difenoconazole (CGA 169374) in or on wheat in the United Kingdom. Syngenta, Jealott´s Hill, United Kingdom RCC Ltd., Itingen, Switzerland. Report No 02-4015 GLP, unpublished. Syngenta File No CGA 169374/2414	Y	Syngenta
IIA 6.3.1/09	Pointurier R.	2000e	Residue study with CGA 293343 + CGA 173506 + CGA 169374 + Tefluthrin in or on spring wheat in south of France. Novartis Crop Protection AG, Basel, Switzerland. ADME – Bioanalyses, Aigues – Vives, France. Report No 9840503. GLP, Unpublished. Syngenta File No CGA 169374/2014	Y	Syngenta
IIA 6.3.1/10	Pointurier R.	2000f	Residue study with CGA 293343 + CGA 173506 + CGA 169374 + Tefluthrin in or on spring wheat in south of France. Novartis Crop Protection AG, Basel, Switzerland. ADME – Bioanalyses, Aigues – Vives, France. Report No 9840504. GLP, Unpublished. Syngenta File No CGA 169374/2015	Y	Syngenta
IIA 6.3.1/11	Pointurier R.	2000g	Residue study with CGA 293343 + CGA 173506 + CGA 169374 in or on spring wheat in south of France. Novartis Crop Protection AG, Basel, Switzerland. ADME – Bioanalyses, Aigues – Vives, France. Report No 9840603. GLP, Unpublished. Syngenta File No CGA 169374/2018	Y	Syngenta
IIA 6.3.1/12	Pointurier R.	2000h	Residue study with CGA 293343 + CGA 173506 + CGA 169374 in or on spring wheat in south of France. Novartis Crop Protection AG, Basel, Switzerland. ADME – Bioanalyses, Aigues – Vives, France. Report No 9840604. GLP, Unpublished. Syngenta File No CGA 169374/2019	Y	Syngenta

Annex point/ reference number	Author (s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N *	Owner **
IIA 6.3.1/13	Krainz A.	2003c	Residue study with difenoconazole (CGA 169374) in or on wheat in France (south). Syngenta, Jealott´s Hill, United Kingdom RCC Ltd., Itingen, Switzerland. Report No 02-4012 GLP, unpublished. Syngenta File No CGA 169374/2411	Y	Syngenta
IIA 6.3.1/14	Krainz A.	2003d	Residue study with difenoconazole (CGA 169374) in or on wheat in France (south). Syngenta, Jealott´s Hill, United Kingdom RCC Ltd., Itingen, Switzerland. Report No 02-4013 GLP, unpublished. Syngenta File No CGA 169374/2412	Y	Syngenta
IIA 6.3.2/01	Pointurier R.	2001a	Residue study with difenoconazole (CGA 169374) in or on apples in France (north). Syngenta Crop Protection AG, Basel, Switzerland. ADME – Bioanalyses, Vergèze, France. Report No 0012101. GLP, unpublished. Syngenta File No CGA 169374/2129	Y	Syngenta
IIA 6.3.2/02	Kühne-Thu H.	2001a	Residue study with difenoconazole (CGA-169374) in or on pears in Switzerland. Syngenta Crop Protection AG, Basel, Switzerland. Novartis Crop Protection AG, Basel, Switzerland. Report No 2089/00. GLP, unpublished. Syngenta File No CGA 169374/2155	Y	Syngenta
IIA 6.3.2/03	Kühne-Thu H.	2001b	Residue study with difenoconazole (CGA-169374) in or on apples in Switzerland. Syngenta Crop Protection AG, Basel, Switzerland. Novartis Crop Protection AG, Basel, Switzerland. Report No 2088/00. GLP, unpublished. Syngenta File No CGA 169374/2156	Y	Syngenta
IIA 6.3.2/04	Kühne-Thu H.	2002a	Residue study with difenoconazole (CGA-169374) in or on apples in Switzerland. Syngenta Crop Protection AG, Basel, Switzerland. Report No 2004/01. GLP, unpublished. Syngenta File No CGA 169374/2213	Y	Syngenta
IIA 6.3.2/05	Kühne-Thu H.	2002b	Residue study with difenoconazole (CGA-169374) in or on pears in Switzerland. Syngenta Crop Protection AG, Basel, Switzerland. Report No 2005/01. GLP, unpublished. Syngenta File No CGA 169374/2214	Y	Syngenta
IIA 6.3.2/06	Pointurier R.	2002a	Residue study with difenoconazole (CGA 169374) in or on apples in France (North). Syngenta Crop Protection AG, Basel, Switzerland. ADME – Bioanalyses, Vergèze, France. Report No 0110501. GLP, unpublished. Syngenta File No CGA 169374/2233	Y	Syngenta

Annex point/ reference number	Author (s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N *	Owner **
IIA 6.3.2/07	Krainz A.	2003e	Residue study with difenoconazole (CGA 169374) in or on apples in France (North). Syngenta, Jealott´s Hill, United Kingdom RCC Ltd., Itingen, Switzerland. Report No 02-2114 GLP, unpublished. Syngenta File No CGA 169374/2386	Y	Syngenta
IIA 6.3.2/08	Krainz A.	2003f	Residue study with difenoconazole (CGA 169374) in or on apples in France (north). Syngenta, Jealott´s Hill, United Kingdom RCC Ltd., Itingen, Switzerland. Report No 02-2083 GLP, unpublished. Syngenta File No CGA 169374/2387	Y	Syngenta
IIA 6.3.2/09	Krainz A.	2003g	Residue study with difenoconazole (CGA 169374) in or on apples in France (North). Syngenta, Jealott´s Hill, United Kingdom RCC Ltd., Itingen, Switzerland. Report No 02-2084 GLP, unpublished. Syngenta File No CGA 169374/2385	Y	Syngenta
IIA 6.3.2/10	Pointurier R.	2001b	Residue study with difenoconazole (CGA 169374) in or on apples in France (South). Syngenta Crop Protection AG, Basel, Switzerland. ADME – Bioanalyses, Vergèze, France. Report No 0012201. GLP, Unpublished. Syngenta File No CGA 169374/2130	Y	Syngenta
IIA 6.3.2/11	Kühne-Thu H.	2001c	Residue study with difenoconazole (CGA-169374) in or on apples in Greece. Syngenta Crop Protection AG, Basel, Switzerland. Report No 2042/00. GLP, unpublished. Syngenta File No CGA 169374/2175	Y	Syngenta
IIA 6.3.2/12	Kühne-Thu H.	2001d	Residue study with difenoconazole (CGA-169374) in or on apples in Italy. Syngenta Crop Protection AG, Basel, Switzerland. Report No 2036/00. GLP, unpublished. Syngenta File No CGA 169374/2176	Y	Syngenta
IIA 6.3.2/13	Kühne-Thu H.	2001e	Residue study with difenoconazole (CGA-169374) in or on apples in Spain. Syngenta Crop Protection AG, Basel, Switzerland. Report No 2025/00. GLP, unpublished. Syngenta File No CGA 169374/2192	Y	Syngenta
IIA 6.3.2/14	Kühne-Thu H.	2001f	Residue study with difenoconazole (CGA-169374) in or on apples in Spain. Syngenta Crop Protection AG, Basel, Switzerland. Report No 2026/00. GLP, unpublished. Syngenta File No CGA 169374/2193	Y	Syngenta

Annex point/ reference number	Author (s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N *	Owner **
IIA 6.3.2/15	Solé C.	2002a	Residue study with difenoconazole (CGA 169374) in or on apples in Italy. Syngenta Crop Protection AG, Basel, Switzerland. ADME – Bioanalyses, Vergèze, France. Report No 2070/01. GLP, Unpublished. Syngenta File No CGA 169374/2229	Y	Syngenta
IIA 6.3.2/16	Solé C.	2002b	Residue study with difenoconazole (CGA 169374) in or on apples in Greece. Syngenta Crop Protection AG, Basel, Switzerland. ADME – Bioanalyses, Vergèze, France. Report No 2019/01. GLP, Unpublished. Syngenta File No CGA 169374/2230	Y	Syngenta
IIA 6.3.2/17	Solé C.	2002c	Residue study with difenoconazole (CGA 169374) in or on pears in Greece. Syngenta Crop Protection AG, Basel, Switzerland. ADME – Bioanalyses, Vergèze, France. Report No 2020/01. GLP, Unpublished. Syngenta File No CGA 169374/2231	Y	Syngenta
IIA 6.3.2/18	Pointurier R.	2002a	Residue study with difenoconazole (CGA 169374) in or on apples in France (south). Syngenta Crop Protection AG, Basel, Switzerland. ADME – Bioanalyses, Vergèze, France. Report No 0110601. GLP, unpublished. Syngenta File No CGA 169374/2234	Y	Syngenta
IIA 6.3.2/19	Solé C.	2002d	Residue study with difenoconazole (CGA 169374) in or on apples in Spain. Syngenta Crop Protection AG, Basel, Switzerland. ADME – Bioanalyses, Vergèze, France. Report No 2096/01. GLP, Unpublished. Syngenta File No CGA 169374/2246	Y	Syngenta
IIA 6.3.2/20	Krainz A.	2003h	Residue study with difenoconazole (CGA 169374) in or on pears in France (south). Syngenta, Jealott´s Hill, United Kingdom RCC Ltd., Itingen, Switzerland. Report No 02-2085 GLP, unpublished. Syngenta File No CGA 169374/2388	Y	Syngenta
IIA 6.3.3/01	Kühne-Thu H.	1988	CGA-169374, 250 EC, carrots, soil, Switzerland. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd. Basel, Switzerland. Report No RR-2005-87. GLP, Unpublished. Syngenta File No CGA 169374/0096	N	Syngenta

Annex point/ reference number	Author (s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N *	Owner **
IIA 6.3.3/02	Kühne-Thu H.	1989	CGA-169374, 250 EC, carrots, soil, Switzerland. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd. Basel, Switzerland. Report No RR-2006-87. GLP, Unpublished. Syngenta File No CGA 169374/0097	N	Syngenta
IIA 6.3.3/03	Maffezzoni M.	1993a	CGA-169374, 250 EC, A-7402A, carrot, France. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy SA, Rueil-Malmaison, France. Report No OF91059. GLP, Unpublished. Syngenta File No CGA 169374/0865	N	Syngenta
IIA 6.3.3/05	Maffezzoni M.	1993b	CGA-169374, 250 EC, A-7402A, carrot, France. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy SA, Rueil-Malmaison, France. Report No OF91089. GLP, Unpublished. Syngenta File No CGA 169374/0866	N	Syngenta
IIA 6.3.3/05 IIA 6.3.3/09	Maffezzoni M.	1993c	CGA-169374, 250 EC, A-7402A, carrot, France. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy SA, Rueil-Malmaison, France. Report No OF92025. GLP, Unpublished. Syngenta File No CGA 169374/0864	N	Syngenta
IIA 6.3.3/06 IIA 6.3.3/10	Maffezzoni M.	1995	CGA-169374, 250 EC, F70464, carrots, France. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy SA, Rueil-Malmaison, France. Report No OF93153. GLP, Unpublished. Syngenta File No CGA 169374/1138	N	Syngenta
IIA 6.3.3/07	Pointurier R.	2001c	Residue study with difenoconazole (CGA 169374) in or on carrots in France (north). Syngenta Crop Protection AG, Basel, Switzerland. ADME – Bioanalyses, Vergèze, France. Report No 0011901. GLP, Unpublished. Syngenta File No CGA 169374/2124	Y	Syngenta
IIA 6.3.3/08	Pointurier R.	2001d	Residue study with difenoconazole (CGA 169374) in or on carrots in France (North). Syngenta Crop Protection AG, Basel, Switzerland. ADME – Bioanalyses, Vergèze, France. Report No 0012001. GLP, Unpublished. Syngenta File No CGA 169374/2127	Y	Syngenta

Annex point/ reference number	Author (s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N *	Owner **
IIA 6.3.3/11	Maffezzoni M.	1999a	Residue study with difenoconazole (CGA-169374) in or on carrots in south of France. Novartis Crop Protection AG, Basel, Switzerland. ADME-Bioanalyses, Aigues-Vives, France. Report No OF96134, Trial LD 77. GLP, Unpublished. Syngenta File No CGA 169374/1826	Y	Syngenta
IIA 6.3.3/12	Maffezzoni M.	1999b	Residue study with difenoconazole (CGA-169374) in or on carrots in south of France. Novartis Crop Protection AG, Basel, Switzerland. ADME-Bioanalyses, Aigues-Vives, France. Report No OF96134, Trial LD BY 18. GLP, Unpublished. Syngenta File No CGA 169374/1827	Y	Syngenta
IIA 6.3.3/13	Maffezzoni M.	1999c	Residue study with CGA-169374 in or on carrots in south of France. Novartis Crop Protection AG, Basel, Switzerland. ADME-Bioanalyses, Aigues-Vives, France. Report No OF96134, Trial LD AC 20. GLP, Unpublished. Syngenta File No CGA 169374/1828	Y	Syngenta
IIA 6.3.3/14	Pointurier R.	2001e	Residue study with difenoconazole (CGA 169374) in or on carrots in France (South). Syngenta Crop Protection AG, Basel, Switzerland. ADME – Bioanalyses, Vergèze, France. Report No 0011902. GLP, Unpublished. Syngenta File No CGA 169374/2125	Y	Syngenta
IIA 6.3.3/15	Pointurier R.	2001f	Residue study with difenoconazole (CGA 169374) in or on carrots in France (South). Syngenta Crop Protection AG, Basel, Switzerland. ADME – Bioanalyses, Vergèze, France. Report No 0011903. GLP, Unpublished. Syngenta File No CGA 169374/2126	Y	Syngenta
IIA 6.3.3/16	Pointurier R.	2001g	Residue study with difenoconazole (CGA 169374) in or on carrots in France (South). Syngenta Crop Protection AG, Basel, Switzerland. ADME – Bioanalyses, Vergèze, France. Report No 0012002. GLP, Unpublished. Syngenta File No CGA 169374/2128	Y	Syngenta
IIA 6.5.1/01	Muir GT.	2003	Difenoconazole: Aqueous hydrolysis at 90, 100 & 120 °C. Syngenta Crop Protection AG, Basel, Switzerland. Syngenta – Jealott´s Hill International, Bracknell, Berkshire, United Kingdom. Report No RJ3360B GLP, Unpublished. Syngenta File No CGA 169374/2312	Y	Syngenta

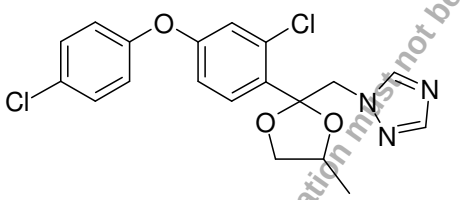
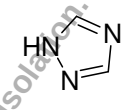
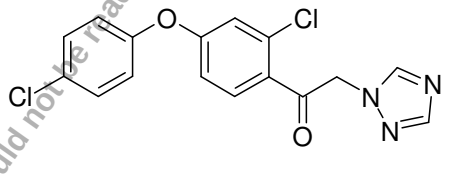
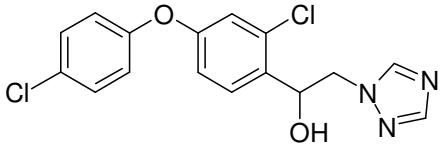
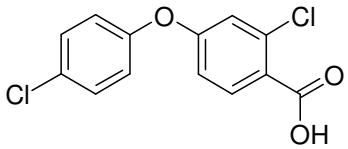
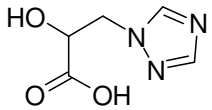
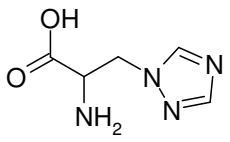
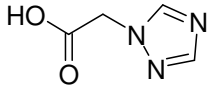
Annex point/ reference number	Author (s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N *	Owner **
IIA 6.5.2/01	Simon P.	2002c	Determination of residues of difenoconazole after application of Score in apples and processed commodities in Germany. Syngenta Crop Protection AG, Basel, Switzerland. Syngenta Agro GmbH, Maintal, Germany. Report No gap82901 GLP, unpublished. Syngenta File No CGA 169374/2282	N	Syngenta
IIA 6.5.2/02	Kühne-Thu H.	1997a	CGA-169374 (and metabolite CGA 205375), 250 EC, A-7402 G, apples and processed fractions, Chile. Novartis Crop Protection AG, Basel, Switzerland. Novartis Crop Protection AG, Basel, Switzerland. Report No 2205/94 GLP, Unpublished. Syngenta File No CGA 169374/1424	N	Syngenta
IIA 6.5.2/03	Kühne-Thu H.	1997b	CGA-169374 (and metabolite CGA 205375), 250 EC, A-7402 G, apples and processed fractions, Chile. Novartis Crop Protection AG, Basel, Switzerland. Novartis Crop Protection AG, Basel, Switzerland. Report No 2204/94 GLP, Unpublished. Syngenta File No CGA 169374/1426	N	Syngenta
IIA 6.5.2/04	Kühne-Thu H.	1997c	CGA-169374 (and metabolite CGA 205375), 250 EC, A-7402/780 A, apples and processed fractions, Brazil. Novartis Crop Protection AG, Basel, Switzerland. Novartis Crop Protection AG, Basel, Switzerland. Report No 2195/94 GLP, Unpublished. Syngenta File No CGA 169374/1431	N	Syngenta
IIA 6.6/01	Walser M.	1994a	Outdoor confined accumulation study on rotational crops after bare ground soil application of [^{14}C-phenyl]-CGA-169374. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Report No 8/94. GLP, Unpublished. Syngenta File No CGA 169374/0924	N	Syngenta
IIA 6.6/02	Walser M.	1994b	Outdoor confined accumulation study on rotational crops after bare ground soil application of [^{14}C-triazole]-CGA-169374. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Ltd., Basel, Switzerland. Report No 4/94. GLP, Unpublished. Syngenta File No CGA 169374/2395	N	Syngenta

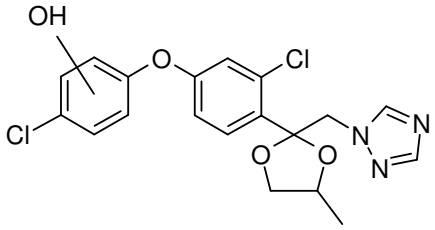
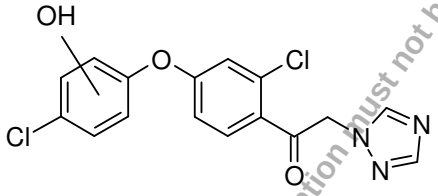
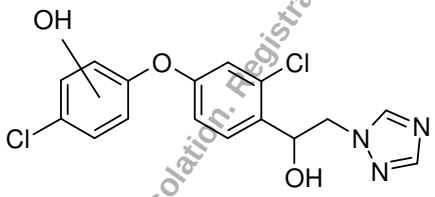
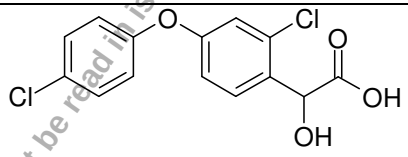
Annex point/ reference number	Author (s)	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N *	Owner **
IIA 6.6/03	Close C.	1995	14C-CGA-169374: Uptake and distribution of residues in confined rotational crops. Novartis Crop Protection AG, Basel, Switzerland. Ciba-Geigy Corp., Greensboro, United States Report No ABR-95057. GLP, Unpublished. Syngenta File No CGA 169374/1118	N	Syngenta
IIA 6.6/04	Heyer R.	1995a	CGA-169374 (metabolite CGA 131013), 250 EC, A-7402 G, rotational crop: carrot, soil, Germany. Novartis Crop Protection AG, Basel, Switzerland. RCC Umweltchemie GmbH & Co. KG, Rossdorf, Germany. Report No 488002. GLP, Unpublished. Syngenta File No CGA 169374/1215	N	Syngenta
IIA 6.6/05	Heyer R.	1995b	CGA-169374 (metabolite CGA 131013), 250 EC, A-7402 G, rotational crop: spinach, soil, Germany. Novartis Crop Protection AG, Basel, Switzerland. RCC Umweltchemie GmbH & Co. KG, Rossdorf, Germany. Report No 488001. GLP, Unpublished. Syngenta File No CGA 169374/1216	N	Syngenta

** Protection for 5 years claimed from date of decision concerning listing in Annex I - the study report has not been submitted to any of the Member States in support of an application for authorization or (though the study report has been submitted) has not been used in any of the Member States as the basis for decision on the initial authorization, or to maintain a given authorization of a plant protection product before the date of submission of the dossier to the Rapporteur Member State.*

*** Owner's code identifications and names (Code identification: SYN, Name: Syngenta.. TDMG, Name: Triazole Derivative Metabolite Group).*

APPENDIX I

Designation Synonyms	Chemical name (IUPAC and/or CA)	Structure	Study Metabolite Identified in
Difenoconazole CGA-169374	1-[2-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-4-methyl-[1,3]dioxolan-2-ylmethyl]-1H-[1,2,4]triazole or 1H-1,2,4-tiazole, 1-[[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]- (CA)		Soil Tomato Wheat Potato Grape Oilseed rape Goat Hen Rat
CGA-71019	1H-[1,2,4]triazole		Wheat Grape Oilseed rape Goat Hen Rat
CGA-205374	1-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-2-[1,2,4]triazol-1-yl-ethanone		Tomato Wheat Potato Grape Oilseed rape Goat Hen Rat
CGA-205375	1-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-2-[1,2,4]triazol-1-yl-ethanol		Tomato Wheat Potato Grape Oilseed rape Goat Hen Rat
CGA-189138	2-chloro-4-(4-chloro-phenoxy)-benzoic acid		Soil Tomato Potato Grape Oilseed rape Goat Hen Rat
Triazole lactic acid CGA 205369	[1,2,4]triazol-1-yl-lactic acid		Rotational crop
Triazole alanine CGA 131013	2-amino-3-[1,2,4]triazol-1-yl-propionic acid		Tomato Wheat Potato Oilseed rape Rotational crop
Triazole acetic acid CGA 142856	[1,2,4]triazol-1-yl-acetic acid		Wheat Potato Grape Oilseed rape Rotational crop

Designation Synonyms	Chemical name (IUPAC and/or CA)	Structure	Study Metabolite Identified in
Hydroxy-CGA-difenoconazole			Tomato Wheat Potato Goat Hen Rat
Hydroxy-CGA-205374			Tomato Wheat Goat
Hydroxy-CGA-205375			Tomato Wheat Potato Goat Hen Rat
NOA 448731	[2-chloro-4-(4-chloro-phenoxy)-phenyl]-hydroxy-acetic acid		Goat